doi: 10.2166/9781789063783_0141

Chapter 8

Controlling organic micropollutants in urban (waste) water treatment by activated carbon adsorption and membrane technology

---

Maria João Rosa, Margarida Campinas, Catarina Silva, Elsa Mesquita and Rui M. C. Viegas

Water Quality and Treatment Laboratory, Urban Water Unit, Hydraulics and Environment Department, LNEC – National Laboratory for Civil Engineering, 1700-066 Lisbon, Portugal
Corresponding author: mjrosa@lnec.pt, mcampinas@lnec.pt, csilva@lnec.pt, emesquita@lnec.pt, rviegas@lnec.pt

ABSTRACT

Population growth and aging, as well as water scarcity driven by climate change call for an enhanced control of organic micropollutants and contaminants of emerging concern (CECs) in the urban water cycle, as progressively entailed in the EU and Portuguese legal framework on drinking water, urban wastewater treatment (UWWT) and water reuse. Multibarrier solutions, grounded on current barriers' improvement, prioritizing low-energy, physical barriers (to minimize byproducts, resources' use, and carbon footprint) and producing fit-for-purpose water(s) are needed. Activated carbon(AC)-based and hybrid powdered activated carbon/low-pressure membrane processes have proven a huge potential for controlling organic micropollutants in drinking water treatment and water reclamation, but there is room for improving their sustainability and cost-efficiency with process optimization. This chapter aims to introduce the CECs' problem in drinking water and UWWT and comprehensively explain the potential for controlling CECs using AC adsorption and hybrid adsorption/low-pressure membrane processes, based on our last decade's lab-, pilot-, and full-scale results herein summarized and discussed.

Keywords: activated carbon adsorption, contaminants of emerging concern, drinking water, hybrid adsorption/membrane process, ceramic microfiltration, organic micropollutants, urban wastewater treatment

ACRONYMS AND ABBREVIATIONS

A254	UV absorbance at 254 nm
AC	activated carbon
AMX	amoxicillin (PhC)
AOC	assimilable organic carbon
AOP	advanced oxidation process
APAP	acetaminophen (PhC)

ARB	antibiotic-resistant bacteria
ARG	antibiotic resistance gene
AS	activated sludge
ATN	atenolol (PhC)
BAC	biologically active carbon
BEI	Beirolas (WWTP)
BET	Brunauer–Emmet–Teller
BOD_5	biochemical oxygen demand at 5 days
BTZ	bentazone (pesticide)
BZ	1-H-benzotriazole (anticorrosive)
BZF	bezafibrate (PhC)
C	carbon
CAF	caffeine (PhC)
CAPEX	capital expenditures
CBZ	carbamazepine (PhC)
CEB	chemically enhanced bakwash
CEC	contaminant of emerging concern
CFA	clofibric acid (PhC)
C/F/S	coagulation/flocculation/sedimentation
CHL	chlortoluron (pesticide)
COD	chemical oxygen demand
CP	chlorophene (biocide)
CTS	cortisone (PhC)
CYP	cyclophosphamide (PhC)
d_{50}	median diameter
D_{pH}	(octanol–water) distribution coefficient at a given pH
DAF	dissolved air flotation
DCF	diclofenac (PhC)
DT	DEET (N,N-diethyl–3-methylbenzamide) (insect repellent)
DES	diethylstilbestrol (hormone)
DIU	diuron (pesticide)
DMT	dimethoate (pesticide)
DOC	dissolved organic carbon
DPR	direct potable reuse
DW	drinking water
DWT	drinking water treatment
DWTP	drinking water treatment plant
E1	estrone (hormone)
E2	17-β-estradiol (hormone)
EE2	17α-ethinylestradiol (hormone)
E3	estriol (hormone)

EDC	endocrine-disrupting compound
EfOM	effluent organic matter
EQS	environmental quality standard
EQSD	environmental quality standard directive
ERY	erythromycin (PhC)
EU	European Union
FLX	fluoxetine (PhC)
F/M	food to microorganisms ratio
FNW	Faro Noroeste (WWTP)
GAC	granular activated carbon
GTD	gestodene (hormone)
HAA	haloacetic acid
HSDM	homogeneous surface diffusion model
IBUP	ibuprofen (PhC)
IND	indomethacin (PhC)
IPR	indirect potable reuse
ISO	International Standardisation Organisation
JRC	Joint Research Centre
k_{bio}	biodegradability coefficient
K_{d}	sorption onto biomass coefficient
K_{ow}	octanol–water partition coefficient
LIN	linuron (pesticide)
LINC	lincomycin (PhC)
lmh	L/(m^2.h)
MBR	membrane bioreactor
MC-LF	microcystin-LF
MC-LR	microcystin-LR
MC-LR$_{\mathrm{eq.}}$	microcystin-LR equivalent (overall concentration of all MC variants)
MC-LY	microcystin-LY
MC-LW	microcystin-LW
MF	microfiltration
μF	micro filter
MIB	2-methylisoborneol
ML	3-methylindole (fragrance)
MM	molar mass
MTPL	metoprolol (PhC)
MXL	mixed liquor
N	nitrogen
NF	nanofiltration
NH	nortriptyline.HCl (antidepressant)
NOM	natural organic matter

NPX	naproxen (PhC)
OPEX	operational expenditures
P	phosphorus
PAC	powdered activated carbon
PFAS	per- and poly-fluoroalkyl substance
pH_{pzc}	point of overall zero charge
PPNL	propranolol
PS	priority substance
PSA	polar surface area
PT	Portugal/Portuguese
RAN	ranitidine (PhC)
RO	reverse osmosis
SDG	sustainable development goal
SDZ	sulfadizine (PhC)
SF	sand filtration
SMX	sulfamethoxazole (PhC)
SPD	sulfapyridine (PhC)
SUVA	specific UV absorbance, given by A254/DOC
T254	transmittance at 254 nm
TCp	treatment capacity
TCZ	tebuconazole (pesticide)
THM	trihalomethane
THMFP	trihalomethane formation potential
TSS	total suspended solids
TTE	testosterone (hormone)
UF	ultrafiltration
UN	United Nations
UV	ultraviolet radiation
UWW	urban wastewater
UWWT	urban wastewater treatment
UWWTD	urban wastewater treatment directive
UWWTP	urban wastewater treatment plant, often referred simply as WWTP – wastewater treatment plant
VOC	volatile organic compound
WFD	Water Framework Directive
WHO	World Health Organisation
WL	Watch List
WTP	water treatment plant
WWTP	wastewater treatment plant

8.1 THE PROBLEM: ORGANIC MICROPOLLUTANTS IN WATER

Water is central to all human activities, to all components of the EU Green Deal and to several United Nations sustainable development goals (UN SDGs), starting with SDG 6 'Clean water and sanitation.' Furthermore, population growth and aging (SDGs 3, 11) and economic growth (SDG 8) are increasing the water demand and contaminants' discharge, while climate change (SDG 13) is decreasing source water availability (droughts and time concentrated rainfall) and increasing water demand (for irrigation, cleaning), thus promoting water exploitation and scarcity, and decreasing source water quality (increased organics, salts, toxic cyanobacterial blooms). These are reasons why we need to 'Take urgent action to combat climate change and its impacts' (SDG 13), 'Build resilient infrastructure' (SDG 9), promote 'Sustainable cities and communities' (SDG 11), carbon neutrality, energy self-sufficiency, resource efficiency, and circular economy, for example, water reuse [SDGs 6, 7 (energy), and 12 (responsible consumption)].

More than ever, effective, and efficient urban water systems are therefore crucial, and the drinking water treatment (DWT) and the urban wastewater treatment (UWWT) are, in addition to the pollution-source control, the key barriers for protecting human health, ecosystems, and their services against contaminants of health–environmental concern. These include regulated contaminants, but barriers must be prepared for addressing also contaminants of emerging concern (CECs).

As clearly proposed by Sauvé and Desrosiers (2014), CECs are naturally occurring or manufactured contaminants present or suspected to be present in various environmental compartments and whose toxicity or persistence are likely to significantly alter the metabolism of a living being; "should remain 'emerging' as long as there is a scarcity of information in the scientific literature or there are poorly documented issues about the associated potential problems they could cause"; 'are expected to be chemicals that show some potential to pose risks to human health or the environment and which are not yet subjected to regulatory criteria or norms for the protection of human health or the environment'; 'not all will actually prove to be evil and have some potential to cause tangible concerns'; 'an already regulated presumed well-known contaminant could certainly regain 'emerging' status as new scientific information becomes available and thus force regulatory agencies to re-evaluate their norms and guidelines'; 'will remain a moving target as new chemical compounds are continuously being produced and science continuously improves its understanding of current and past contaminants.'

CECs include synthetic chemicals, naturally produced metabolites and biological hazards. Among the latter, currently, antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), and viruses and virions are of top concern. Naturally occurring CECs include natural hormones excreted in water (Ternes & Joss, 2006) and cyanotoxins (hepato-, neuro-, and dermatotoxins) produced by toxic cyanobacterial blooms in surface waters. These blooms (in fresh and transitional waters) are generally triggered by phosphorus, temperature, and solar light conditions, and the most commonly occurring cyanotoxin is microcystin-LR (Chorus & Welker, 2021; Menaia & Rosa, 2006). MC-LR was therefore included in the Portuguese legislation relative to drinking water quality standards (Decree-law 306/2007, Decree-law 152/2017) following the World Health Organisation (WHO) guidelines (Chorus & Welker, 2021, the 2nd edition of the WHO Guide published in 1999) and some Portuguese or EU research projects developed in Portugal (e.g., TOXIC and CIANOTOX projects), and later included in the EU Drinking Water Directive (Directive (EU) 2020/2184).

Industrial chemicals, such as pharmaceutical compounds (PhCs) (human and veterinary drugs), synthetic hormones, cosmetics and personal care products, pesticides, flame-retardants, plasticizers, food additives, per- and poly-fluoroalkyl substances (PFAS), and nano- and microplastics, raise considerable toxicological concerns for the aquatic environment since they may be transported into waterbodies in some stage of their lifecycle (Mestre *et al.*, 2022a). Actually, most of these chemicals have been already detected in surface and groundwater in pg/L to μg/L worldwide (Geissen *et al.*, 2015), as illustrated for PhCs in Figure 8.1 (Aus der Beek *et al.*, 2016) and Figure 8.2 (Silva *et al.*, 2021), whereas in urban wastewaters they occur in ng/L to dozens in μg/L. Figure 8.2 compares the concentrations of 13 PhCs found by others in urban wastewaters in Portugal and beyond with those found in BEI and FNW WWTPs' influents in 2016–2019 (within LIFE Impetus project). We have statistically found the PhC occurrence seasonality is well-defined by the maximum air temperature, with a turning point of 20°C in Lisbon and 22°C in Faro. Some PhCs showed lower concentrations in colder (wet) months due to rainwater dilution, whereas others showed higher concentrations, reflecting an increased seasonal consumption and/or slower transformation due to lower air temperatures and/or shorter hydraulic retention times. Seasonal studies should therefore focus on temperature and rainfall rather than on calendar seasons, increasingly uncertain due to climate change (Silva *et al.*, 2021).

On the contrary, a review of pesticides' monitoring studies of surface waters worldwide (Souza *et al.*, 2020) showed a critical occurrence of atrazine and its metabolites, metalochlor, chlorpyrifos, and tebuconazole, and high concentrations and frequency of diuron (0.03–22,770 ng/L) and of the insecticide dimethoate (0.57–61,200 ng/L).

Domestic wastewater, hospital and industrial effluents, stormwater runoff, rural runoff, and manure media are the main sources of CECs entering the aquatic environment (Eggen *et al.*, 2014; Luo *et al.*, 2014). So far, urban wastewater treatment plants (UWWTPs) were essentially designed to remove total suspended solids (TSS) and reduce the chemical and the biochemical oxygen demands (COD and BOD_5, respectively) in the biological secondary treatment, and, when the receiving water requires so, also to remove nutrients (nitrogen and or phosphorus) to avoid eutrophication, or to disinfect to control water microbiological quality. CECs' control in such UWWTPs starts with a good quality secondary effluent since biodegradation and sorption onto particulate matter and biomass are the two major elimination mechanisms (Rosa *et al.*, 2019; Siegrist & Joss, 2012; Silva *et al.*, 2023; Ternes & Joss, 2006). In turn, the non-biodegradable and polar CECs can easily escape the conventional secondary treatment (Eggen *et al.*, 2014; Luo *et al.*, 2014; Rosa *et al.*, 2019; Siegrist & Joss, 2012; Silva *et al.*, 2022; Ternes & Joss, 2006), being released into the water bodies, where they may accumulate in biota (Cravo *et al.*, 2022). The same may happen with the CEC metabolites (e.g., PhCs produced and excreted by human and veterinary metabolism) that, although typically less studied (Pourchet *et al.*, 2020), may be as toxic or more than their parent compounds (Golovko *et al.*, 2021). The continuous discharge of CECs on ecosystems may affect their health, biodiversity, and

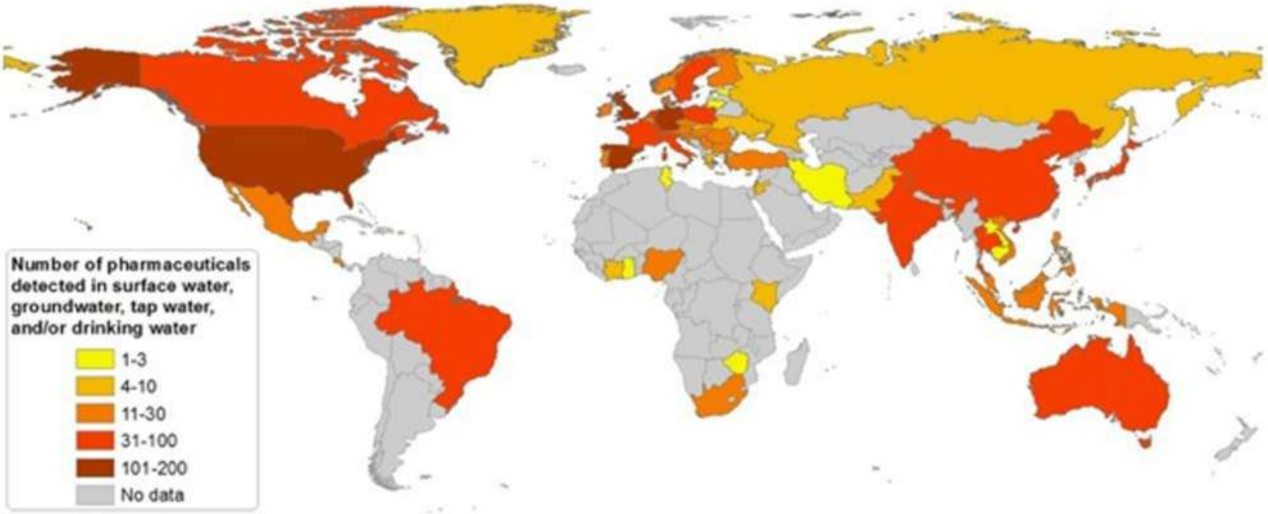


Figure 8.1 Country survey on the number of PhCs detected in surface waters, groundwater, or tap/drinking water. *Source*: From Aus der Beek *et al.* (2016).

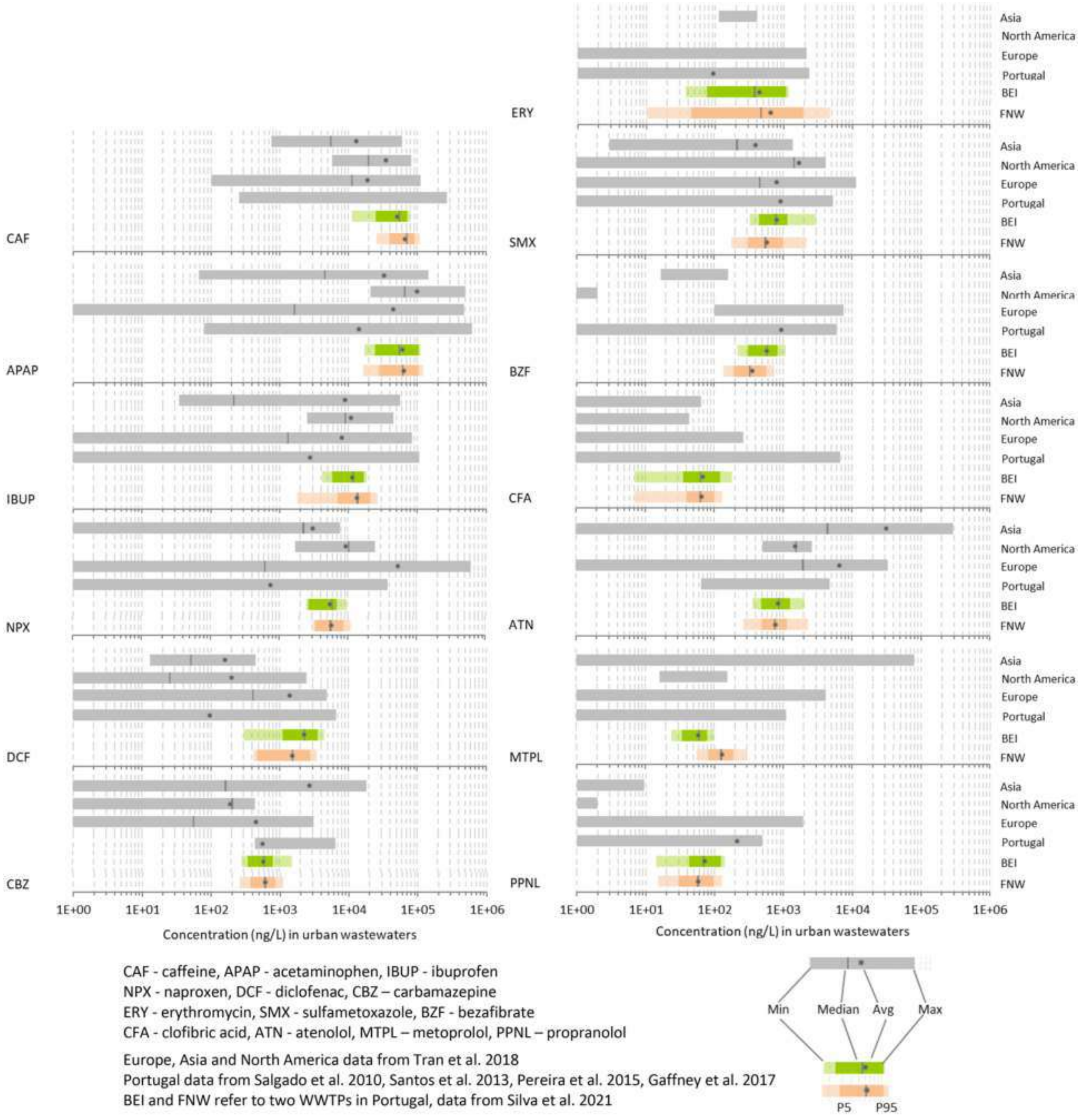


Figure 8.2 Examples of pharmaceuticals' concentration in urban wastewater. *Source*: Adapted from Silva *et al.* (2021).

eventually the quality of raw water for human consumption, particularly where highly populated and industrialized urban areas have closed water cycles and/or the dilution factors of rivers or receiving waters are not enough to disregard the risk for aquatic environment, as in some cities in Central Europe (Eggen *et al.*, 2014).

The EU water legislation evolution in the last two decades has been reflecting the CEC monitoring and mitigation needs, as illustrated in Figure 8.3, starting by monitoring and more recently addressing treatment. The Water Framework Directive (WFD) 2000/60/EC

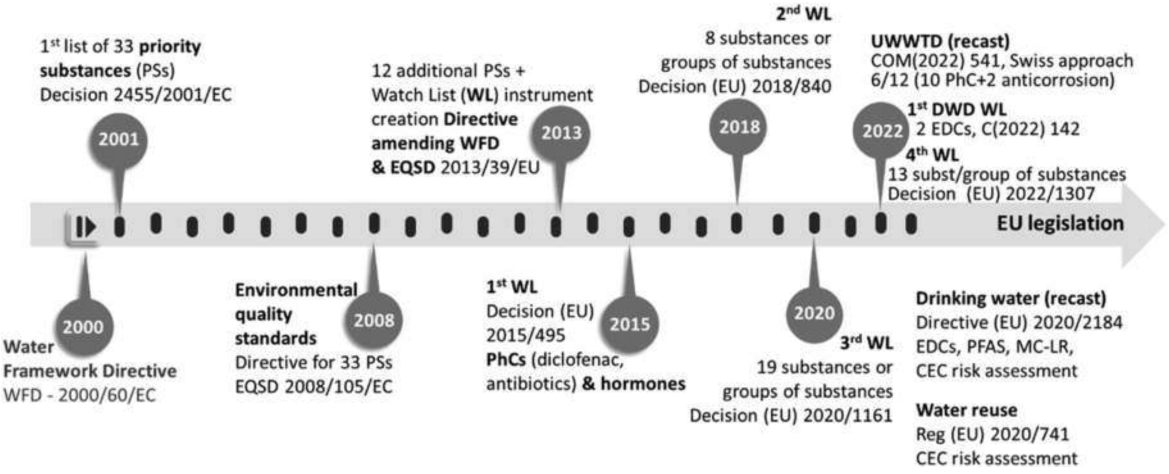


Figure 8.3 Evolution of water-related European legislation concerning CECs. *Source*: Adapted from Viegas *et al.* (2021b) and Mestre *et al.* (2022a and updated).

and subsequent directives and decisions established environmental quality standards (EQS) for 45 priority substances (specific herbicides, pesticides, insecticides, industrial chemicals, hydrocarbons, polyaromatic hydrocarbons, and heavy metals) and in 2013 (Directive 2013/39/EU) a new mechanism, the Watch List (WL), was introduced to support the identification of priority substances for regulation under WFD. A WL comprises CECs not yet regulated but that may pose significant risk due to their potential toxicological effects. Four WLs have been published since 2015, comprising PhCs, hormones, and pesticides/herbicides (Mestre *et al.*, 2022a), the 4th WL including seven new substances or groups (Gomez Cortes *et al.*, 2022). The same mechanism was recently adopted in drinking water, with the publication of the 1st WL (C(2022) 142) entailing that the potential presence of two endocrine-disrupting substances (17-β-estradiol and nonylphenol) will have to be monitored at relevant points throughout the whole water supply chain and indicating guidance values for each substance (CID, 2022).

In drinking water and in water reuse (namely for the highest quality reclaimed water for agriculture irrigation), there are no fixed limits for CECs, but additional requirements may have to be adopted for pharmaceuticals and other CECs depending on the outcome of the monitoring according to the 1st DWD WL (C(2022) 142) or of the mandatory risk assessment (Directive (EU) 2020/2184 and Regulation (EU) 2020/741). The same takes place in the national legislation, namely Decree-law 152/2017 (drinking water quality) and Decree-law 119/2019 (water reuse), the latter (as well as the Regulation (EU) 2020/741) fully aligned with the ISO standards on water reuse (ISO 16075 series – Guidelines for treated wastewater use for irrigation projects, ISO 16075 series (2015–2021) – Guidelines for treated wastewater use for irrigation projects, ISO 20426:2018 – Guidelines for health risk assessment and management for non-potable water reuse).

Regarding wastewater, the recently proposed revised text for the Urban Wastewater Treatment Directive (UWWTD recast, COM (2022)) adopts the Swiss approach, with whom the German Federal Centres for Trace Substances (KomS) in Baden-Württemberg, North Rhine-Westphalia, and Berlin (Central Europe) agree. This is, UWWTD (recast) introduces the obligation to apply additional treatment to urban wastewater (quaternary treatment) in order to eliminate the broadest possible spectrum of micropollutants, that is, 80% removal (contrasting with the typical WFD approach of limit concentrations) of 6 CECs of a list of 12 in all UWWTPs treating ≥100,000 p.e. by December 31, 2035 (at the latest) and, by 2040, in all facilities ≥10,000 p.e. in areas where the concentration or accumulation of micropollutants poses a risk to human health or the environment according to the specified criteria (areas to be identified by the member states). Furthermore, it introduces the extended producer (including importers) responsibility (EPR) to contribute to support the costs of the quaternary treatment, such financial contribution being established based on the quantities and toxicity of the products the producer (including importers) places on the market. In Germany, late 2019, more than 20 plants have been expanded with advanced treatment technologies and put into operation to provide 80% reduction of selected indicator substances, and further 27 facilities were planned or under construction (Metzger *et al.*, 2019).

Once a CEC is regulated it becomes a micropollutant, as the new UWWTD refers to the 12 PhCs from which six must be selected for minimum 80% removal in UWWTP and the EU & Portuguese drinking water standards refer to microcystin-LR and pesticides.

8.2 MICROPOLLUTANTS TARGETED AND KEY PROPERTIES FOR REMOVAL

This chapter aims to raise the awareness and the technical and scientific preparedness of the readers to support transforming the latest regulation in DWT and UWWT into practical impact, namely by comprehensively explaining a solution for controlling organic micropollutants of different molar mass (MM), charge, hydrophobicity/hydrophilicity, biodegradability, and sorption onto biomass – the key properties for removal by the targeted solutions.

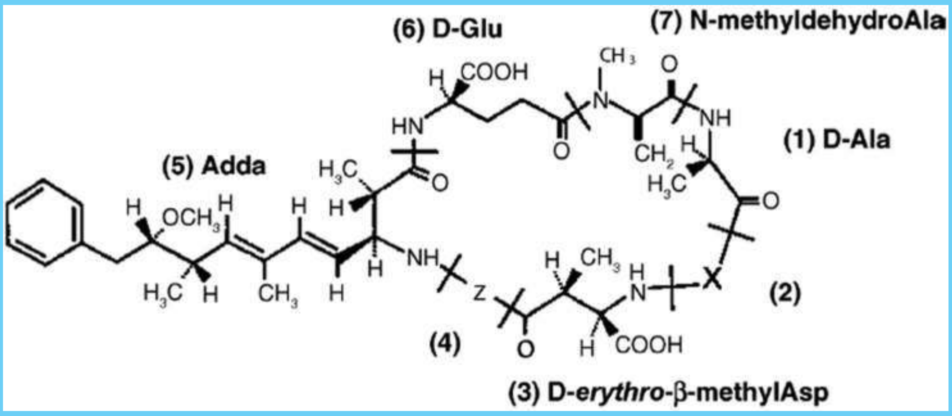
Amongst the large spectrum of organic micropollutants, many may be controlled at source or its use limited or prohibited, while others will likely be naturally produced in surface waters (e.g., cyanotoxins) or inevitably (partially) discharged in urban wastewaters (e.g., pharmaceuticals). Therefore, for DWTPs, the selected organic micropollutants are MC-LR (and other microcystin variants naturally occurring with MC-LR, Table 8.1, often overall expressed as MC-LR$_{eq}$) and pesticides, regulated contaminants, as well as representative PhCs, considered via the risk assessment.

For UWWTPs, the selected organic micropollutants for advanced adsorption/membrane treatment are representative recalcitrant PhCs (i.e., of low biodegradability (k_{bio}) and sorption onto biomass (K_d), Figure 8.4), escaping the secondary (for C removal) or tertiary (for C, N and P removal) treatment, that is, poorly removed or exhibiting variable and unreliable removal by secondary/tertiary treatment. Considering their distinct physical–chemical properties, namely charge at pH 7.4 and hydrophobic/hydrophilic character (expressed by Log K_{ow} (the logarithm of the n-octanol/water partition coefficient) or Log D_{pH} (the logarithm of the distribution coefficient, which corrects the Log K_{ow} for the pK_a of each compound by quantifying the amount of both the ionized and non-ionized forms of the PhCs in octanol and water at the pH values under study; Viegas *et al.*, 2022), the higher the more hydrophobic), our studies have been often conducted with carbamazepine (CBZ), diclofenac (DCF), and sulfamethoxazole (SMX) (Table 8.2), though not limited to (other examples in Table 8.3 and Sections 8.6, 8.7, and 8.8).

8.3 AVAILABLE TECHNOLOGIES FOR CONTROLLING ORGANIC MICROPOLLUTANTS

As introduced earlier, conventional UWWTPs essentially target TSS, COD, and BODs reduction via physical unit operations and biological processes, as well as nitrogen and phosphorus reduction or disinfection (*Escherichia coli* inactivation) when needed, respectively, to avoid eutrophication or protect bathing waters/aquaculture. In turn, as described in Rosa *et al.* (2009), conventional DWTPs include (a) preoxidation, for controlling biological growth, taste, and odor compounds, ferrous and manganese ions, (b) coagulation/flocculation/sedimentation and rapid sand filtration for removing turbidity and reducing the natural organic matter (NOM), particularly the more hydrophobic fraction (expressed by SUVA > 4–5 L/(mgC m), where SUVA represents the specific UV absorbance), the major precursor of organochlorinated by-products, and (c) final chlorination for water disinfection and for providing a disinfectant residual in the water distribution system (to

Table 8.1 Properties of microcystin variants.

	MC-LR	MC-LY	MC-LW	MC-LF
				
Amino acids (X, Z)	Leucine, arginine	Leucine, tyrosine	Leucine, tryptophane	Leucine, phenylalanine
Molar mass (g/mol)	994	1001	1024	985
Net charge at pH 7	−1	−2	−2	−2
		Increasing hydrophobicity		

Source: Adapted from Campinas and Rosa (2006).

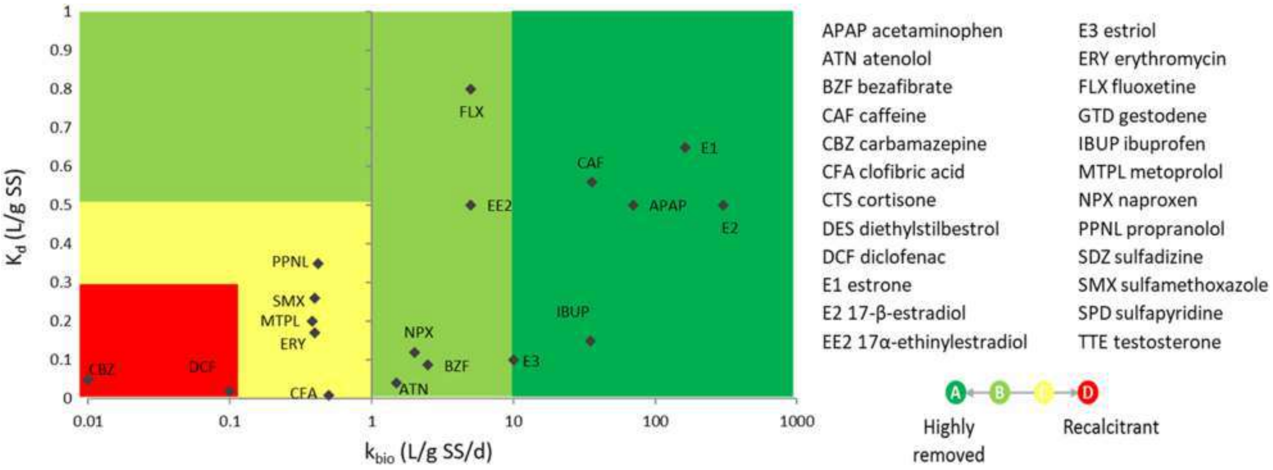
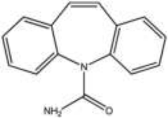
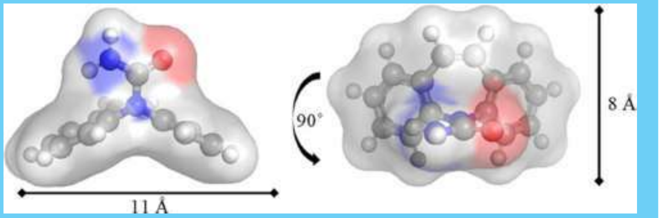
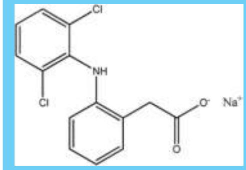
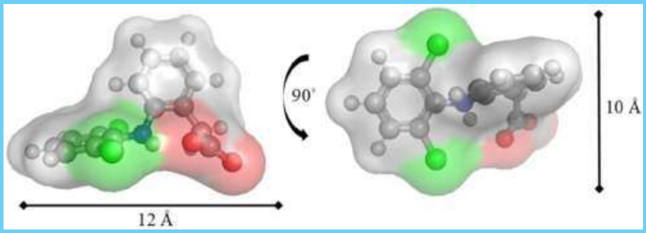
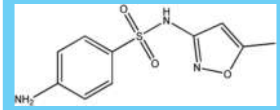
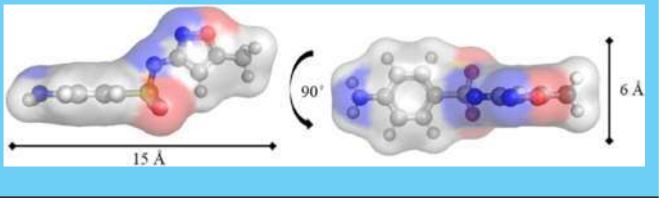


Figure 8.4 K_d vs k_{bio} for selected organic micropollutants and CECs. *Source*: From Rosa *et al.* (2019) and Silva *et al.* (2022).

prevent biological regrowth). Consequently, the conventional treatment plants were not designed as full barriers against organic micropollutants. The strategy to control them must therefore start by assessing and improving the performance of the existing barriers and then upgrade them with advanced treatment, as needed.

Operational, low-investment measures for improving current UWWTPs include providing conditions for the build-up of the slowly growing nitrifying bacteria, which can excrete enzymes

Table 8.2 Carbamazepine, diclofenac, and sulfamethoxazole key properties for removal by adsorption and adsorption/membrane filtration.

PhC Molecular Structure and Therapeutic Class	Physical–Chemical Properties	Optimized Geometries and Dimensions
Carbamazepine/CBZ  Anti-epileptic and psychiatric drug	MM = 236.3 g/mol $\text{Log } K_{ow} = 2.67$ $\text{Log } D_{7.4} = 2.28$ $\text{p}K_a = 13.9$ Neutral and hydrophobic	
Diclofenac/DCF  Non-steroidal analgesic and anti-inflammatory drug	MM = 318.1 g/mol $\text{Log } K_{ow} = 4.06$ $\text{Log } D_{7.4} = 1.37$ $\text{p}K_a = 4.0$ Anionic and relatively hydrophobic	
Sulfamethoxazole/SMX  Antibiotic	MW = 253.3 g/mol $\text{Log } K_{ow} = 0.89$ $\text{Log } D_{7.4} = -0.56$ $\text{p}K_{a1} = 1.7$; $\text{p}K_{a2} = 5.6$ Anionic and hydrophilic	

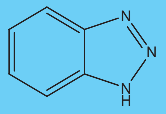
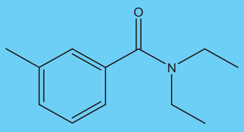
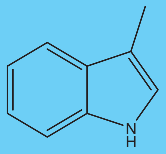
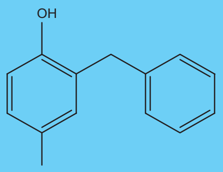
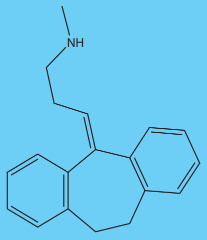
Source: From Mestre *et al.* (2022b) and Viegas *et al.* (2022).

and, by co-metabolism, may break down some low degradable molecules (Clara *et al.*, 2005; Ternes & Joss, 2006). These conditions include sludge retention times of, at least, 10–15 days (Clara *et al.*, 2005) or F/M (food to microorganisms) ratios below 0.08 d^{-1} that were associated with N-removal > 80% and alkalinity reduction > 40% (due to nitrification) (Rosa *et al.*, 2019; Silva *et al.*, 2022).

Improvement measures in conventional DWTP include adjusting preoxidation and C/F/S operating conditions for promoting NOM removal by enhanced coagulation and implementing C/F/S assisted with powdered activated carbon (PAC) addition. For instance, an enhanced control of microcystins (cyanobacteria and other cyanotoxins) may be achieved using low ozone doses in preoxidation (to avoid cell disruption prior to C/F/S and maximize intracellular MC-LR_{eq} removal), adequate PAC and coagulant doses, dissolved air flotation (DAF) instead of sedimentation, smooth start-up/shut-down of sand filtration cycles, and multilayer filtration (Hall *et al.*, 2005; Raspati *et al.*, 2015; Ribau Teixeira *et al.*, 2020; Rosa *et al.*, 2009; Smeets *et al.*, 2015).

DWTP and UWWTP upgrade relies on implementing advanced treatment (quaternary treatment according to the proposed recasted UWWTD). Established advanced (waste)water treatment options for enhanced control of organic micropollutants and CECs include (Baresel *et al.*, 2017; Campinas *et al.*, 2017, 2022; Chorus & Welker, 2021; Eggen *et al.*, 2014; Hall *et al.*, 2005; Hillenbrand *et al.*, 2016; Luo *et al.*, 2014; Mestre *et al.*, 2022a, b; Metzger *et al.*, 2019; Mulder *et al.*, 2015; Pesqueira *et al.*, 2020; Rizzo *et al.*, 2019, 2020; Rosa *et al.*, 2009; Rosa *et al.*, 2019; Smeets

Table 8.3 Other selected organic micropollutants and their key properties for removal by AC adsorption and membrane filtration.

PPCP	Structure	Molecular Formula	MM, g/mol	V_{molar}, cm^3 (1)	Log K_{ow}	Charge at pH 7	Log *D* at pH 7–7.5	Category
1-H-Benzotriazole (BZ)		$C_6H_5N_3$	119.1	88.3	1.44	Neutral	1.42–1.39	Anticorrosive
DEET (DT)		$C_{12}H_{17}NO$	191.3	194.0	2.18	Neutral	2.18	Insect repellent
3-Methylindole (ML)		C_9H_9N	131.3	118.1	2.60	Neutral	2.60	Fragrance
Chlorophene (CP)		$C_{13}H_{11}ClO$	218.7	179.2	4.18	Neutral	4.18	Biocide
Nortriptyline. HCl (NH)	 HCl	$C_{19}H_{21}NO\cdot HCl$	299.8	242.9	4.51	Positive	1.40–1.80	Antidepressant

(1) Calculated by ChemSketch

Source: From Rodriguez *et al.* (2016).

et al., 2015; Ternes & Joss, 2006; UMWELT.NRW, 2019; Viegas *et al.*, 2021b; Zietzschmann *et al.*, 2014; Zietzschmann, 2020):

- Ozonation and advanced oxidation processes (AOPs, e.g., O_3/H_2O_2, UV/ H_2O_2, Fenton).
- Adsorption-based systems, including PAC, granular activated carbon (GAC) filtration or biologically active GAC filters (BAC);
- Membrane filtration, that is, microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO);
- Adsorption/membrane hybrid processes, for example, PAC/MF, PAC/UF, PAC/NF.

The technology applicability depends on its separation mechanisms vs the target contaminants' properties (e.g., charge, hydrophobicity/hydrophilicity, solubility, volatility, chemical resistance, biodegradability, sedimentability/floatability, colloidal character, release of intracellular metabolites), as illustrated in Table 8.4 for macro and microcontaminants (Rosa *et al.*, 2009).

Each option has its pros and cons, as extensively compared in literature (e.g., in Luo *et al.*, 2014; Pesqueira *et al.*, 2020; Rizzo *et al.*, 2019, 2020). Regarding adsorption-based treatment, during the last decade, several projects addressed its introduction in an increasing number of WWTPs in

Table 8.4 Effectiveness of alternative and advanced processes for macro and microcontaminant control.

Contaminant	C/F + DAF	UV	GAC	BAC	MF	UF	PAC/UF	NF[a]	RO
Protozoa (cysts, oocysts)	+/-	+	+/-		+	+	+	+	+
Bacteria (vegetative forms)	-/+	+	-		+	+	+	+	+
Bacteria (endospores)	-/+	-	-		+	+	+	+	+
Helminth eggs	+/-	-	+/-		+	+	+	+	+
Cyanobacteria	+	- [b]	+/-		+	+	+	+	+
Enteroviruses	-	-	+/-		+/-	+	+	+	+
NOM_SUVA < 3 L/(mgC.m) [c]	-/+	+/-	-/+		+	-	-	+/-	+
NOM_SUVA > 4 L/(mgC.m) [c]	+/-		+/-		+/-	-/+	+/-	+	+
Assimable organic carbon (AOC)	-	+/-	-/+	+	-	-	-/+	+/-	+
Trihalomethanes (THMs)	-		+		-	-	+	+/-	+
Haloacetic acids (HAAs)	-		+		-	-	+	+/-	+
Bromate	-		+/-	+/-	-	-	-/+	+	+
Bromide	-				-	-	-	+/-	+
Chlorate	-				-	-	-	+	+
Chloride	-				-	-	-	+/-	+
Nitrate	-				-	-	-	+	+
Sodium	-				-	-	-	+/-	+
Sulfate	-				-	-	-	+	+
Microcystins	+ or -/+[d]		+ or +/- [e]		-	-	+ or +/- [e]	+	+
Taste and odour compounds (MIB, geosmin)	-/+		+		-	-	+	+	+
Volatile organic compounds (VOCs)	+[f]		+		-	-	+/-	-/+	+
EDCs and pharmaceuticals (hydrophobic and chemically resistant)	-/+		+		-	-	+	+	+
Pesticides (including chemically resistant)	-/+		+ or +/- [e]		-	-	+	+	+

- Not adequate.
-/+ Limited effectiveness.
+/- Partial control if adequate operation conditions are guaranteed.
+ Effective provided adequate operation conditions are guaranteed.
No information available.
[a] Considering 200 Da molecular cut-off.
[b] UV should not be used to control cyanobacteria, since it leads to cell rupture and cyanotoxin release.
[c] SUVA < 3 L/(mgC. m) indicates mainly hydrophilic NOM. SUVA > 4 L/(mgC.m) indicates mainly hydrophobic NOM and especially aromatic compounds.
[d] Effective removal of intracellular toxins; no significant removal of dissolved toxins.
[e] Depends on chemical characteristics of the target compound.
[f] There are volatilization conditions in C/F/DAF.
Source: From Rosa *et al.* (2009) and Smeets *et al.* (2015).

German-speaking countries, mainly because of Swiss and German governmental requirements or encouragement to accomplish CEC abatement from urban wastewater. As reviewed in Mestre *et al.* (2022a), results on the different technologies implemented have been published in reference journals, but the practical knowledge on the ACs' selection for pilot-, large-, and full-scale implementation can only be consulted in project reports and other documents written mostly in German. An exception is the Zietzschmann's (2020) book chapter, providing valuable information on the adsorptive removal of organic microcontaminants from wastewater, covering practical aspects of PAC and GAC processes and operation fundamentals, tools for their performance prediction and general considerations for an informed selection of an AC product.

This chapter focuses on PAC adsorption and on PAC/low-pressure membrane technology. These are two best available technologies, flexible and resilient to severe, climate change-driven variations in raw water composition, particularly suited as seasonal/sporadic (for episodes) barriers. Moreover, these technologies rely on physical separation, which allows minimizing the health and environmental risks associated with the potential formation of undesired by-products. Low-pressure-driven membranes are preferentially addressed to minimize the energy use and carbon footprint.

8.4 AC ADSORPTION

In water treatment, adsorption is the physical (or chemical) mechanism by which dissolved molecules (adsorbates or solutes) are captured on a solid interface (adsorbent). AC is the most used adsorbent in DWT and UWWT due to its nanoporous structure, responsible for an outstanding surface area, typically from hundreds to 2000 m^2/g (Mestre *et al.*, 2022a, b).

Most carbon-rich materials may be used as raw materials for AC production, but a large-scale production requires large amounts of precursors with reproducible features, controlled ash content, and cost. Commercially available products are mainly produced from coal, wood, or coconut shell; water treatment applications represent around one-third of the market share and are expected to remain the largest throughout 2020–2027, being the current driver for market growth (Figure 8.5, Mestre *et al.*, 2022a).

The precursors are first carbonized, and the chars are then physically or chemically activated (using high temperatures under controlled atmosphere, e.g., steam activation) to increase the porous network (of macro-, meso-, and micropores, Figure 8.6) yielding a high surface area. Given their relevance for PhC adsorption, the micropores' classification is further broken down into supermicropores (0.7–2 nm wide) and ultramicropores (<0.7 nm width). Manufacturers commonly report the iodine number and BET (Brunauer–Emmet–Teller) area values as indicators of the apparent surface area of a given AC, with both parameters usually presenting close values despite their distinct units (mg/g and m^2/g, respectively) (Mestre *et al.*, 2022a; Zietzschmann *et al.*, 2014).

ACs are industrially applied as PACs, average particle size <100 μm or as GAC filters (average particle size >100 μm and median $d_{50} > 1$ mm) (Figure 8.5), the lower the particle size, the lower the

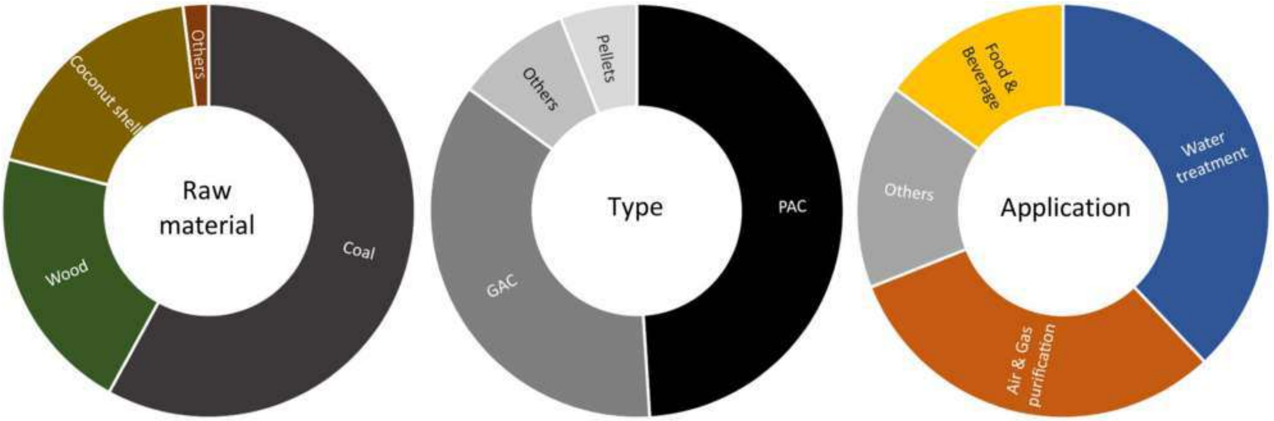


Figure 8.5 Global AC market share by raw material and type. *Source*: Data adapted from Inkwood Research (2019) and application (data adapted from Fortune Business Insights, 2019). From Mestre *et al.* (2022a).

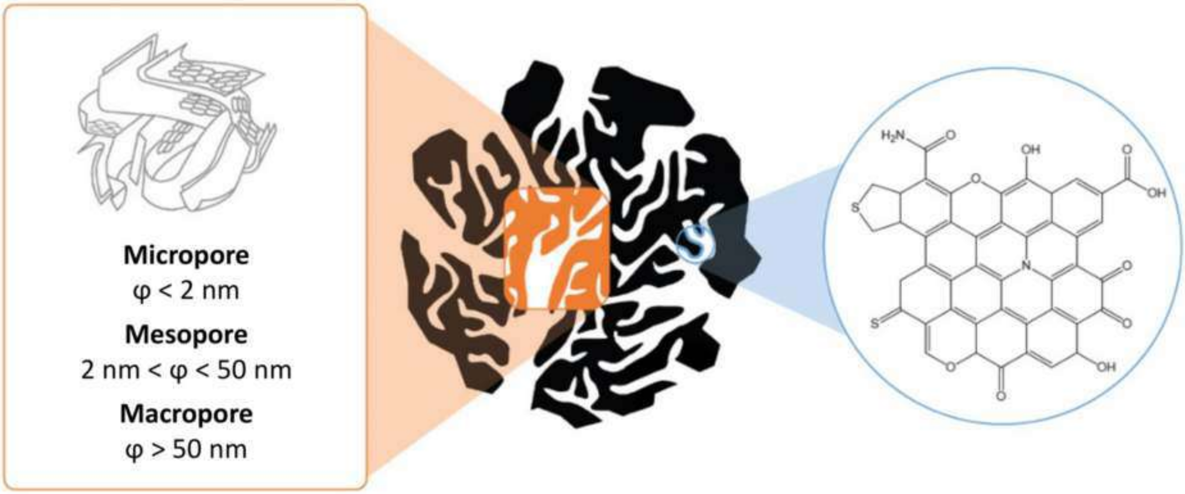
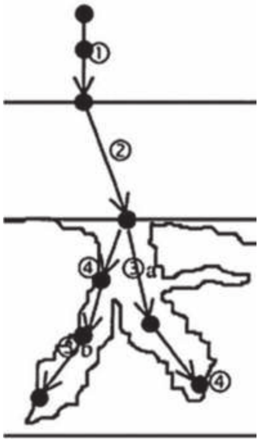


Figure 8.6 Representation of nanoporous structure and surface chemistry groups on AC. *Source*: From Mestre *et al.* (2022a).

adsorption path and therefore the faster the adsorption kinetics to the active sites for a similar porous structure (Figure 8.7). Super fine PACs (sPAC) with enhanced kinetics are emerging for applications not requiring particle settleability, that is, for PAC/membrane applications (Section 8.6). After exhaustion, PACs are usually discharged in landfill or incinerated, while GACs can be reactivated (or regenerated) and reused.

AC adsorption capacity (adsorbate/adsorbent mass ratio, often determined through Freundlich or Langmuir isotherms) and kinetics (HSDM (homogeneous surface diffusion model) vs Boyd's model, Viegas *et al.*, 2014) depend on (external and intraparticle) diffusion and mass transfer and affinity (Campinas *et al.*, 2013), that is, adsorbent–adsorbate hydrophobic and electrostatic interactions. These are determined by the AC textural properties (the closer the active-site pore size to the solute's size the better; Zietzschmann, 2020) and surface chemistry (e.g., hydrophobicity and net surface charge, expressed by $\mathrm{pH_{pzc}}$ – examples in Figure 8.8), as well as by the solutes' properties (e.g., size, hydrophobicity, and net charge; examples in Tables 8.2 and 8.3) and the water background organics and inorganics.



1. CEC bulk diffusion/convection to stagnant boundary layer surrounding the AC particle

2. CEC external mass transfer across the boundary layer (external mass transfer)

3. CEC porous diffusion (in water filled pores) or ***surface diffusion*** (series of hops with adsorption-desorption along the pore surface) or both (intraparticle diffusion).

4. CEC adsorption, physical or chemical attachment onto the surface of the adsorbent surface site

Figure 8.7 AC adsorption representation. *Source*: Adapted from Viegas *et al.* (2014).

BET surface area (m^2/g)	1112
Ash content (%)	8.17
Moisture (%)	2
Apparent density (g/cm^3)	0.16
Geometric mean diameter (μm)	6
Primary micropore (<7 Å) volume (cm^3/g)	0.343
Secondary micropore (7-20 Å) volume (cm^3/g)	0.194
Mesopore (20-500 Å) volume (cm^3/g)	0.357
Micropore surface area (m^2/g)	733
Mesopore surface area (m^2/g)	379

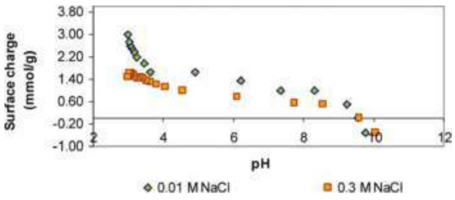


Figure 8.8 Key characteristics (textural, left; surface charge, right) of an AC illustrated for a high performing (mesoporous and microporous, alkaline, i.e., pH$_{pzc}$ $\sim$ 9.5) commercial PAC for CEC control (PAC Norit SA-UF). *Source*: Adapted from Campinas and Rosa (2006).

Water background organics determine the competitive adsorption by (a) direct competition for the adsorption sites (the closer the NOM/EfOM (effluent organic matter) size and hydrophobicity to the target solute the stronger the competition; Campinas *et al.*, 2013) or (b) pore blockage and are often characterized by DOC content and character (e.g., Rodriguez *et al.*, 2016). This is the reason why, in practical applications, one uses the DOC-normalized PAC dose (mg PAC/mg DOC). Background inorganic matrix may be easily characterized by two bulk parameters (i) the water pH, determining the AC and solute's ionization/net charge, and (ii) the electrical conductivity (expressing the ionic strength), determining the electrostatic repulsions' shielding effect and so the solute's hydrodynamic size (Campinas & Rosa, 2006).

The development of high-performing, environmental-friendly (biomass-derived, steam activated) PACs from local biomasses is very important for the sake of the technology's sustainability (Mestre *et al.*, 2022b; Viegas *et al.*, 2020b), as well as of fine sPAC and magnetic PACs allowing their recovery and regeneration (as ongoing within EMPOWER + project).

8.5 MEMBRANE FILTRATION

In membrane filtration for water treatment, an applied pressure forces water to pass through a semi-permeable membrane, which retains the solutes larger than the membrane pores, the narrower the pores, the higher the pressure, as illustrated in Figure 8.9 for MF, UF, NF, and RO (and also for sand filters, for comparison).

Membrane filtration covers a broad spectrum of options in terms of membrane pore/selectivity/applied pressure, but also in terms of (a) membrane materials and modules, (b) filtration mode, dead-end, or crossflow, in/out or out/in, pressurized or immersed, and (c) number of stages (Figure 8.10) (Pinho & Rosa, 1995). Common applications include (i) 1-stage cross-flow polymeric spiral-wound RO membranes for seawater desalination, (ii) polymeric spiral-wound NF membranes for DWT, that is, groundwater softening and NOM and CEC removal from surface water, (iii) pressurized in/out polymeric hollow-fibre UF membranes for DWT and water reclamation, (iv) immersed out/in polymeric hollow-fiber or flat sheet UF membranes for membrane bioreactors (MBRs) for UWWT/water reclamation, and (v) the emerging ceramic in/out dead-end tubular MF membranes for surface water treatment. All membranes (from MF to RO) are full physical barriers against particles (TSS and turbidity) and bacteria; UF membranes are also effective against viruses and macromolecules; NF membranes further remove small organics (usually $>$ 300 Da), divalent salts, and partially monovalent salts, which are highly removed by RO. This is, all membranes are adequate for water (physical) disinfection, but the low-pressure MF and UF are not able to remove the organic micropollutants targeted.

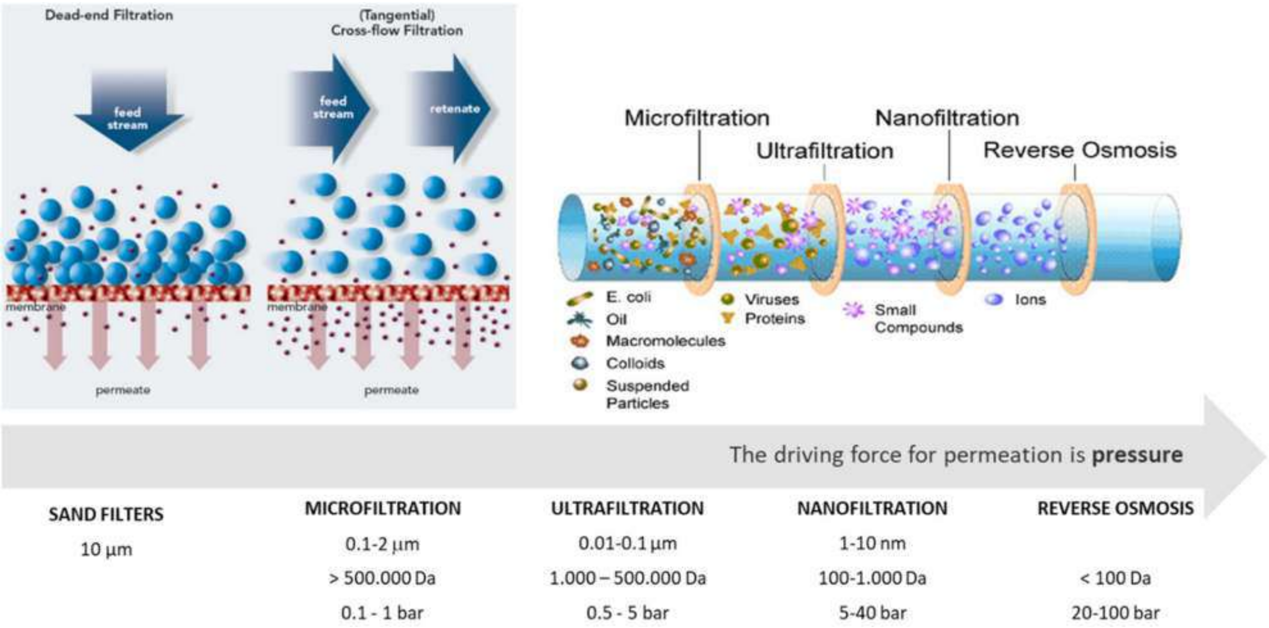


Figure 8.9 Membrane filtration in water treatment – MF, UF, NF, and RO.

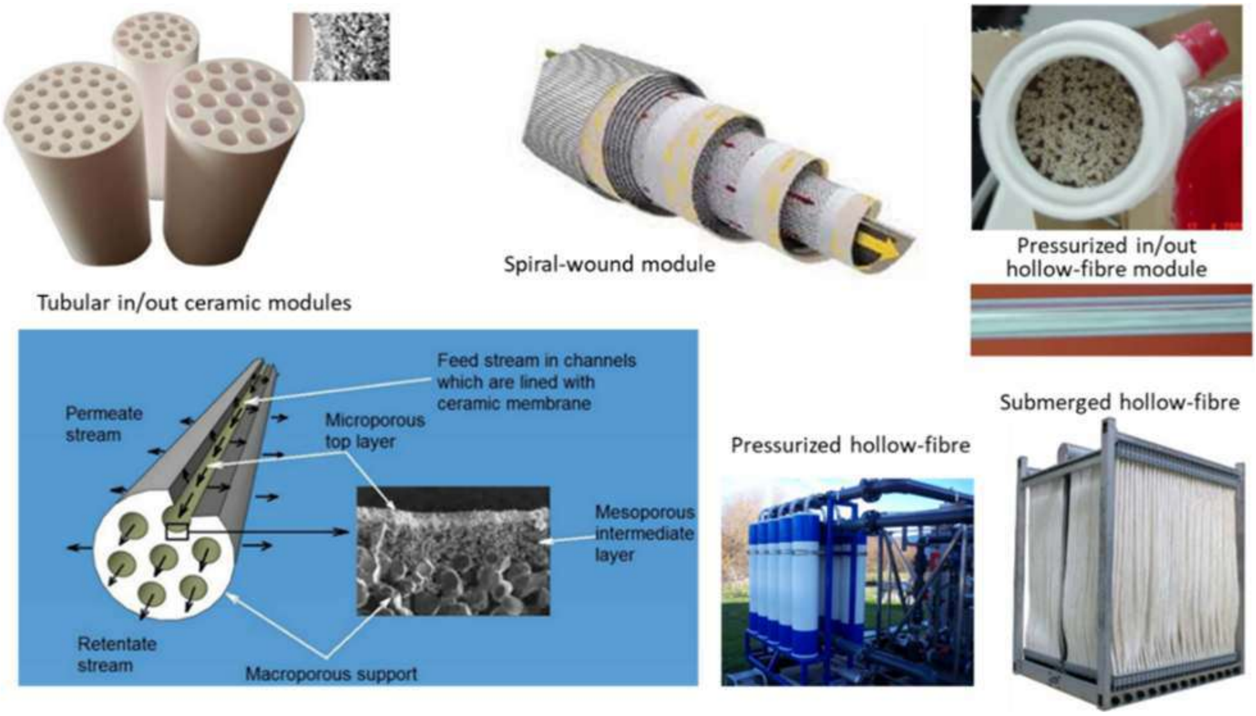


Figure 8.10 Membranes and modules.

As ceramic membranes have so far involved higher initial capital costs than polymeric membranes, they represented only 2–3% of the membrane market in 2015 and 12% of the membrane materials used until 2016 for DWT by membrane processes with backwashing. However, ceramic MF application is expected to grow over the next years due to their many advantages over polymeric membranes and also due to their significant cost decrease (Wise *et al.*, 2020). Ceramic membranes have higher chemical, thermal, and mechanical stability, making them easily backwashed and allowing more aggressive physical and chemical cleaning that can extend their lifetime. Ceramic membranes are also better candidates for hybrid adsorption/membrane processes than polymeric membranes as they can be significantly resistant to deterioration by biofilm growth and to surface abrasion by coarse particles circulation, two concerns mostly related with PAC long-term use (Campinas *et al.*, 2021c), which have driven our pioneering research on PAC/ceramic MF and/or PAC/ceramic UF at pilot scale for water reclamation (within EU-FP7 TRUST project developed in 2012–2013; Viegas *et al.*, 2015, 2020a) and drinking water production (LIFE Hymemb project, 2014–2016; Campinas *et al.*, 2021b, c).

Membrane performance (fluxes, selectivity, water recovery, and fouling rate over time) greatly depends on the intake water quality and on the membrane operation conditions and cleaning, which control the membrane (reversible and irreversible, bio- and chemical) fouling, scaling, and aging, ultimately determining the membrane productivity, lifespan and the energy costs (Figure 8.11). A new indicator was developed for performance assessment and optimization, the treatment capacity (TCp), that is, the design flowrate normalized to membrane area and intake pressure. TCp ($m^3/(m^2.d.bar)$) is the effective volume of permeate produced by unit time, membrane area, and intake feed pressure, deducting the permeate volume used for backwashing and chemically enhanced backwashing (CEB), and considering the productive time (filtration time) and the time a unit is off-line for cleaning procedures (backwashing and CEB). TCp integrates all key aspects of process productivity and therefore constitutes a useful indicator to balance flux, energy consumption, backwash frequency, and chemical cleaning frequency (Campinas *et al.*, 2021c).

The intake water background organics play a key role on membrane fouling, usually requiring coagulation pre-treatment for NOM control (Campinas *et al.*, 2021c; Ribau Teixeira & Rosa, 2006), whereas the water inorganics (pH, ions) affect the membrane scaling (inorganic precipitation) but also the flux and selectivity of NF membranes due to charge (pH) and shielding (ions) effects (higher pH corresponding to higher electrostatic repulsions, narrower pores, lower flux, and higher solute retention; deleterious effects partially balanced by the ions' shielding effect) (Ribau Teixeira *et al.*, 2005).

8.6 HYBRID AC ADSORPTION/MEMBRANE PROCESSES

The hybrid adsorption/membrane process aims at taking the best of each technology (i.e., PAC adsorption and membrane filtration) while overcoming their limitations. PAC (type, concentration, and dosing conditions, e.g., continuous or pulse addition) can be easily adjusted to the organic micropollutants (or other CECs) targeted and a finer PAC, with faster kinetics, and higher efficiency may be used with no risk of PAC release into the treated water. In turn, a low-pressure (low energy) membrane may be used to fully retain the fine PAC particles (unlike PAC conventional addition; Campinas *et al.*, 2021a), whereas PAC may help controlling the membrane fouling by NOM (Campinas & Rosa, 2010a) (Figure 8.12).

This combination ('hybridization') yields a flexible, compact, and modular solution consisting of a safe and reliable barrier for disinfection and organic CEC control, able to cope with severe water quality changes driven by climate change. MF, UF, and loose-NF membranes may be used, with or without coagulation, depending on the upstream treatment – PAC/(C)/MF (Campinas *et al.*, 2021b, c; Viegas *et al.*, 2020a, 2021a), PAC/UF (Campinas & Rosa, 2010a, b, c, d; Rodriguez *et al.*, 2016), or PAC/NF (Viegas *et al.*, 2018). Despite its potential, when we published our first results on the hybrid adsorption/membrane process for the removal of cyanotoxins from drinking water, back in 2010, only one published study was known on PAC/UF for such application (Campinas & Rosa, 2010b).

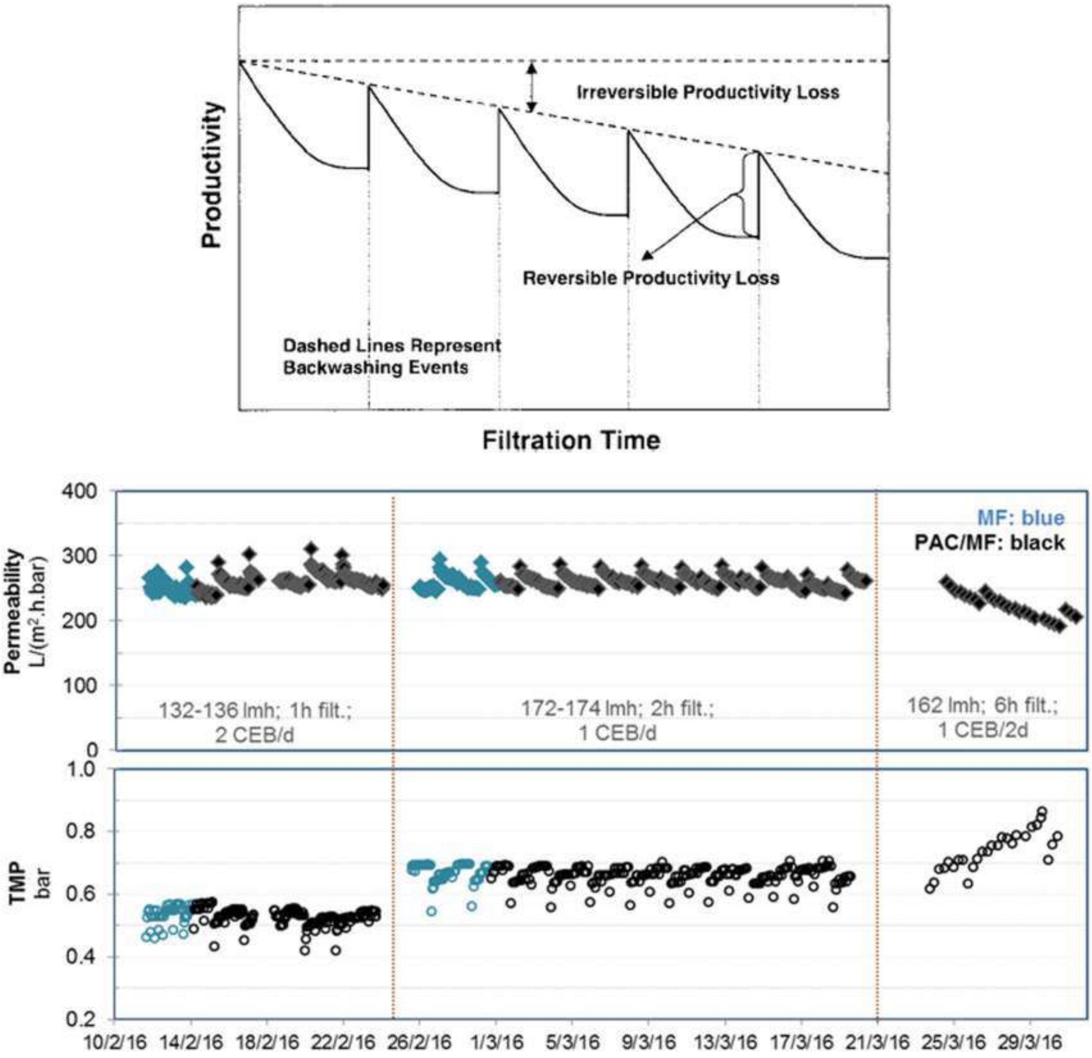
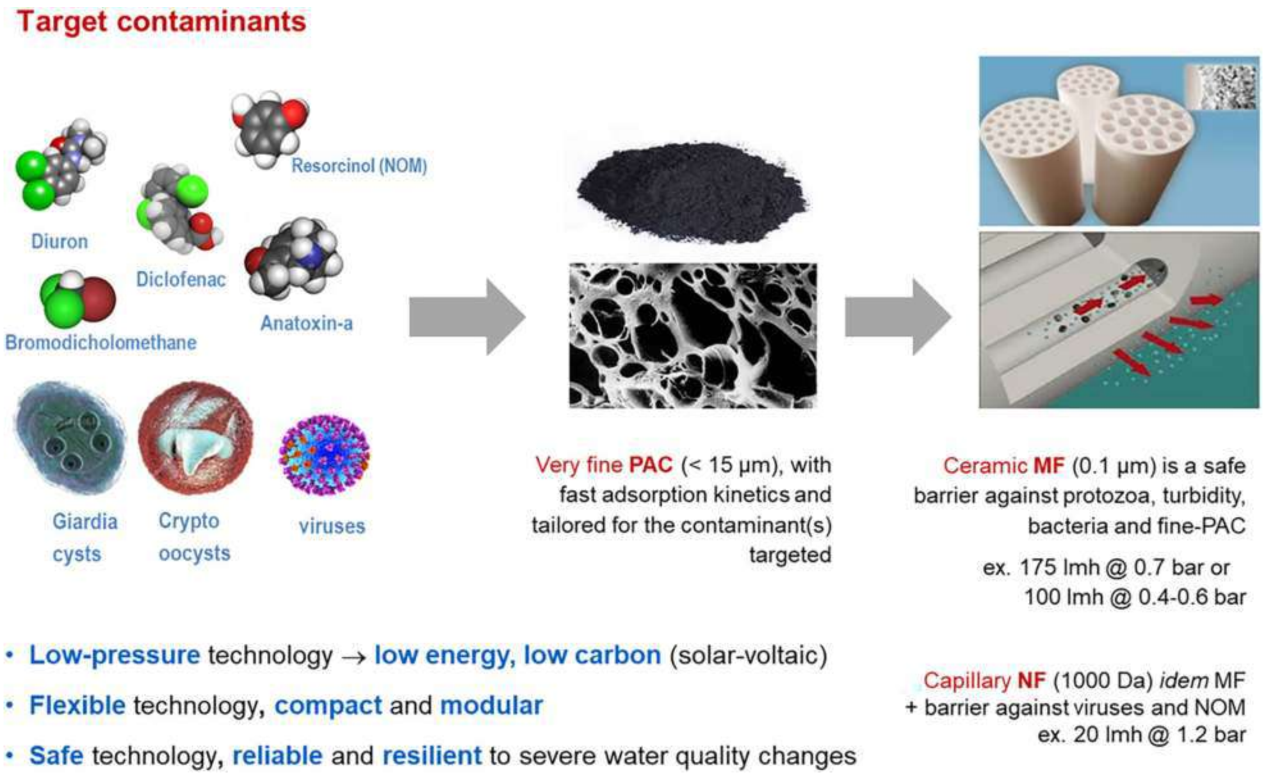


Figure 8.11 Membrane productivity over time/filtration cycles – conceptual scheme (left) and PAC/MF pilot results (right). *Source*: From Campinas *et al.* (2021c).

In addition, and as introduced in Section 8.5, ceramic membranes are particularly suited for hybrid adsorption/membrane processes due to their lower abrasion by PAC and subsequent longer lifetime (2–5 times the lifetime of polymeric membranes), their low energy consumption (<0.1 kWh/m^3) and higher water recovery rate (>95%) both in DWT (Campinas *et al.*, 2021c) and in water reclamation (Viegas *et al.*, 2020a). Again, in 2012, when our studies with pressurized hybrid low-pressure ceramic membrane processes started (we started investigating ceramic MF and PAC/MF for water reclamation in 2012 (FP7 TRUST, 2011–2014), the same year we submitted for funding LIFE Hymemb for benchmarking PAC/ceramic MF against PAC conventional addition in drinking water production (executed in 2014–2016, awarded as a best LIFE ENV project 2016–2017).), most research on PAC/MF was mainly dedicated to conventional polymeric membranes and submerged configuration. Moreover, many studies focused on membrane fouling and traditional water quality parameters conducted at the lab scale, sometimes with synthetic waters. Pilot studies of pressurized PAC/coagulation/ceramic MF envisaging organic microcontaminants, NOM, and microorganisms' removal under real scenarios, with real waters and quality variations were, and still are, scarce.



Very fine **PAC** (< 15 μm), with fast adsorption kinetics and tailored for the contaminant(s) targeted

Ceramic **MF** (0.1 μm) is a safe barrier against protozoa, turbidity, bacteria and fine-PAC
ex. 175 lmh @ 0.7 bar or
100 lmh @ 0.4-0.6 bar

Capillary **NF** (1000 Da) *idem* MF
+ barrier against viruses and NOM
ex. 20 lmh @ 1.2 bar

- **Low-pressure** technology → **low energy, low carbon** (solar-voltaic)
- **Flexible** technology, **compact** and **modular**
- **Safe** technology, **reliable** and **resilient** to severe water quality changes

Figure 8.12 Hybrid adsorption/membrane process.

8.7 APPLICATIONS IN DWT

Complementing the former sections, which included a short critical state of the art on the problem and the technologies targeted, this and Sections 8.8 relative to the applications are focused on our last decade's results, presenting our main contributions to this research area. The applications studied are illustrated in Figure 8.13 and aimed at answering the following questions: 'to what extent & why?', 'where, how & at what cost?' PAC conventional application (PAC/C/F/S) and the innovative PAC/MF (or PAC/UF) can control organic micropollutants and CECs in surface water treatment for drinking water production. Our strategy was to start by optimizing the current barriers (option 1), then to find the best (tailored) solution to upgrade the treatment, if needed, by investigating the several alternative points where to apply the advanced treatment solution proposed (PAC/MF or UF, options 2–5). Such strategy involved studies at the lab-, but also at pilot- and full-scales (Figure 8.14), which allowed developing cost analysis, and were carried out within PhD (Campinas & Rosa, 2006, 2010a, b), postdoc (Campinas *et al.*, 2013), and demonstration projects (LIFE Hymemb; Campinas *et al.*, 2017, 2021a, b, c; Viegas *et al.*, 2021a).

Regarding the 'to what extent & why', our results allowed understanding and modelling the adsorption process. In brief:

- All options (1–5) achieve good removal of the pesticides, PhCs and hormones, and MC-LR$_{eq}$ targeted (Campinas & Rosa, 2006, 2010b; Campinas *et al.*, 2017, 2021a, b).
- Charge, hydrophobicity (Log K_{ow}, Log D), molar mass (MM), and aromaticity are the adsorbate's key factors for adsorption (Figure 8.15, Campinas *et al.*, 2021a), with a turning point for Log K_{ow} 2.2, above which the compounds are very amenable to adsorption (Figure 8.16, Campinas *et al.*, 2021a), as found earlier in a UWWT study (Rodriguez *et al.*, 2016). Positively charged functional

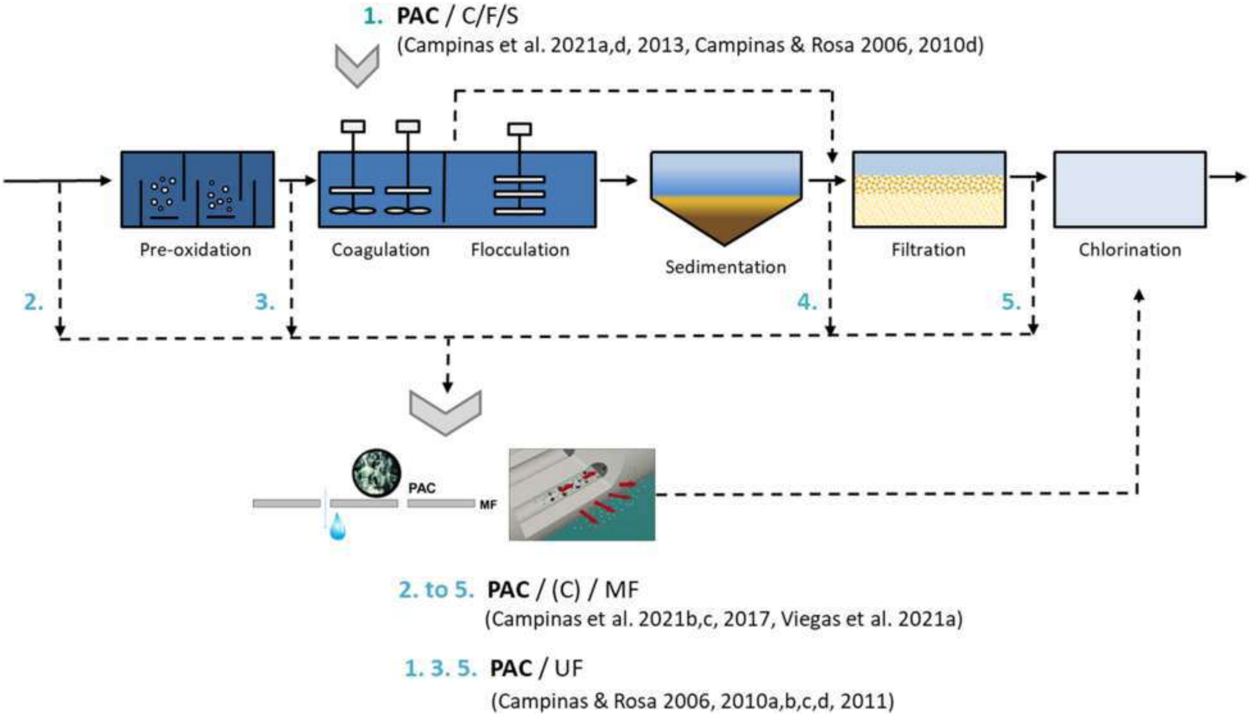
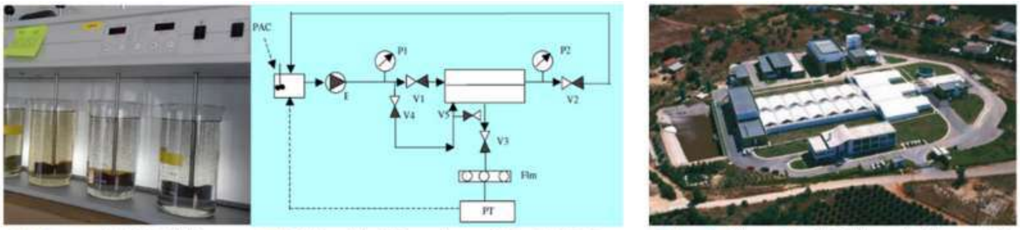


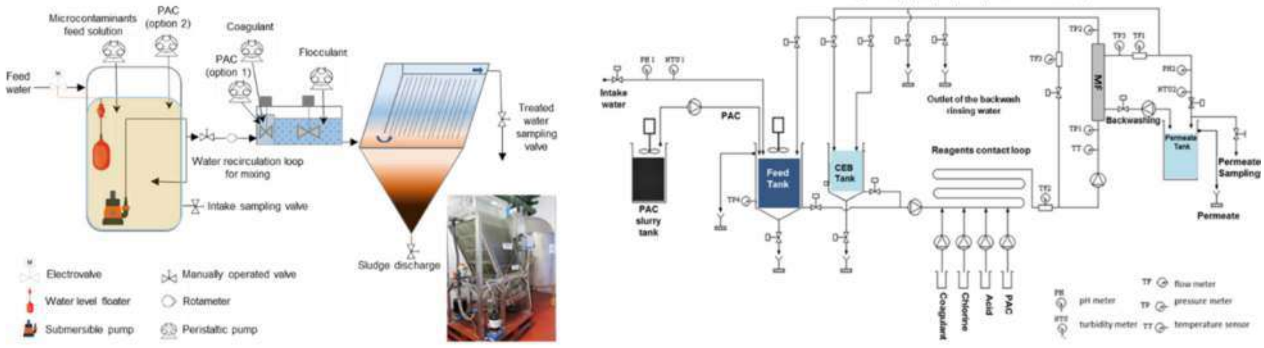
Figure 8.13 PAC application for controlling organic micropollutants in DWT (the conventional sequence represents Alcantarilha WTP).



Lab. Jar tests for PAC/C/F/S

Lab. Hollow-fibre UF membrane, 0.05 m², 100 kDa (Campinas et al. 2010a,b)

Full-scale. Alcantarilha WTP (Águas do Algarve, S.A.) 3 m³/s PAC/C/F/S (Campinas et al. 2017)



Pilot. PAC/C/F/S at Alcantarilha WTP (Campinas et al. 2021a)

Pilot. PAC / (C)/MF, ceramic 0.75 m², 0.1 µm, dead-end, 80-330 L/(m².h) constant flux at Alcantarilha WTP (Campinas et al. 2021c)

Figure 8.14 Lab, pilot, and full-scale tests of PAC application in DWT.

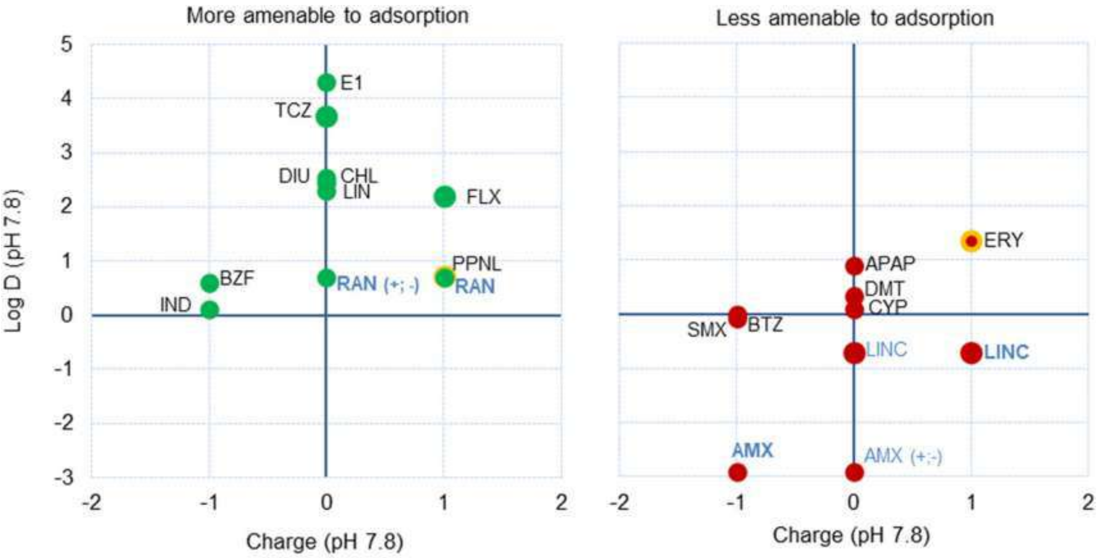


Figure 8.15 Log *D* vs charge for selected PhCs and pesticides. *Source*: From Campinas *et al.* (2021a) (very amenable to adsorption (green), relatively amenable (yellow), and less amenable (red). Compounds in blue indicate they have a distribution of species between two charges at the working pH, the dominant charge emphasized in bold letter. For neutral compounds, (+,-) highlights they have positive and negative functional groups, despite the overall neutral charge.

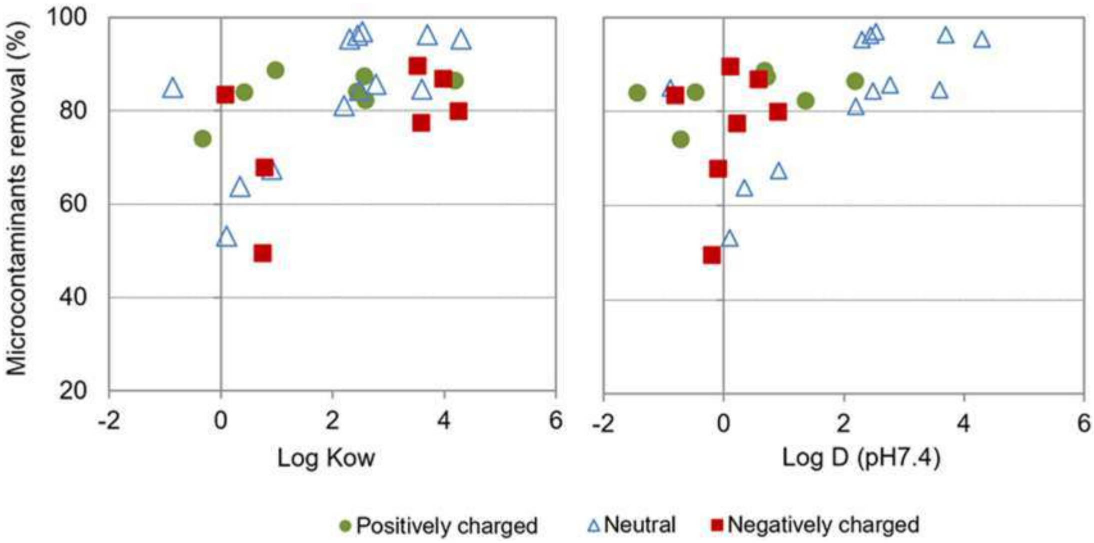


Figure 8.16 Microcontaminants' removal vs hydrophobicity measured by Log K_{ow} (left) or Log $D_{7.4}$ (right). *Source*: From Campinas *et al.* (2021a).

groups, low surface polar area (PSA) and/or high aromaticity acted as adsorption enhancers of low-hydrophobicity compounds (Log $K_{ow} < 2.2$) (Figure 8.17). Coherently with other studies, a better removal of the positively charged compounds was observed, the difference depending on the PAC net charge, that is, better with the negatively charged PAC (Campinas *et al.*, 2021a). A multiple linear regression allowed deriving correlations integrating the key variables of PhC

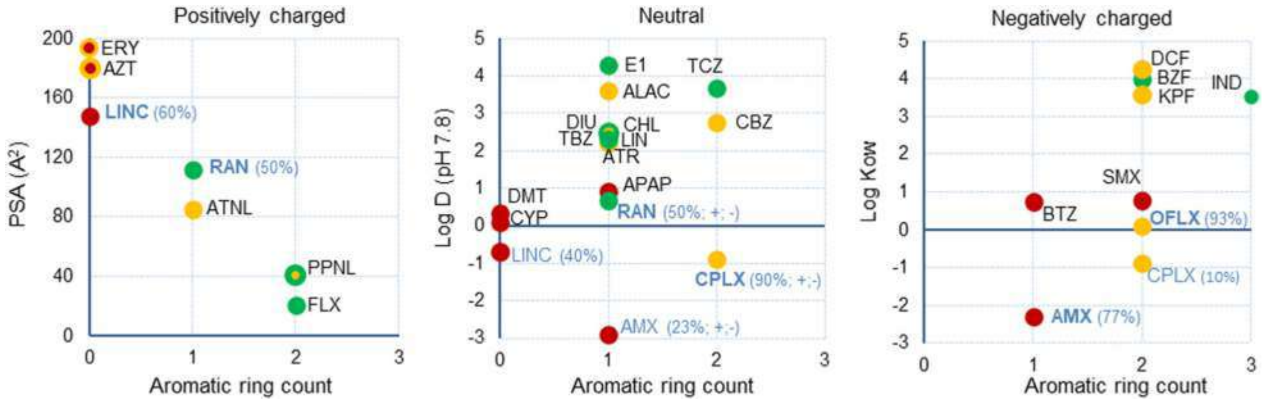
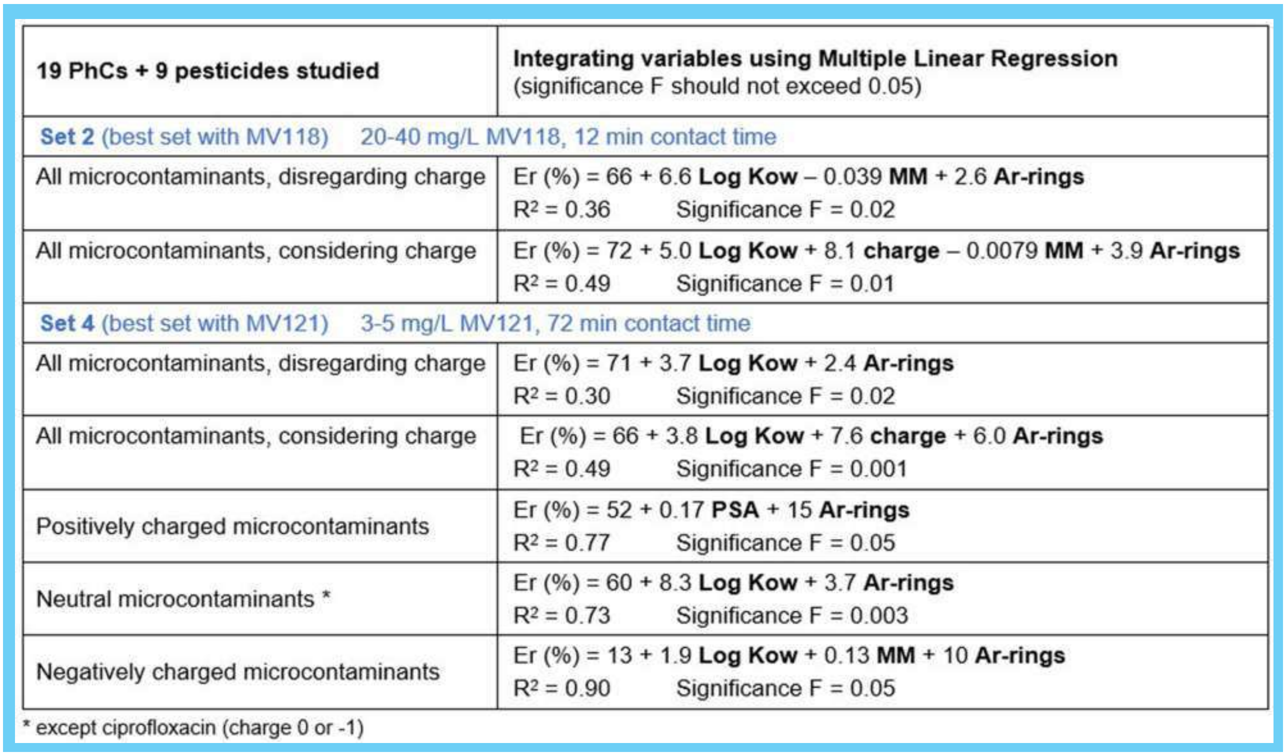


Figure 8.17 PSA, aromatic ring count, Log $D_{7.8}$ and Log K_{ow} role on the adsorption of positively charged (left), neutral (center) and negatively charged (right) PhCs and pesticides (compounds very amenable to removal in green, relatively amenable in yellow, and less amenable in red). *Source*: From Campinas *et al.* (2021a).

and pesticides' removal (Table 8.5, Campinas *et al.*, 2021a). Log *D* vs charge plot (Figure 8.15, Campinas *et al.*, 2021a) may be used to select the indicator CECs (surrogates) – an exhaustive monitoring is technically and economically unfeasible.

- Water background organics increase the PAC dose for balancing background NOM competition, which is stronger for the compounds less-amenable to adsorption and for similar-size NOM,

Table 8.5 Correlations between PAC/C/F/S removal efficiencies (Er) and the properties of 19 PhCs and nine pesticides.



19 PhCs + 9 pesticides studied	**Integrating variables using Multiple Linear Regression** (significance F should not exceed 0.05)
Set 2 (best set with MV118) 20-40 mg/L MV118, 12 min contact time	
All microcontaminants, disregarding charge	Er (%) = 66 + 6.6 **Log Kow** – 0.039 **MM** + 2.6 **Ar-rings** R² = 0.36 Significance F = 0.02
All microcontaminants, considering charge	Er (%) = 72 + 5.0 **Log Kow** + 8.1 **charge** – 0.0079 **MM** + 3.9 **Ar-rings** R² = 0.49 Significance F = 0.01
Set 4 (best set with MV121) 3-5 mg/L MV121, 72 min contact time	
All microcontaminants, disregarding charge	Er (%) = 71 + 3.7 **Log Kow** + 2.4 **Ar-rings** R² = 0.30 Significance F = 0.02
All microcontaminants, considering charge	Er (%) = 66 + 3.8 **Log Kow** + 7.6 **charge** + 6.0 **Ar-rings** R² = 0.49 Significance F = 0.001
Positively charged microcontaminants	Er (%) = 52 + 0.17 **PSA** + 15 **Ar-rings** R² = 0.77 Significance F = 0.05
Neutral microcontaminants *	Er (%) = 60 + 8.3 **Log Kow** + 3.7 **Ar-rings** R² = 0.73 Significance F = 0.003
Negatively charged microcontaminants	Er (%) = 13 + 1.9 **Log Kow** + 0.13 **MM** + 10 **Ar-rings** R² = 0.90 Significance F = 0.05

* except ciprofloxacin (charge 0 or -1)

Source: Adapted from Campinas *et al.* (2021a).

for example, tannic acid and MC-LR (Campinas *et al.*, 2013). Background inorganics affect the extent and mechanisms of NOM-target adsorption (via shielding mechanisms) and may have a positive effect on PAC adsorption depending on the PAC overall charge (Campinas & Rosa, 2006; Campinas *et al.*, 2013) – for example, for a solute and PAC with opposite charges, for low surface concentrations (low X/M), ions shield PAC-solute electrostatic attraction resulting in an ionic strength-driven adsorption reduction, whereas for high X/M ratios, ions shield solute–solute intra- and intermolecular repulsions resulting in an ionic strength-driven adsorption enhancement.

- PAC (10 mg/L in Campinas and Rosa, 2010b; 6–24 mg/L in Campinas *et al.*, 2021c) does not impair the membrane flux, rather it helps controlling the irreversible membrane fouling, minimizing the chemical cleaning frequency. It enhances NOM retention by UF/MF, including algogenic organic matter (AOM) (Campinas & Rosa, 2010a), though not significantly for the highly hydrophilic compounds whose membrane foulant behavior may increase driven by water divalent salts (Campinas & Rosa, 2010a).

Regarding the 'where, how & at what cost', the main results are:

- **All options** (1–5) allow an effective control of the target micropollutants and CECs as long as an adequate PAC type and dose is applied. The differences are more related with (a) the risk of PAC particles escaping the treatment (in PAC/C/F/S the dose appears to be limited to 10 mg/L) and (b) the energy consumption and the cost, for the upgrading solution or overall (Campinas *et al.*, 2021c).
- **Option 1. PAC/C/F/S** (Campinas *et al.*, 2021a) – removals of 65–79% for total-pharmaceuticals (19) and 73–83% for total-pesticides (9) were achieved with 3–9 mg/L of a mesoporous negatively charged PAC or with 20–24 mg/L of a microporous positively charged PAC. For hydrophilic low NOM waters, PAC textural properties and PAC surface chemistry were both important for adsorption. Half to one third of PAC dose would be enough as long as a PAC with adequate pore-size distribution is used, this means using a PAC with high secondary microporous volume but also with high volume of mesopores to avoid microcontaminants' size exclusion and minimize PAC pore blocking phenomenon by NOM. Similar PAC savings are possible providing an extra 1-h PAC contact time to the very short time observed in the regular operation of the WTP where the PAC/CFS pilot was installed (around 15 min), with benefits particularly for the compounds of higher molar mass and/or less amenable to adsorption. A high vulnerability of clarification effectiveness to PAC dosing was observed with the low-turbidity waters tested, with a higher risk of negative impact for PAC doses above 10 mg/L towards residual turbidity due to PAC fines (with particulate microcontaminants), residual aluminum and aerobic endospores, the latter used as surrogates of biological forms resistant to chemical disinfection, as *Cryptosporidium* oocysts and *Giardia* cysts. This result may compromise the microcontaminants' removal reliability unless a downstream filtration safely retains the PAC fines.
- **Options 2–5. PAC/MF** (Campinas *et al.*, 2021b) – PAC/(Alum)/MF (conditions below) achieved 75% to complete removal of total microcontaminants (pesticides, PhCs, or microcystins) with 4–18 mg/L of a mesoporous PAC and 2 h contact time (Figure 8.18), with a reliable particle separation (turbidity < 0.03 NTU) and low aluminum residuals. Microcontaminants showed different amenabilities to PAC adsorption, depending on their charge, hydrophobicity (Log K_{ow}), polar surface area and aromatic rings count. Compounds less amenable to adsorption showed higher vulnerability to NOM competition (higher A254 waters), greatly benefiting from DOC-normalized PAC dose increase (Figure 8.19, Campinas *et al.*, 2021b). PAC/Alum/MF also attained 29–47% NOM median removal as DOC, decreasing THMFP by 26%. PAC complemented NOM removal by coagulation (+15% to +19%, percentage points), though with no substantial improvement toward THMFP and membrane fouling. Furthermore, PAC/Alum/MF was a full barrier against aerobic endospores, and PAC dosing was crucial for $\geq$ 1.1-log reduction in bacteriophages.

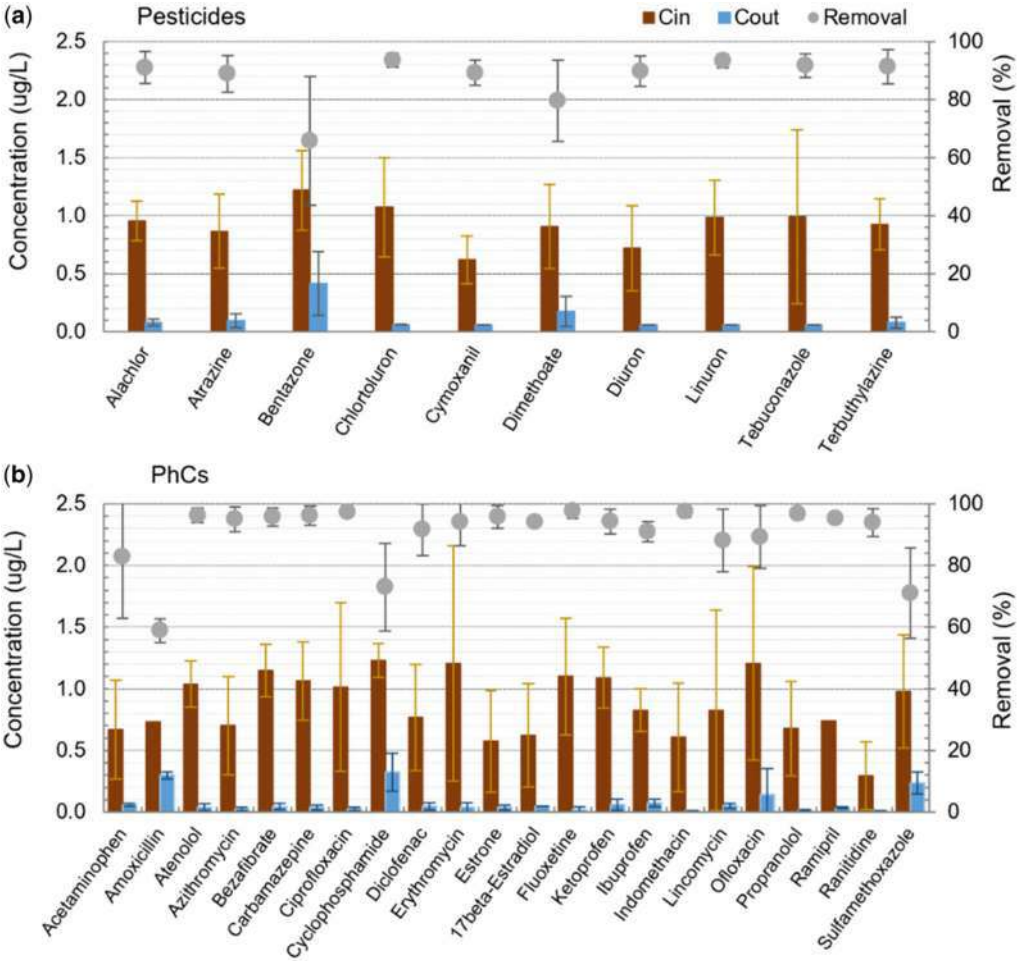


Figure 8.18 Average intake (brown bars) and permeate (blue bars) concentrations and removals (circles) of pesticides (a) and pharmaceuticals (b) after PAC/(Alum)/MF (average values of all spiking trials; error bars represent standard deviations between trials). *Source*: From Campinas *et al.* (2021b).

- **Options 2–5. PAC/MF** with 15 µm particle PAC, pH_{pzc} 10–11, 1126 m^2/g BET area and 0.83 cm^3/g total pore volume with 53% of mesopores (2–50 nm diameter); pressurized module, with three tubular MF (0.1 µm) ceramic (ZrO_2/TiO_2) membranes (0.75 m^2), operated in a continuous dead-end mode with constant permeate flowrate in the 80–330 L/(m^2.h) range (Campinas *et al.*, 2021c) – PAC addition (6–24 mg/L) did not promote membrane fouling and, for all conditions tested, TCp was kept constant or slightly increased with PAC dosing. Membrane fouling observed during the 1.5-year demonstration period was essentially reversible, since no significant change in clean membrane permeability was observed. As expected, a higher treatment capacity was obtained with filtered water (9.6 m^3/(m^2.d.bar), option 5), followed by ozonated/pre-coagulated water (5.6 m^3/(m^2.d.bar), option 4), with similar results for raw water and ozonated water with recirculated filter-backwash waters (4.7–4.9 m^3/(m^2.d.bar), options 2 and 3). The non-clarified waters tested (options 2 and 3) required pretreatment with in-line alum coagulation to minimize membrane fouling (PAC/C/MF). Water recovery was 97–99%. PAC/MF energy consumption was 0.045–0.053 kWh/m^3 (options 5–3, 0.02 kWh/m^3 for PAC dosing), inversely varying with TCp. The cost analysis showed MF total production costs

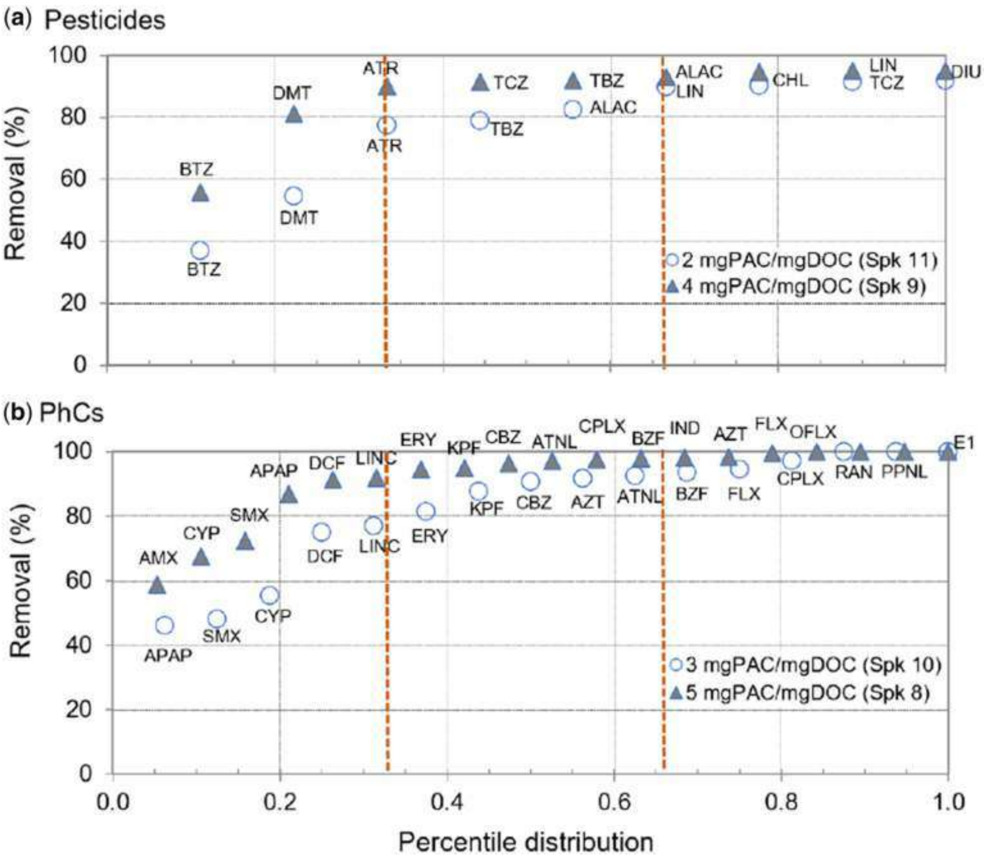


Figure 8.19 DOC-normalized PAC dose effect on the percentile distribution of removal efficiencies of nine pesticides (a) and 19 PhCs (b) during two spiking trials (those with the highest number of microcontaminants and the lowest PAC doses). *Source*: From Campinas *et al.* (2021b).

(CAPEX and OPEX) of 0.07 and 0.04€/m^3 for treating 1,00,000 m^3/d raw and filtered waters, respectively; PAC dosing increases costs 0.03€/m^3 for both waters. Figure 8.20 shows PAC/MF total costs' breakdown.

- **Options 2–5. PAC/MF** idem (Viegas *et al.*, 2021a) showed the way PAC is dosed matters – two pilot trials were conducted to compare continuous 10–12 mg/L PAC inline dosing with 8–10 mg/L dosing to a 2 h-contact tank. Two low turbidity/low natural organic matter (NOM <2 mgC/L) surface waters spiked with 7.2–10.3 μg/L total pesticides were tested. Removal differences between the two PAC dosing options depended on pesticides' amenability to adsorption and NOM characteristics (A254) (Figure 8.21). Waters with low A254-absorbing NOM and only pesticides amenable to adsorption showed very high removals (all pesticides ≥93%) and no significant differences between the two PAC dosing options. Waters containing higher A254-absorbing NOM and high loads of pesticides less amenable to adsorption (dimethoate, bentazone) required higher inline PAC dose. Those or more severe conditions may require PAC doses higher than tested to comply with the Drinking Water Directive limits for pesticides. Cost analysis showed PAC inline dosing is more cost-effective than PAC dosing to the contact tank when identical PAC dose is sufficient or when the doses are low, even if 50% higher for inline dosing, and the plant is small.

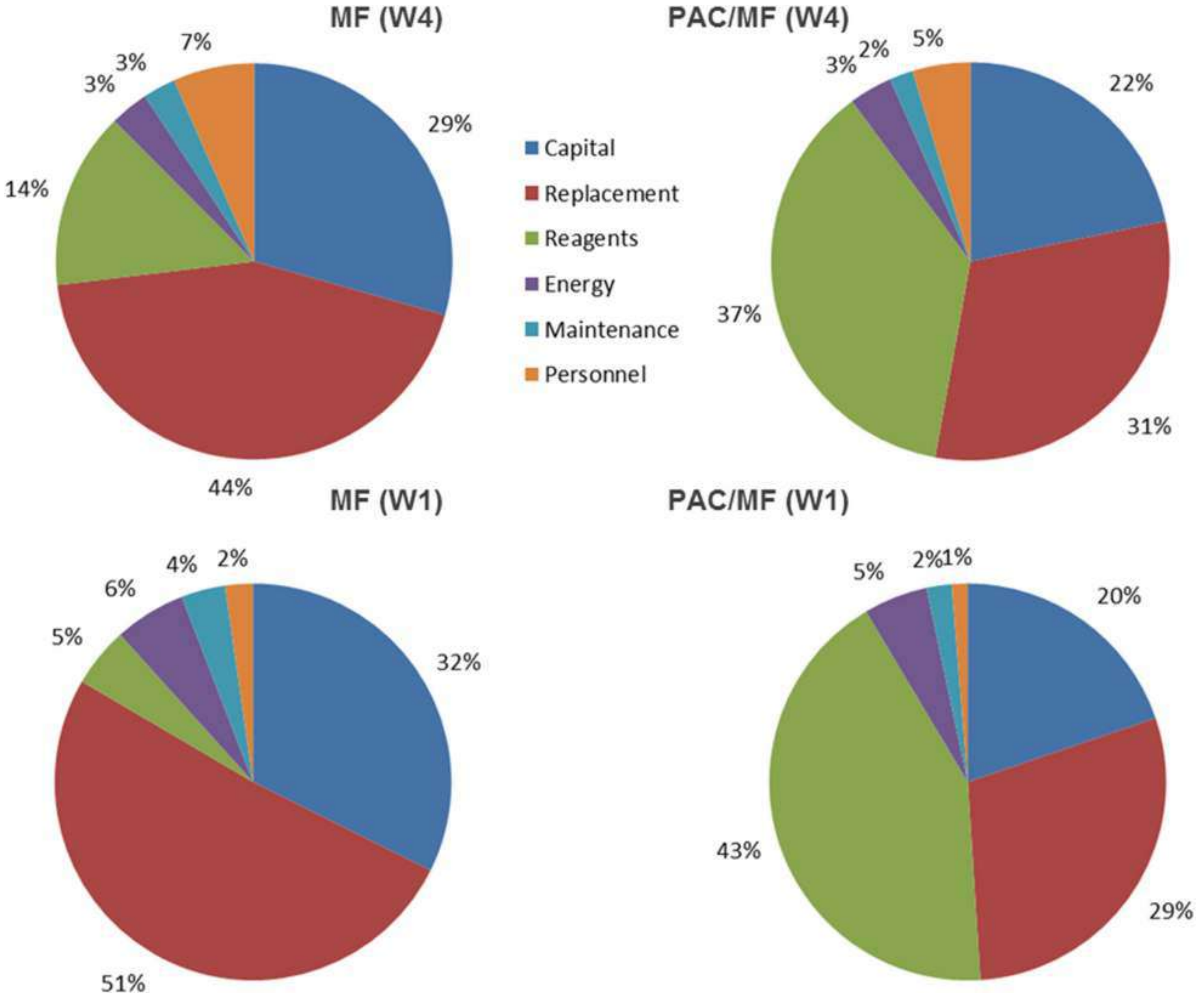


Figure 8.20 Cost structure of ceramic MF (left) and PAC/MF (right) for option 2 (W4) and option 5 (W1). *Source*: From Campinas *et al.* (2021c).

- **Option 5. PAC/UF** with mesoporous and microporous positively charged (alkaline) fine PAC and hydrophilic hollow-fiber UF membrane, 100 kDa cut-off, in/out cross-flow filtration (Campinas & Rosa, 2010a, b) – in the absence of background NOM, PAC/UF with 10 mg/L PAC and up to 20 μg/L MC-LR$_{eq}$ feed concentration achieved 93–98% MC-LR$_{eq}$ removal and a cycle-averaged permeate concentration below the WHO drinking water guideline-value for microcystin-LR variant. NOM type and concentration and MC initial concentration determined the PAC dose to be used. While 10 mg/L PAC effectively controlled ca. 5 μg/L MC-LR$_{eq}$ in a model water with 2.5 mg/L NOM or with *M. aeruginosa* culture (cells and algogenic organic matter), 15 mg/L PAC were unable to achieve the WHO quality with a water containing higher concentrations of NOM (5 mg/L) and microcystins (ca. 20 μg/L MC-LR$_{eq}$) (Campinas & Rosa, 2010c, 2011). UF is a safe barrier against cyanobacteria, ensuring an absolute removal of *M. aeruginosa* single cells, the smallest cyanobacterial cells and hence the most difficult to remove. An increased cell lysis was observed with cell aging, although it did not necessarily degrade permeate quality, as in parallel to cell damage an enhancement of microcystin rejection by the

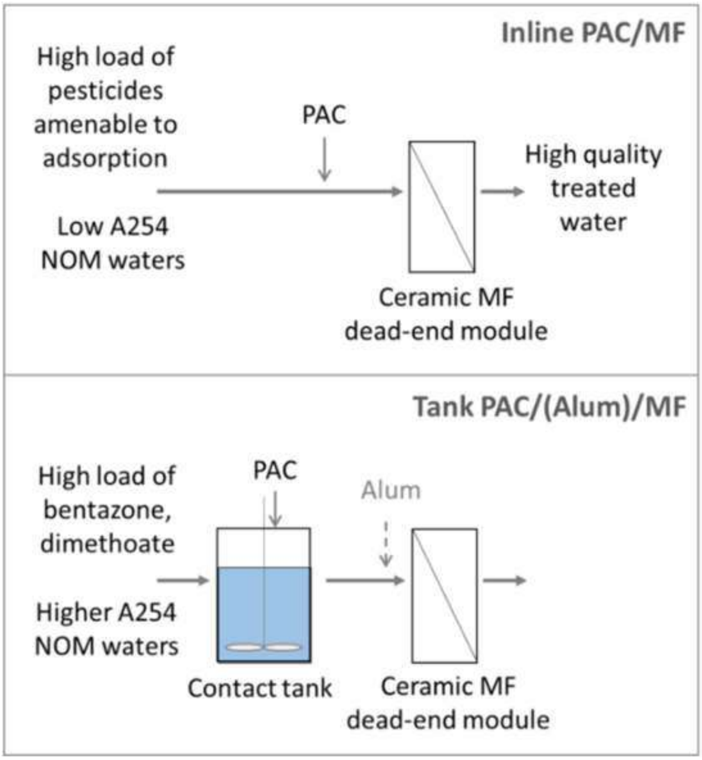


Figure 8.21 Inline vs tank PAC dosing in PAC/MF for pesticides and NOM removal in DWT. *Source*: From Viegas *et al.* (2021a).

UF hydrophilic membrane was observed with cell aging, most probably due to AOM-driven microcystin adsorption on the membrane, connected to the greater content of the older cultures in segregated AOM (mucopolysaccharides) and/or protein lysed AOM (Campinas & Rosa, 2010c, 2011).

8.8 APPLICATIONS IN UWWT AND WATER RECLAMATION

The applications we have been studying over the last decade are illustrated in Figure 8.22 and aimed at answering the following questions: 'to what extent and why?', 'where, how and at what cost?' PAC different set-ups, PAC dosing directly into activated sludge reactor (PAC-AS) and PAC dosing downstream the biological treatment in a hybrid adsorption/membrane process (PAC/MF, or PAC/UF, or PAC/loose NF) can control organic micropollutants and CECs in UWWT/resource recovery or water reclamation. Our strategy is to start by optimizing the current barriers (option 1), then to find the best (tailored) solution to upgrade the treatment if needed by investigating several alternatives (options 2–5), and finally to look ahead to more demanding needs likely to occur in the (near) future, namely the direct potable reuse (DPR). Such strategy involved studies at lab-, pilot-, and full-scales (Figure 8.23), which allowed developing cost analysis, and were conducted within PhD (Rodriguez *et al.*, 2016), postdoc (Viegas *et al.*, 2018), and R&I projects – LIFE Impetus (Campinas *et al.*, 2022; Mestre *et al.*, 2022a; Rosa *et al.*, 2019; Silva *et al.*, 2022; Viegas *et al.*, 2022), FP7 TRUST (Viegas *et al.*, 2020a), LIFE aWARE (Viegas *et al.*, 2018), and H2020 B-WaterSmart (Galego *et al.*, 2022). We also investigated the development of new PACs, framed within a circular economy approach (Mestre *et al.*, 2022a, b; Viegas *et al.*, 2020b).

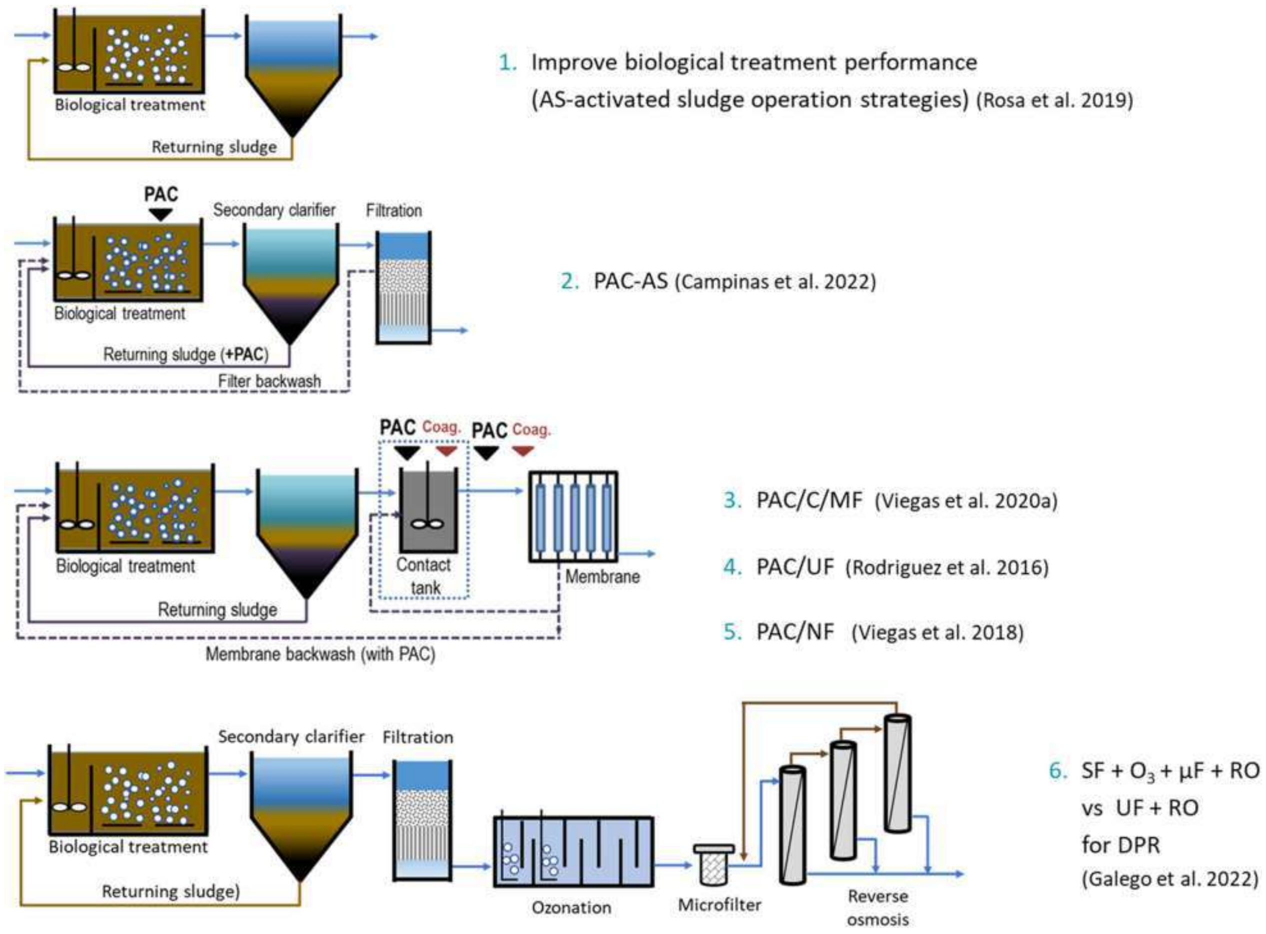
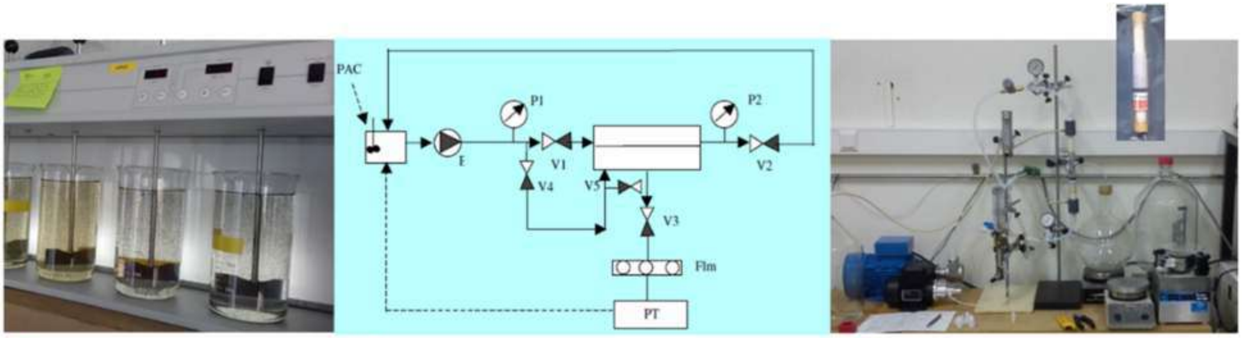


Figure 8.22 PAC application for controlling organic micropollutants in UWWT/water reclamation.

Regarding 'to what extent & why' and 'where, how & at what cost', briefly, our results showed:

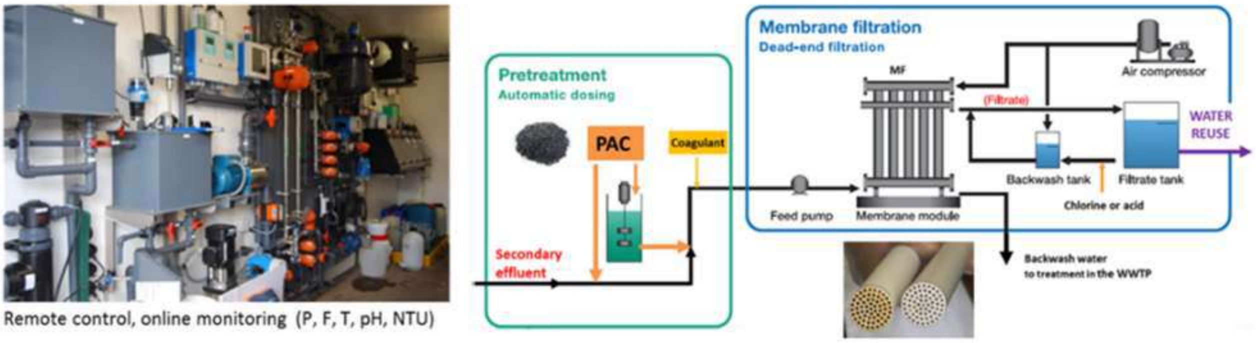
- **All options**, including option 1, achieve some or good removal of the organic micropollutants targeted. So, if possible, it is worth starting by implementing option 1 (no/low investment) and then upgrade as needed.
- **Option 1. AS – activated sludge performance improvement** (Rosa *et al.*, 2019; Silva *et al.*, 2022) – the control of a representative range of PhCs (24) was studied in two activated sludge UWWTPs with nitrification/ denitrification (oxidation ditch in FNW, anaerobic/anoxic/aerobic system in BEI), involving 55 sampling campaigns during 2.5 years. The results show similar removals in both plants and, as earlier found, some of the most abundant PhCs at the WWTP inlet were highly removed during treatment (caffeine, acetaminophen, ibuprofen, and naproxen), others presented intermediate and variable removals (the antibiotics erythromycin, sulfamethoxazole, sulfapyridine, and sulfadiazine; the beta-blockers metoprolol and propranolol) and some compounds were recalcitrant to treatment, as the antiepileptic/ anticonvulsant carbamazepine and the anti-inflammatory diclofenac. The PhC removals in both plants highly correlated with the PhCs' biodegradation constants (k_{bio}), with a turning point at 1 L/(g SS.d), above which the median removals were above 86% in BEI and above 74% in FNW (Figure 8.24). A 4-class (from A – easily removed to D – recalcitrant) biodegradation/sorption framework is proposed for interpreting and predicting PhC control in urban AS WWTPs (Figure 8.4), as well as for



Lab. Jar tests for PAC/C/F/S

Lab. Hollow-fibre UF membrane, 0.05 m^2, 100 kDa (Campinas et al. 2010a,b)

Lab. Capillary NF, 0.075 m^2, 1000 Da (Viegas et al. 2018)



Remote control, online monitoring (P, F, T, pH, NTU)

Pilot. PAC / (C)/MF, ceramic 0.8 m^2, 0.1 μm, dead-end constant flux at BEI WWTP (Viegas et al. 2020a)

Full-scale WWTP
Beirolas (Águas Tejo Atlàntico, S.A.)
50000 m^3/d, AS system A2O
(Rosa et al. 2019)

Full-scale WWTP
Faro NW (Águas do Algarve, S.A.)
5000 m^3/d, AS system oxidation ditch

PAC dosing
@ oxidation ditch

Figure 8.23 Lab, pilot, and full-scale tests of PAC application in UWWT.

selecting the indicator CECs (surrogates, as proposed in UWWTD recast). A statistical analysis indicated significantly higher removals in BEI associated with F/M values below 0.08 d^{-1}, and in FNW associated with N-removals > 80% and alkalinity reductions > 40% (indirect effect of nitrification) and were associated with an effluent transmittance (T254) of 67% (coherent with Zietzschmann *et al.* (2014) and Zietzschmann (2020), who have found A254 to be a reasonable/good surrogate parameter). Though expressed by different proxys, these results are

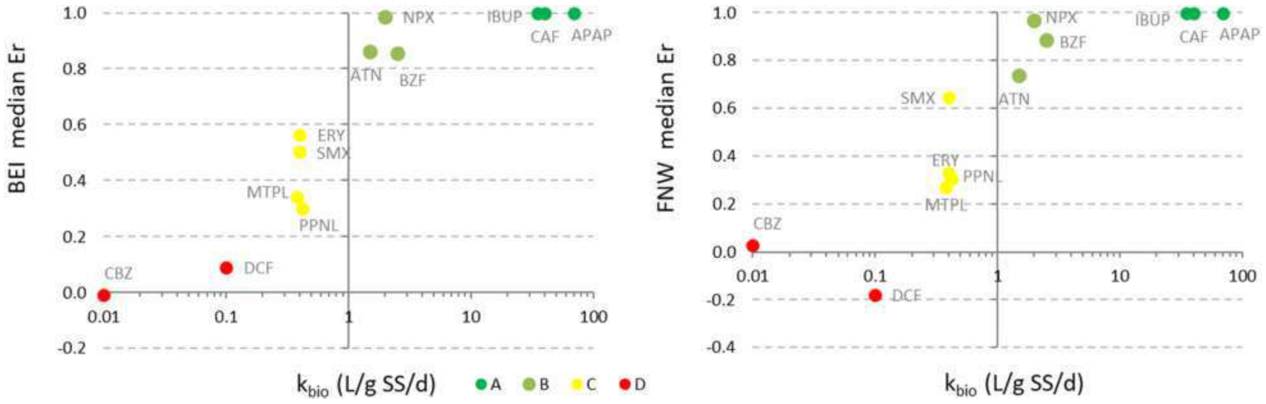
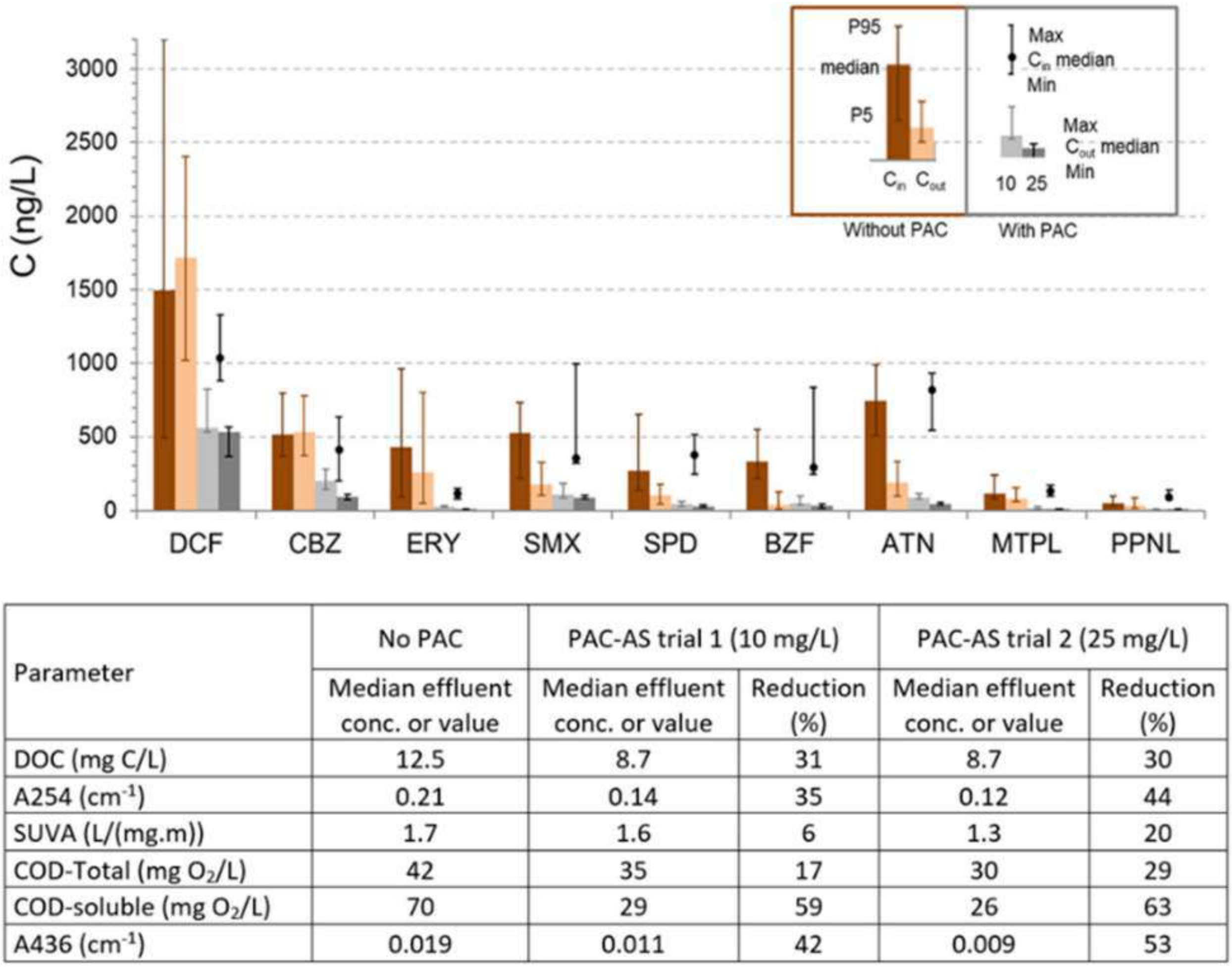


Figure 8.24 PhC median removal vs kbio of each PhC in BEI and FNW UWWTPs (the colours correspond to the A–D class of the PhC in Figure 8.4). *Source*: From Silva *et al.* (2022).

consistent with an enhanced elimination of some low biodegradable PhCs when conditions for the build-up of the slowly growing nitrifying bacteria (which can excrete enzymes and, by co-metabolism, break down some low degradable molecules) are provided, as found by Clara *et al.* (2005) for sludge retention times of, at least, 10–15 days. Promoting nitrification is therefore an operational low-investment measure for improving current UWWTPs towards PhC elimination.

- **Option 2. PAC-AS** (Campinas *et al.*, 2022) – PAC dosing to a conventional AS reactor is a low-investment option for controlling PhCs in UWWT, but its advantages and limitations in real operating environments are not fully assessed. A 3-week PAC-AS full-scale test was conducted in an oxidation ditch plant to assess PAC impact on effluent quality (PhCs, DOC, and other parameters), energy consumption, sludge production, and direct costs. DOC-normalized PAC doses of 0.7–2.6 mgPAC/mgDOC significantly reduced recalcitrant PhC discharged (e.g., by 63–84% for carbamazepine and 63–70% for diclofenac), the higher dose yielding a more reliable effluent quality (Figure 8.25). Effluent quality for total phosphorus, color, organic matter and transmittance was also enhanced and no interference with nitrification, oxidation–reduction potential or dissolved oxygen in the oxidation ditch was observed, resulting in no energy consumption increase. PAC had no impact on effluent turbidity and mixed liquor (MXL) suspended solids settleability, showing a positive effect on dewatered sludge dry weight and ultimately a 7–9% increase on final sludge production. After stopping PAC dosing, PAC remaining in the recirculated sludge presented adsorption capacity for some PhCs until it was completely out of system. Estimated direct costs (CAPEX + OPEX) for PAC addition to AS-reactor [0.064–0.055 €/m^3 for 5000–50,000 m^3/d, for 20 mg/L PAC dosed 12 months/year] are almost plant-size independent and compare favorably with literature data for PAC and GAC post-treatment (Baresel *et al.*, 2017; Rizzo *et al.*, 2019) and similarly with post-ozonation (Abegglen & Siegrist, 2012; Baresel *et al.*, 2017; Hillenbrand *et al.*, 2016; Mulder *et al.*, 2015).
- **AC adsorption UWWT vs DWT** – UWWT results are very consistent with the analogous ones found for DWT, though requiring more demanding conditions (higher PAC doses, lower membrane productivity) to balance the more concentrated water composition, so the removal mechanisms are essentially the same in both types of water. Charge, hydrophobicity, and aromaticity are again the adsorbate's key factors for PAC adsorption, as well as the water background organics and inorganics, the former with a stronger competing effect on the compounds less amenable to adsorption and the latter via the same shielding effect, as thoroughly analyzed in option 4 investigation (conducted at lab scale). A partial least-squares (PLS) regression with 25 descriptors



Parameter	No PAC	PAC-AS trial 1 (10 mg/L)		PAC-AS trial 2 (25 mg/L)	
	Median effluent conc. or value	Median effluent conc. or value	Reduction (%)	Median effluent conc. or value	Reduction (%)
DOC (mg C/L)	12.5	8.7	31	8.7	30
A254 (cm^{-1})	0.21	0.14	35	0.12	44
SUVA (L/(mg.m))	1.7	1.6	6	1.3	20
COD-Total (mg O_2/L)	42	35	17	30	29
COD-soluble (mg O_2/L)	70	29	59	26	63
A436 (cm^{-1})	0.019	0.011	42	0.009	53

Figure 8.25 Example of PAC-AS performance in PhC (left) and organic matter (right) control. *Source*: Adapted from Campinas *et al.* (2022).

(10 water-related (8 OM, 2 ion matrix), 7 PhC-related, and 8 PAC-related descriptors) yielded the following correlations of PhC removal from urban wastewater (Viegas *et al.*, 2022), which are overall coherent with those found in DWT (Table 8.5):

- Describing the adsorption capacity

$$\%\,removal\ (21\,h) =$$

$$-0.147 \cdot sHB\ \left(mg\,\frac{C}{L}\right) - 1.45x10^{-4} \cdot EC\ \left(\frac{uS}{cm}\right) + 6.73x10^{-3} \cdot Solv.energy\ \left(\frac{kJ}{mol}\right)$$

$$+0.0978 \cdot \log Kow + 2.04x10^{-4} \cdot A_{BET}\ \left(\frac{m^2}{g}\right) + 0.709$$

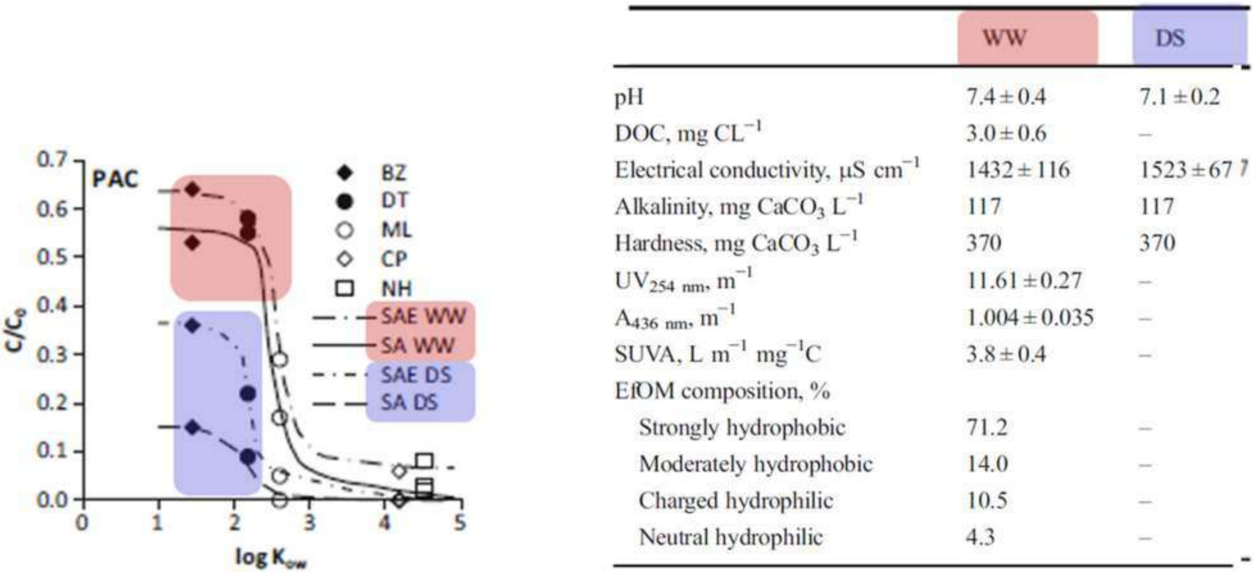
- Describing the short-term adsorption

$$\%\,removal\ (1\,h) = -0.0469 \cdot cHF\ \left(mg\,\frac{C}{L}\right) + 4.49x10^{-3} \cdot Solv.energy\ \left(\frac{kJ}{mol}\right) +$$

$$0.0190 \cdot \log P + 0.190 \cdot V_{super}\left(\frac{cm^3}{g}\right) - 1.24x10^{-3} \cdot PAC_{dens}\ \left(\frac{kg}{cm^3}\right) + 0.928$$

These correlations show the existence of complex interactions that a univariate analysis is not sufficient to describe and a good descriptive capability. Namely, it shows (a) the hydrophobic PhC–PAC interactions play a major role in the adsorption process, with the solvation energy and log K_{ow} being the most suitable descriptors, (b) the slightly hydrophobic fraction of the water DOM impact the adsorption capacity whereas its charged hydrophilic compounds impact the short-term adsorption, (c) the water inorganic matrix appears to impact only the PAC adsorption capacity and not the short-term adsorption, (d) for the pool of PACs tested, the results point to the BET area as a good descriptor of the PAC capacity, while the short-term adsorption kinetics appears to be better related to its supermicropore volume and density, the latter inversely related with the particle size, that is, the solute's intraparticle path.

- **Option 4. PAC/UF** (Rodriguez *et al.*, 2016) – for the compounds in Table 8.3, hydrophobicity is the adsorption key property for neutral adsorbates, with a turning point at Log K_{ow} 2.2–2.6, above which the compounds are very amenable to adsorption, that is, present low residual normalized concentrations (Figure 8.26). The uptake of the positively charged nortriptyline by the positively charged, meso- and microporous PACs exceeded the expected from Log *D* due to its high aromaticity and the background ions, which partially shielded PAC-nortriptyline electrostatic repulsions. Adsorption capacity depended on the solute's hydrophobicity, whereas the kinetics further depended on its charge. Hydrophobic EfOM was preferentially adsorbed and a stronger competitor, particularly for microcontaminants with Log $K_{ow} < 2.6$. The highly microporous PAC better adsorbed these target compounds and the hydrophobic EfOM, and it attenuated the EfOM competition. So, in real applications, PACs with higher volume of secondary micropores or small mesopores (SA vs SAE) might be a strategy for attenuating EfOM competition. For all waters, PAC had no effect on UF-flux, and it significantly improved the microcontaminants' and EfOM removal by PAC/UF over standalone PAC and UF; PAC/UF was more effective and efficient than PAC/sedimentation; PAC dose should target the contaminants with Log $K_{ow} < 2.6$, the weaker adsorbates, as found by others (e.g., Zietzschmann, 2020).



	WW	DS
pH	7.4 ± 0.4	7.1 ± 0.2
DOC, mg CL^{-1}	3.0 ± 0.6	–
Electrical conductivity, µS cm^{-1}	1432 ± 116	1523 ± 67
Alkalinity, mg $CaCO_3$ L^{-1}	117	117
Hardness, mg $CaCO_3$ L^{-1}	370	370
$UV_{254\ nm}$, m^{-1}	11.61 ± 0.27	–
$A_{436\ nm}$, m^{-1}	1.004 ± 0.035	–
SUVA, L m^{-1} mg^{-1}C	3.8 ± 0.4	–
EfOM composition, %		
Strongly hydrophobic	71.2	–
Moderately hydrophobic	14.0	–
Charged hydrophilic	10.5	–
Neutral hydrophilic	4.3	–

Figure 8.26 Log K_{ow} role on organic microcontaminants' control by PAC (SA and SAE) adsorption and the effect of water background organics (wastewater – WW and deionized water with equivalent inorganic matrix – DS) (compounds in Table 8.3). *Source*: Adapted from Rodriguez *et al.* (2016).

- **Option 3. PAC/C/MF** (Viegas *et al.*, 2020a) – pilot assays (100 L/(m^{2}h), 10 mg Fe/L) were conducted with sand-filtered secondary effluent spiked with four chemically diverse PhCs (ibuprofen, carbamazepine, sulfamethoxazole, and atenolol; Table 8.2, Figure 8.4, Figure 8.17) and 15 mg/L PAC dosed inline or to a 15-min contactor. The results showed no PAC-driven membrane fouling and +15 to +18% added removal with PAC contactor, reaching significant removals of CBZ and ATN (59–60%), SMX (50%), color (48%), A254 (35%), and DOC (28%). Earlier long-term demo tests with the same pilot proved PAC/Fe/ceramicMF to consistently produce highly clarified (monthly median $<$ 0.1 NTU) and bacteria-free water, regardless of the severe variations in its intake (Figure 8.27, Viegas *et al.*, 2015). A detailed cost analysis points to total production costs of 0.21 €/m^3 for 50,000 m^3/day and 20 years membrane lifespan, mainly associated with equipment/membranes replacement, capital and reagents, the energy having the smallest share (for a specific flux of 261 L/(m^2.h.bar) and an energy consumption of 0.026 kWh/m^3).
- **Option 5. PAC/NF** (Viegas *et al.*, 2020a) – bench-scale results with a loose-NF membrane (capillary NF, 1000 Da cut-off) showed high removal efficiencies of four target PhCs (ATN, CBZ, DCF, SMX; 100 μg/L each spiked in secondary sand-filtered effluent) and the PAC continuous dosing to be more efficient than the pulse dosing, 50 mg/L PAC achieving a total-PhC removal of 68% (ranging from 58% to 89%), 58% DOC removal and 90% color removal. NF fluxes of 20 L/(m^2.h) were achieved with 1.2 bar transmembrane pressure, 1 m/s crossflow velocity, with no pressure increase up to 100 mg/L PAC. The results were successfully used in the design and operation of the PAC/NF pilot for the technology demonstration in El Prat WWTP (Barcelona). Compared to UF/RO (50/50), PAC/NF at pilot scale yielded comparable EfOM and PhC removals, PAC costs similar to UF/RO reagent costs, 40% less energy. Furthermore, PAC/NF concentrate recirculation to MBR showed synergic effects (not possible for UF/RO concentrate) with economic (lower cost) and operational advantages – PAC in the MBR improved the removal of refractory microcontaminants (diuron and carbamazepine) and metals (Cu, Ni, Zn, Pb) (Martin *et al.*, 2016).
- **PAC adsorption modelling** (integrating adsorption kinetics and isotherms) has the predictive ability to forecast the contaminant removal as a function of PAC dose, contact time and adsorbate concentration (Figure 8.28, Rosa *et al.*, 2019); it is therefore a powerful tool to support the design and operation of real systems. Under real scenarios with multisolute competitive adsorption, sound modelling involves integrating models describing the competitive adsorption

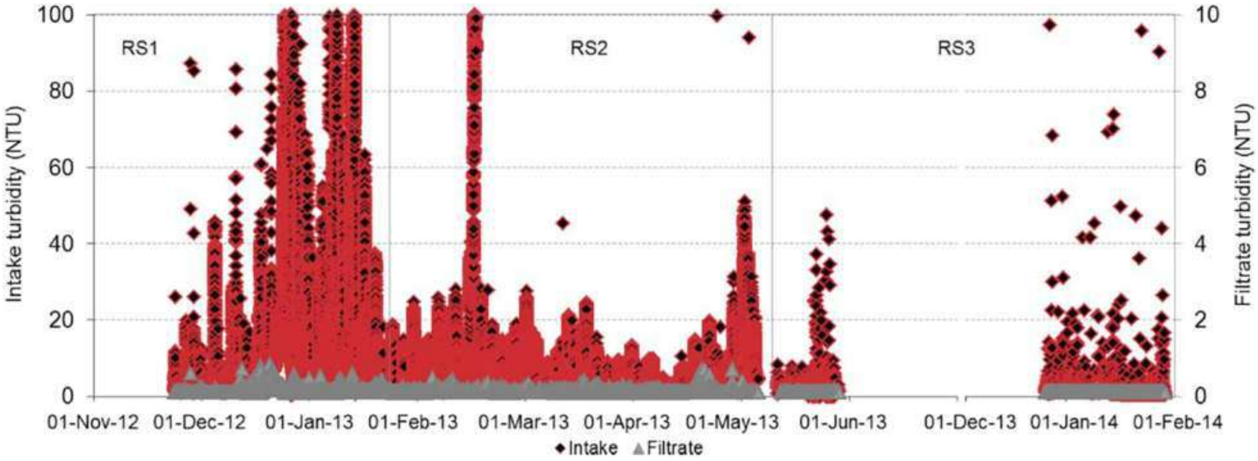


Figure 8.27 In-line records of water turbidity in PAC/MF intake (left axis) and filtrate (right axis). *Source*: From Viegas *et al.* (2015).

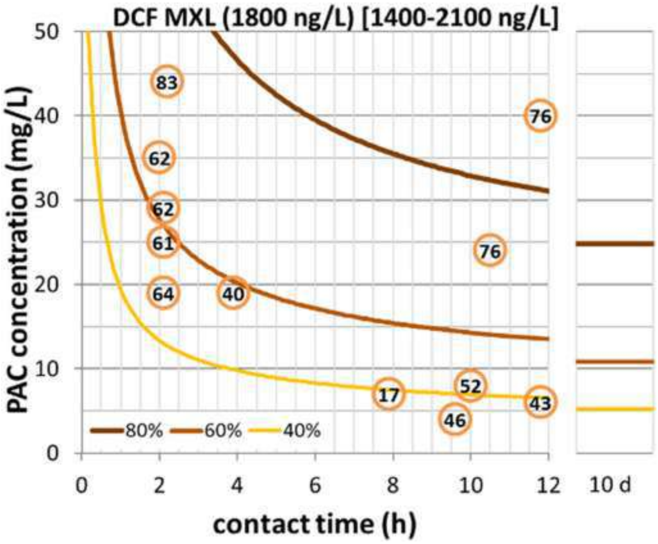


Figure 8.28 Model (IAST/EBCM, HSDM) predicted PAC adsorption removals (solid lines for 80%, 60%, 40%) of diclofenac from FNW WWTP as a function of PAC dose and contact time compared to field results from pilot-scale trials (circles). *Source*: From Rosa *et al.* (2019) (the 10-day prediction corresponds to the PAC full capacity use due to PAC recirculation; the values inscribed in the circles are the removals obtained in the pilot trials).

isotherms (e.g., the IAST/EBCM – ideal adsorption solution theory/equivalent background compound model) and kinetics (e.g., HSDM) (Campinas *et al.*, 2013; Rosa *et al.*, 2019; Viegas *et al.*, 2014; Worch, 2012).

- **Options 3–5.** As in DWT, these options with MF, UF, or NF membranes ensure a reliable water **disinfection** up to class A of reclaimed water (the highest water quality in Reg (EU) 741/2020, DL 119/2019), which is adequate for unrestricted water reuse, an extra feature which should be considered while comparing alternative options for PhC control. The pressure increase from MF to UF and NF [e.g., from 261 L/(m^2.h.bar), 0.40 bar for ceramic MF (Viegas *et al.*, 2020a) to 17 L/(m^2.h.bar), 1.2 bar for NF (Viegas *et al.*, 2018)] corresponds to an energy-intensity increase.
- **Option 6. direct potable reuse** – building on an earlier experience on batch production (Galego *et al.*, 2022), tests with a 24/7 automated pilot are starting to demonstrate the safety of potable water production by post-ozonation and or three-stage reverse osmosis, after sand-filtration or UF, and to develop the protocol to produce reclaimed water for artisanal beer production.

8.9 CLOSING REMARKS

Population growth and aging, as well as water scarcity driven by climate change call for an enhanced control of organic micropollutants and CECs in the urban water cycle, as progressively entailed in the EU and Portuguese legal framework on drinking water, UWWT and water reuse. In this context, there is a crucial need for multibarrier solutions, grounded on current barriers' improvement, prioritizing low-energy, physical barriers (to minimize by-products, resources' use, and carbon footprint) and producing fit-for-purpose water(s).

AC-based and hybrid PAC/membrane processes have proven a huge potential for controlling CECs in water treatment and water reclamation, but there is room for improving their sustainability and cost-efficiency with PAC development and process optimization. For instance, new high-performing environmental-friendly (biomass-derived, steam-activated) PACs from local biomasses (circular economy approach), as well as of more efficient finer sPAC and magnetic PACs allowing their recovery and regeneration are very important. Each application is water and target contaminant-specific and requires methodologies for selecting representative contaminants, the characterization of the inorganic and organic matrices of the waters, and to understand and anticipate/overcome competing factors, namely through modelling. Pilot demonstration and mathematical modelling are therefore success factors for process design and operation.

Strategic planning, multiobjective-driven (e.g., organic micropollutant control, ARB&ARGs control, and disinfection for water reuse) and performance-based decisions taken by skilled and competent human resources are required to successfully address the moving targets that the CECs constitute.

FUNDING AND ACKNOWLEDGMENTS

This work has received financial support from the European Union LIFE ENV Programme under Grant Agreements LIFE12 ENV/PT/001154 (www.life-hymemb.eu), LIFE14 ENV/PT/000739 (www.life-impetus.eu), and LIFE11 ENV/ES/000606 (LIFE aWARE), from EU FP7 contract 265122 (TRUST) and H2020 contract 869171 (https://b-watersmart.eu/), as well as from the Portuguese Fundação para a Ciência e Tecnologia (FCT) through PTDC/QUE-QUE/6024/2020 (EMPOWER+). This publication reflects only the authors' views and the European Union and FCT are not liable for any use that may be made of the information contained therein. We also acknowledge Metawater, Pentair, and Salmon for kindly providing membranes and ACs.

REFERENCES

Abegglen C. and Siegrist H. (2012). Mikroverunreinigungen aus kommunalem Abwasser. Verfahren zur weitergehenden elimination auf Kläranlagen. (Micropollutants from municipal wastewater. Process for further elimination in sewage treatment plants.) (In German). Bundesamt für Umwelt, Bern, Umwelt-Wissen. *Nr*, **1214**, 210 S.

Aus der Beek T., Weber F.-A., Bergmann A., Hickmann S., Ebert I., Hein A. and Küster A. (2016). Pharmaceuticals in the environment – global occurrences and perspectives. *Environmental Toxicology and Chemistry*, **35**(4), 823–835, https://doi.org/10.1002/etc.3339

Baresel C., Magnér J., Magnusson K. and Olshammar M. (2017). Tekniska lösningar för Avancerad Rening av Avloppsvatten. (Technical Solutions for Advanced Wastewater Treatment.) (In Swedish). IVL Svenska Miljöinstitutet AB, Stockholm

Campinas M. and Rosa M. J. (2006). The ionic strength effect on microcystin and natural organic matter surrogate adsorption onto PAC. *Journal of Colloid and Interface Science*, **299**, 520–529, https://doi.org/10.1016/j.jcis.2006.02.042

Campinas M. and Rosa M. J. (2010a). Assessing PAC contribution to the NOM fouling control in PAC/UF systems. *Water Research*, **44**(5), 1636–1644, https://doi.org/10.1016/j.watres.2009.11.012

Campinas M. and Rosa M. J. (2010b). Removal of microcystins by PAC/UF. *Separation and Purification Technology*, **71**(1), 114–120, https://doi.org/10.1016/j.seppur.2009.11.010

Campinas M. and Rosa M. J. (2010c). Evaluation of cyanobacterial cells removal and lysis by ultrafiltration. *Separation and Purification Technology*, **70**, 345–353, https://doi.org/10.1016/j.seppur.2009.10.021

Campinas M. and Rosa M. J. (2010d). Comparing PAC/UF and conventional clarification with PAC for removing microcystins from natural waters. *Desalination and Water Treatment*, **16**, 120–128, https://doi.org/10.5004/dwt.2010.1092

Campinas M. and Rosa M. J. (2011). PAC/UF for removing cyanobacterial cells and toxins from drinking water. Chap 12 In: Expanding Issues in Desalination, R. Y. Ning (ed.), InTech. Rijeka, Croatia, pp. 233–252. ISBN: 978-953-307-624-9.

Campinas M., Viegas R. and Rosa M. J. (2013). Modelling and understanding the competitive adsorption of microcystins and tannic acid. *Water Research*, **47**, 5690–5699, https://doi.org/10.1016/j.watres.2013.06.048

Campinas M., Viegas R. M. C., Silva C. and Rosa M. J. (2017). Technical Guidelines of PAC/MF – Powdered Activated Carbon/Ceramic Microfiltration for Drinking Water Production. Deliverable of LIFE Hymemb Project (LIFE12 ENV/PT/001154). LNEC, Lisboa, ISBN: 978-972-49-2299-7. 32 pp.

Campinas M., Silva C., Viegas R. M. C., Coelho R., Lucas H. and Rosa M. J. (2021a). To what extent may pharmaceuticals and pesticides be removed by PAC conventional addition to low-turbidity surface waters and what are the potential bottlenecks? *Journal of Water Process Engineering*, **40**, 101833, https://doi.org/10.1016/j.jwpe.2020.101833

Campinas M., Viegas R. M. C., Coelho R., Lucas H. and Rosa M. J. (2021b). Adsorption/coagulation/ceramic microfiltration for treating challenging waters for drinking water production. *Membranes*, **11**(2), 91, https://doi.org/10.3390/membranes11020091

Campinas M., Viegas R. M. C., Silva C. and Rosa M. J. (2021c). Operational performance and cost analysis of PAC/ceramic MF for drinking water production: exploring treatment capacity as a new indicator for performance assessment and optimization. *Separation and Purification Technology*, **255**, 117443, https://doi.org/10.1016/j.seppur.2020.117443

Campinas M., Viegas R. M. C., Almeida C. M. M., Martins A., Silva C., Mesquita E., Coelho M. R., Silva S., Cardoso V. V., Benoliel M. J. and Rosa M. J. (2022). Powdered activated carbon full-scale addition to the activated sludge reactor of a municipal wastewater treatment plant: pharmaceutical compounds control and overall impact on the process. *Journal of Water Process Engineering*, **49**, 102975, https://doi.org/10.1016/j.jwpe.2022.102975

Chorus I. and Welker M. (eds.) (2021). Toxic Cyanobacteria in Water. A Guide to Their Public Health Consequences, Monitoring and Management. 2nd ed. World Health Organisation, CRC Press, London. eBook ISBN 9781003081449. 859 pp.

CID (2022). Commission Implementing Decision of 19.1.2022 establishing a watch list of substances and compounds of concern for water intended for human consumption as provided for in Directive (EU) 2020/2184 of the European Parliament and of the Council. C(2022) 142 final.

Clara M., Kreuzinger N., Strenn B., Gans O. and Kroiss H. (2005). The solids retention time – a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research*, **39**(1), 97–106, https://doi.org/10.1016/j.watres.2004.08.036

COM (2022). Proposal for a Directive of the European Parliament and of the Council concerning urban wastewater treatment (recast). 2022/0345 (COD). COM(2022) 541 final.

Cravo A., Silva S., Rodrigues J., Cardoso V. V., Benoliel M. J., Correia C., Coelho M. R., Rosa M. J. and Almeida C. M. M. (2022). Understanding the bioaccumulation of pharmaceutical active compounds by clams *Ruditapes decussatus* exposed to a UWWTP discharge. *Environmental Research*, **208**, 112632, https://doi.org/10.1016/j.envres.2021.112632

Eggen R. I. L., Hollender J., Joss A., Schärer M. and Stamm C. (2014). Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. *Environmental Science & Technology*, **48**, 7683–7689, https://doi.org/10.1021/es500907n

Fortune Business Insights (2019). Activated carbon market size, trends, growth & report [2020–2027]. Retrieved from https://www.fortunebusinessinsights.com/activated-carbon-market-102175 (accessed 4 May 2021)

Galego J., Lourinho R., Figueiredo D., Costa C., Viegas R. and Rosa M. J. (2022). Protocol for water reclamation by ozone/reverse osmosis for safe direct potable reuse in beer production. IWA World Water Congress & Exhibition, September 11–15, Copenhagen, Denmark. 4 pp. (poster)

Geissen V., Mol H., Klumpp E., Umlauf G., Nadal M., van der Ploeg M., van de Zee S. E. A. T. M. and Ritsema C. J. (2015). Emerging pollutants in the environment: A challenge for water resource management. *International Soil and Water Conservation Research*, **3**, 57–65, https://doi.org/10.1016/j.iswcr.2015.03.002

Golovko O., Örn S., Sörengård M., Frieberg K., Nassazzi W., Lai F. Y. and Ahrens L. (2021). Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. *Science of the Total Environment*, **754**, 142122, https://doi.org/10.1016/j.scitotenv.2020.142122

Gomez Cortes L., Marinov D., Sanseverino I., Navarro Cuenca A., Niegowska Conforti M., Porcel Rodriguez E., Stefanelli F. and Lettieri T. (2022). Selection of Substances for the 4th Watch List under the Water Framework Directive. EUR 31148 EN, Publications Office of the European Union, Luxembourg. ISBN 978-92-76-55021-1. JRC130252. https://doi.org/10.2760/01939

Hall T., Schmidt W., Codd G. A., von Gunten U., Kaas H., Acero J. L., Heijman B., Meriluoto J., Rosa M. J., Manckiewicz J. (2005). Best Practice Guidance for Management of Cyanotoxins in Water Supplies. TOXIC 5FP project (EVK1-CT-2002-00107). Abo Akademi University (Finland), DHI (Denmark), DVGW-TZW (Germany), EAWAG (Switzerland), KIWA (The Netherlands), University of Algarve (Portugal), University of Dundee (UK), University of Extremadura (Spain), University of Lodz (Poland), WRc plc (UK). Water Research Centre, UK. Sept., 136 pp.

Hillenbrand T., Tettenborn F., Fuchs S., Toshovski S., Metzger S., Tjoeng I., Wermter P., Hecht D., Kersting M., Werbeck N. and Wunderlin P. (2016). Maßnahmen zur Verminderung des Eintrages von Mikroschadstoffen in die Gewässer – Phase 2 (Measures to Reduce the Input of Micropollutants Into Water Bodies – Phase 2.) (In German), Texte 60/2016, Umweltsbundesambt, Dessau-Roßlau, Germany, 235 pp. ISSN 1862-4804. Retrieved from https://www.umweltbundesamt.de/sites/default/files/medien/377/publikationen/mikroschadstoffen_in_die_gewasser-phase_2.pdf (accessed 16 May 2019)

Inkwood Research (2019). Global activated carbon market forecast 2019–2028: growth, size, opportunity, share. Retrieved from https://inkwoodresearch.com/reports/activated-carbon-market/ (accessed 4 May 2021).

Luo Y., Guo W., Ngo H. H., Nghiem L. D., Hai F. I., Zhang J., Liang S. and Wang X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*, **473–474**, 619–641, https://doi.org/10.1016/j.scitotenv.2013.12.065

Martin I., Echevarría C., Mesa C., Lefèvre B., Bernat X., Viegas R. M. C., Mesquita E., Rosa M. J., Borràs J., Martínez B., Vega A., Aguiló P. and Castellví E. (2016). LIFE aWARE project: development of hybrid wastewater treatment and reclamation schemes to enhance removal of micro pollutants and promote water reuse. LIFE aWARE Lisbon Workshop, June 6, Lisbon, LNEC.

Menaia J. and Rosa MJ. (2006). Cianobactérias e Água Para Abastecimento Público. Manual de Apoio ao Curso AdP/LNEC 'Florescências de algas e cianobactérias nas águas de Captação' Carvoeiro, Algarve, nov. (Cyanobacteria and Public Water Supply. Course Support Manual AdP/LNEC 'Algal blooms and cyanobacteria in catchment water') (In Portuguese). Laboratório Nacional de Engenharia Civil, Portugal. 14 pp.

Mestre A. S., Campinas M., Viegas R. M. C., Mesquita E., Carvalho A. P. and Rosa M. J. (2022a). Activated carbons in full-scale advanced wastewater treatment. Chap. 17 In: Advanced Materials for Sustainable Environmental Remediation: Terrestrial and Aquatic Environments, D. A. Giannakoudakis, L. Meili and I. Anastopoulos (eds.), Elsevier, MA, USA, pp. 433–474. eBook ISBN: 9780323904865. https://doi.org/10.1016/B978-0-323-90485-8.00001-1

Mestre A. S., Viegas R. M. C., Mesquita E., Rosa M. J. and Carvalho A. P. (2022b). Engineered pine nut shell derived activated carbons for improved removal of recalcitrant pharmaceuticals in urban wastewater treatment. *Journal of Hazardous Materials*, **437**, 129319, https://doi.org/10.1016/j.jhazmat.2022.129319

Metzger S., Alt K., Benstoem F., Biebersdorf N., Boehler M., Bornemann C., Christian H., Jedele K., Jekel M., Lyko S., Andreas N., Wintgens T., Krahnstöver T., Mätzig H., Remy C., Rößler A., Zietzschmann F. and Wilhelm C. (2019). Aktivkohleeinsatz auf Kommunalen Kläranlagen zur Spurenstoffentfernung – Verfahrensvarianten, Reinigungsleistung und Betriebliche Aspekte (Activated Carbon Application on Municipal Wastewater Treatment Plants for Trace Compound Removal – Process Variants, Treatment Performance and Operational Aspects.) (In German). Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e.V. (DWA), Hennef (Germany).

Mulder M., Antakyali D. and Ante S. (2015). Costs of Removal of Micropollutants From Effluents of Municipal Wastewater Treatment Plants – General Cost Estimates for The Netherlands Based on Implemented Full Scale Post Treatments of Effluents of Wastewater Treatment Plants in Germany and Switzerland. STOWA and Waterboard the Dommel, The Netherlands. 55 pp.

Pesqueira J. F. J. R., Pereira M. F. R. and Silva A. M. T. (2020). Environmental impact assessment of advanced urban wastewater treatment technologies for the removal of priority substances and contaminants of emerging concern: a review. *Journal of Cleaner Production*, **261**, 121078, https://doi.org/10.1016/j.jclepro.2020.121078

Pinho M. N. N. C. and Rosa M. J. F. (1995). Membranas e processos de Separação com Membranas. Instituto Superior Técnico, Lisboa, dezembro, 56 pp.

Pourchet M., Debrauwer L., Klanova J., Price E. J., Covaci A., Caballero-Casero N., Oberacher H., Lamoree M., Damont A., Fenaille F., Vlaanderen J., Meijer J., Krauss M., Sarigiannis D., Barouki R., Le Bizec B. and Antignac J.-P. (2020). Suspect and non-targeted screening of chemicals of emerging concern for human biomonitoring, environmental health studies and support to risk assessment: from promises to challenges and harmonisation issues. *Environment International* **139**, 105545, https://doi.org/10.1016/j.envint.2020.105545

Raspati G., Menaia J., Raat K., Rosa M. J., Gonzalez B. L. and Sivertsen E. (2015). Assessment of current treatment works to handle climate change related pollutants and options to make current multi-barrier systems climate change proof. Chap. 4.7 In: Climate Change, Water Supply and Sanitation: Risk Assessment, Management, Mitigation and Reduction, A. Hulsmann *et al.* (eds.), IWA Publishing, London, pp. 284–287. ISBN: 9781780405001 (eBook). https://doi.org/10.2166/9781780405001

Ribau Teixeira M. and Rosa M. J. (2006). The impact of the water background inorganic matrix on the natural organic matter removal by nanofiltration. *Journal of Membrane Science*, **279**, 513–520, https://doi.org/10.1016/j.memsci.2005.12.045

Ribau Teixeira M., Rosa M. J. and Nystrom M. (2005). The role of membrane charge on nanofiltration performance. *Journal of Membrane Science*, **265**, 160–166, https://doi.org/10.1016/j.memsci.2005.04.046

Ribau Teixeira M., Rosa M. J., Sorlini S., Biasibetti M., Christophoridis C. and Edwards C. (2020). Removal of cyanobacteria and cyanotoxins by conventional physical-chemical treatment. Chap. 3 In: Water Treatment for Purification From Cyanobacteria and Cyanotoxins, A. E. Hiskia, T. M. Triantis, M. G. Antoniou, T. Kaloudis and D. D. Dionysiou (eds.), 1st edn, Wiley, Hoboken, NJ, USA, pp. 69–97. Online ISBN: 9781118928677. https://doi.org/10.1002/9781118928677.ch3

Rizzo L., Malato S., Antakyali D., Beretsou V. G., Dolić M. B., Gernjak W., Heath E., Ivancev-Tumbas I., Karaolia P., Lado Ribeiro A. R., Mascolo G., McArdell C. S., Schaar H., Silva A. M. T. and Fatta-Kassinos D. (2019). Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. *Science of the Total Environment*, **655**, 986–1008, https://doi.org/10.1016/j.scitotenv.2018.11.265

Rizzo L., Gernjak W., Krzeminski P., Malato S., McArdell C. S., Perez J. A. S., Schaar H. and Fatta-Kassinos D. (2020). Best available technologies and treatment trains to address current challenges in urban wastewater reuse for irrigation of crops in EU countries. *Science of the Total Environment*, **710**, 136312, https://doi.org/10.1016/j.scitotenv.2019.136312

Rodriguez E., Campinas M., Acero J. L. and Rosa M. J. (2016). Investigating PPCP removal from wastewater by powdered activated carbon/ultrafiltration. *Water Air & Soil Pollution*, **227**(6), 1, https://doi.org/10.1007/s11270-016-2870-7

Rosa M. J., Vieira P. and Menaia J. (2009). O Tratamento de água Para Consumo Humano Face à Qualidade da água de Origem. Guia Técnico 13. IRAR, LNEC, Lisboa. ISBN 978-989-95392-7-3. 82 pp.

Rosa M. J., Campinas M., Viegas R. M. C. and Silva C. (2019). LIFE IMPETUS Technical Guidelines for Improved Control of Pharmaceutical Compounds in Urban Activated Sludge WWTPs. Deliverable of LIFE Impetus project (LIFE14 ENV/PT/000739). LNEC, Lisboa. 49 pp.

Sauvé S. and Desrosiers M. (2014). A review of what is an emerging contaminant. *Chemistry Central Journal*, **8**(1), 15, https://doi.org/10.1186/1752-153X-8-15

Siegrist H. and Joss A. (2012). Review on the fate of organic micropollutants in wastewater treatment and water reuse with membranes. *Water Science and Technology*, **66**(6), 1369–1376, https://doi.org/10.2166/wst.2012.285

Silva C., Almeida C. M. M., Rodrigues J. A., Silva S., Coelho M. R., Martins A., Alves R., Cardoso E., Cardoso V. V., Benoliel M. J. and Rosa M. J. (2021). Occurrence and seasonality of pharmaceutical compounds in urban wastewaters in two Portuguese regions. *Urban Water Journal*, **18**(6), 465–478, https://doi.org/10.1080/1573062X.2021.1893365

Silva C., Almeida C. M. M., Rodrigues J. A., Silva S., Coelho M. R., Martins A., Lourinho R., Cardoso E., Cardoso V. V., Benoliel M. J., Mesquita E., Ribeiro R. and Rosa M. J. (2023). Improving the control of pharmaceutical compounds in activated sludge wastewater treatment plants: Key operating conditions and monitoring parameters. *Journal of Water Process Engineering*, **54**, 103985, 214–7144, https://doi.org/10.1016/j.jwpe.2023.103985

Smeets P., Kardinaal E., Eikebrokk B., Menaia J., Mesquita E. and Rosa M. J. (2015). Optimised operation of drinking water systems. Chap. 4.14 In: Climate Change, Water Supply and Sanitation: Risk Assessment, Management, Mitigation and Reduction. A. Hulsmann G. Grützmacher, G. van den Berg, W. Rauch, A. L. Jensen, V. Popovych, M. R. Mazzola, L. S. Vamvakeridou-Lyroudia and D. A. Savic (eds.), IWA Publishing, London, pp. 332–337. ISBN: 9781780405001 (eBook). https://doi.org/10.2166/9781780405001

Souza R. M., Seibert D., Quesada H. B., Bassetti F. J., Fagundes-Klen M. R. and Bergamasco R. (2020). Occurrence, impacts and general aspects of pesticides in surface water – a review. *Process Safety and Environmental Protection*, **135**, 22–37. https://doi.org/10.1016/j.psep.2019.12.035

Ternes T. A. and Joss A. (eds.) (2006). Human Pharmaceuticals, Hormones and Fragrances. The Challenge of Micropollutants in Urban Water Management. IWA Publishing, Cornwall, UK. ISBN 10 1843390930. 453 pp.

UMWELT.NRW (2019). Entwicklung und Stander Abwasserbeseitigungin Nordrhein-Westfalen (Development and Standing of Wastewater Disposal in North Rhine-Westphalia.) (In German). Report of the Ministry for Environment, Agriculture, Conservation and Consumer Protection of the State of North Rhine-Westphalia, Germany.

Viegas R. M. C., Campinas M., Costa H. and Rosa M. J. (2014). How do the HSDM and Boyd's model compare for estimating intraparticle diffusion coefficients in adsorption processes. *Adsorption*, **20**(5–6), 737–746, https://doi.org/10.1007/s10450-014-9617-9

Viegas R. M. C., Mesquita E., Inocêncio P., Teixeira A. P., Martins J. and Rosa M. J. (2015). Water reclamation with hybrid coagulation–ceramic microfiltration: first part of a long-term pilot study in Portugal. *Journal of Water Reuse and Desalination*, **5**(4), 550–556, https://doi.org/10.2166/wrd.2015.122

Viegas R. M. C., Mesquita E., Campinas M., Almeida C. M. M. and Rosa M. J. (2018). Designing a PAC/NF advanced process for controlling pharmaceutical compounds in reclaimed water. In: Sanitation Approaches and Solutions and the Sustainable Development Goals, J. S. Matos and M. J. Rosa (eds.), Technical course 08. ERSAR, EWA, APESB, Lisbon, pp. 97–108. ISBN: 978-972-98996-8-3.

Viegas R. M. C., Mesquita E., Campinas M. and Rosa M. J. (2020a). Pilot studies and cost analysis of hybrid powdered activated carbon/ceramic microfiltration for controlling pharmaceutical compounds and organic matter in water reclamation. *Water*, **12**(1), 33, https://doi.org/10.3390/w12010033

Viegas R. M. C., Mestre A. S., Mesquita E., Campinas M., Andrade M., Carvalho A. P. and Rosa M. J. (2020b). Assessing the applicability of a new carob waste-derived powdered activated carbon to control pharmaceutical compounds in wastewater treatment. *Science of the Total Environment*, **743**, 140791, https://doi.org/10.1016/j.scitotenv.2020.140791

Viegas R. M. C., Campinas M., Coelho R., Lucas H. and Rosa M. J. (2021a). Hybrid process of adsorption/coagulation/ceramic MF for removing pesticides in drinking water treatment – inline vs. contact tank PAC dosing. *Membranes*, **11**(2), 72, https://doi.org/10.3390/membranes11020072

Viegas R. M. C., Ribeiro R., Rosa M. J., Campinas M., Mesquita E. and Silva C. (2021b). Controlo de contaminantes de interesse emergente em ETA e ETAR. Dossier 'Contaminantes emergentes na Água'. *Indústria e Ambiente*, **126**, 16–19.

Viegas R. M. C., Mestre A. S., Mesquita E., Machuqueiro M., Andrade M. A., Carvalho A. P. and Rosa M. J. (2022). Key factors for activated carbon adsorption of pharmaceutical compounds from wastewaters: a multivariate modelling approach. *Water*, **14**(2), 166, https://doi.org/10.3390/w14020166

Wise B. L., Shih W. and Smith S. (2020). Ceramic Ultrafiltration Membranes with Improved Economics, Operability, and Process Design Flexibility. Nanostone Water Inc., Minnesota, USA, IWC 17–34.

Worch E. (2012). Adsorption Technology in Water Treatment. Fundamentals, Processes, and Modeling. Walter de Gruyter GmbH & Co. KG, Berlin/Boston. ISBN 978-3-11-024022-1

Zietzschmann F. (2020). Adsorptive removal of pharmaceutically active compounds from wastewater. In: Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment, S. Rodriguez-Mozaz, P. B. Cano and M. S. Adroguer (eds.), Springer Berlin Heidelberg, Berlin, Heidelberg, Vol. **2020**, pp. 1–29. https://doi.org/10.1007/698_2020_687

Zietzschmann F., Altmann J., Ruhl A. S., Dünnbier U., Dommisch I., Sperlich A., Meinel F. and Jekel M. (2014). Estimating organic micro-pollutant removal potential of activated carbons using UV absorption and carbon characteristics. *Water Research*, **56**, 48–55, https://doi.org/10.1016/j.watres.2014.02.044

doi: 10.2166/9781789063783_0181

Chapter 9

Constructed wetlands: an approach toward phytoremediation for wastewater treatment

*Sahail Alvi[1], Sai Shankar Sahu[1], Vivek Rana[2] and Subodh Kumar Maiti[1,]**

[1]Department of Environmental Science and Engineering, Indian Institute of Technology (Indian School of Mines), Dhanbad, 826004, Jharkhand, India
[2]Central Pollution Control Board, New Delhi, India
**Corresponding author: subodh@iitism.ac.in*

ABSTRACT

Constructed wetlands (CWs) have been used as a proven efficient technology for wastewater treatment since the early 1950s. However, the first full-scale operation was conducted in the late 1960s. CW is an engineered system that utilizes various macrophytes to treat wastewater. It is cost effective, easy to operate, and maintain as compared to other conventional wastewater treatment technologies. It works with the mechanisms of different phytoremediation processes (i.e., phytoextraction, phytostabilization, phytovolatization, phytodegradation) and sedimentation, agglomeration and biodegradation of wastewater. CW can be classified on the basis of vegetation types (submerged, emergent, free-floating, and floating leaved), hydrological properties (free water, surface flow, and subsurface flow). Subsurface flow wetlands can be further classified as vertical or horizontal depending on the flow direction. Hybrid systems such as CWs clubbed with tube settler for hospital wastewater, biochar-based CW for manure wastewater are reported to have better treatment efficiency. However, the performance of CWs depends on several factors like vegetation type, type of wetland, type of wastewater treated, design specification of CWs, substrate used, microbiology of CW, climate and hydrological factors, and so on. Therefore, this chapter mainly focuses on all these aspects to fill the knowledge gaps and gives an insight to future research challenges and opportunities to achieve sustainability in the field of wastewater treatment.

Keywords: constructed wetland, wastewater treatment, phytoremediation, sustainability

9.1 INTRODUCTION

Constructed wetland (CW) is an artificial wetland, mainly dominated by emergent plants (*Typha*, *Sagittaria*, *Canna indica*, etc.), which is a low-cost natural treatment used commonly for sewage and graywater treatment but currently industrial wastewater is also treated. The CW uses natural functions of vegetation, soil, organisms, and mechanisms of removal of pollutants through combination of physical, chemical, and biological actions. In many cases, CWs are also used as secondary treatment to treat

The chapter is from the book *Clean Technologies Towards a Sustainable Future: Physicochemical, Biochemical and Biotechnological Approaches*, Pradeep Verma, Maulin P. Shah (Editors).

wastewater. Based on type and characteristics of wastewater to be treated, the design of CW is varied. Research studies recommended that CWs can also be used as primary treatment, when concentration of suspended solids (SS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) is higher.

9.2 PHYTOREMEDIATION

Phytoremediation is a process to restore the ecosystem by using plants and related microorganisms. They are used to reduce the concentration of contaminants and their impacts on the system. Some of the popular accumulator plants for cleaning up the contaminated water are duckweed (*Lemna minor*), water lettuce (*Pistia stratiotes*), water hyacinth (*Eichhornia crassipes*), and so on. In addition to this, various novel technologies such as microbial intervention, use of genetic innovation and pharmaceutical additives, and so on are applied for enhancing the remediation potential. Different mechanisms of phytoremediation are shown in Figure 9.1.

9.2.1 Mechanisms of phytoremediation

Phytoremediation technology can be applied on a range of contaminants by using different habitats. They entail various mechanisms including in-situ stabilization, degradation and retrieval of pollutants.

9.3 PHYTOEXTRACTION

Phytoextraction is the removal of heavy metals or inorganic contaminants from water by using metal-tolerant plants. The plants uptake the contaminants by their roots and concentrate them in their above-ground biomass. They can further be harvested, recovered, disposed, or recycled. Plants that have a high metal accumulating potential are called hyperaccumulators.

9.4 PHYTODEGRADATION

Phytodegradation is the breakdown of organic pollutants either directly, by the release of enzymes from the roots, or by biochemical processes occurring within plant cells. Organic pollutants are absorbed by roots and converted into less toxic compounds in plant cells during phytodegradation. Organic pollutants that are hydrophobic have been especially effective at being biodegraded by plants. A few research studies show that successful phytodegradation of hazardous and resistant organic substances has been achieved using poplar plants (*Populus* spp.).

9.5 PHYTOVOLATIZATION

Phytovolatization is the process by which pollutants are uptaken by plant roots, transformed into gaseous form and released into the environment. Evapotranspiration process of plants enhances phytovolatization. So, the plants having strong evapotranspiration rates are desired. In this process, plants actively take up volatile organic pollutants, particularly, volatile organic compounds (VOCs).

9.6 PHYTOSTABILIZATION

Phytostabilization is the phenomenon by which metals or contaminants are immobilized within the profile of soil, so that the pollutant has minimum pollutant escape and biological exposure. Long-term stabilization and containment of pollutants are desired.

9.7 RHIZODEGRADATION

Rhizodegradation is the biodegradation of organic pollutants by soil microorganisms into less toxic compounds within the rhizosphere. Root exudates, which are organic compounds that support soil

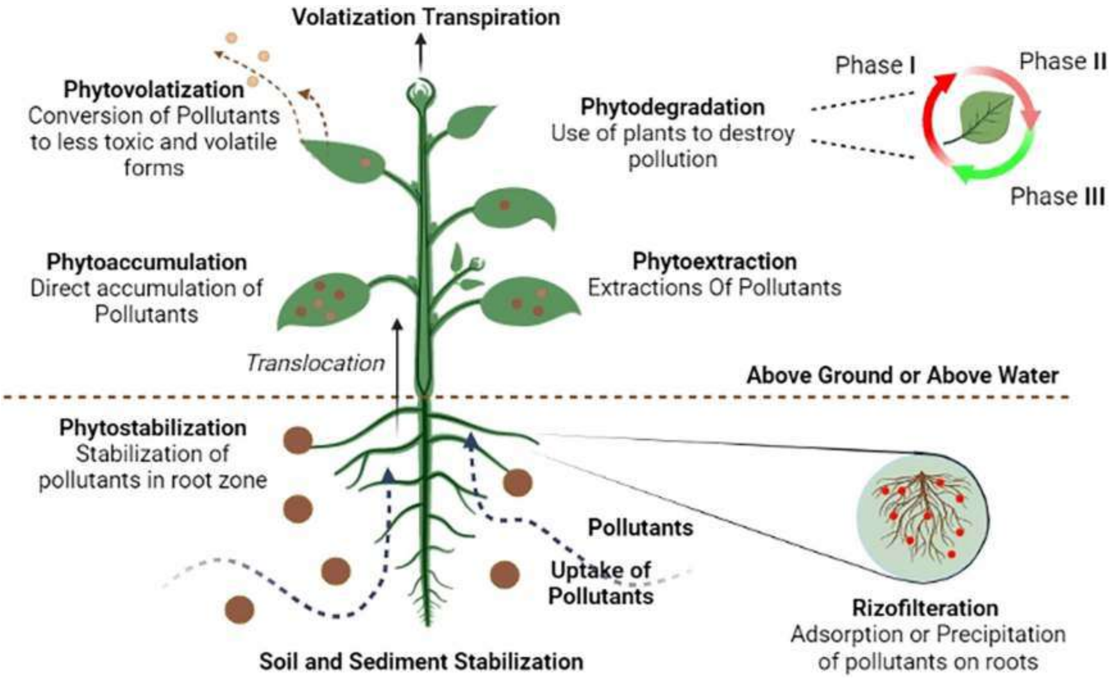


Figure 9.1 Mechanisms of phytoremediation.

microbial communities, aid in this process. The inocula of a particular bacterium can be introduced into contaminated soils to speed up this process. According to Campbell and Greaves (1990), plants discharge about 20% of the total photosynthates, including sugars, organic acids, and amino acids, to the rhizosphere, promoting the development of microorganisms. The area of about 1–3 mm from the root surface is known as the rhizosphere, and it is here that soil microbes can proliferate up to three or four orders of magnitude more than in nonvegetated soils (Schnoor & Huang, 1993). Some soil microbes co-metabolistically break down or mineralize organic pollutants such as PCBs and PAHs (Donnelly & Fletcher, 1994).

9.8 CONSTRUCTED WETLAND

CWs comprise of engineered systems that use biological processes to treat a wide array of wastewaters emanating from municipal and industrial sources. CWs utilize natural sources such as plants, soils, and associated microbial assemblages, which result in wastewater treatment through various physical, chemical, and biological processes that work individually and with each other. Helophytes, which are used in CWs, play a vital role in the treatment of wastewater. The CW is composed of one or more substrate-filled beds with a specific flow regime that promote the development of plants and microorganisms. The bed is reinforced by an impermeable layer at the bottom. The substrate actively removes contaminants by physio-chemical processes such as filtration and sedimentation of suspended particles, filtration of pathogenic organisms, and sorption of organic materials, nitrogen, phosphorus, and heavy metals.

In developing countries, there is a huge gap between wastewater generation and treatment due to which untreated wastewater is discharged into natural water bodies and lead to their contamination. To meet the increasing gap between wastewater generation and treatment, there is a strong need to reduce the load on existing wastewater treatment infrastructures. CWs, as a green technology, may be employed to treat wastewater. CWs are an inexpensive technology than conventional and advanced wastewater treatment systems, which require less operation and maintenance cost. In the past few decades, CWs, a green wastewater treatment method that mimics natural wetlands, has been used extensively to treat a variety of wastewaters, including sewage, agrarian effluent, industrial effluents, mine drainage, landfill leachate, stormwater runoff, polluted river water, and urban runoff (Vymazal, 2014). The design, development, and performance of CWs have been the subject of numerous studies recently, and it has also been suggested that CWs may be effective at removing a variety of pollutants from wastewater, including pathogens, pharmaceutical contaminants, organic matter, nutrients, and trace elements (Vymazal, 2014).

9.8.1 Merits of CW systems

It is only reliant on solar energy and is visually appealing. Environmental disturbance are kept to a minimum, and in situ treatment protects topsoil. It works best at locations with modest, minimal contamination levels but with specialized plants and process modifications its use can even be applied to industrial wastewaters. It is effective for treating a variety of environmental pollutants and is less pricey than traditional physico-chemical techniques by 60–80% or even more.

9.8.2 Demerits of CW systems

Cleaning up a location is a time-intensive procedure that could take at least several growing seasons. Animals may be harmed by plants that extract harmful heavy metals or persistent toxins, which can also pollute the food chain. Additionally, those organic and inorganic pollutants may produce intermediates that are cytotoxic to people, plants, and other creatures. The key to demonstrating the viability of phytoremediation is to conduct analyses on the mass balance and metabolic destiny of contaminants in plants.

9.8.3 Types of CW systems

CW systems may be categorized on the basis of hydrology (surface flow and subsurface flow in open water), macrophytic growth types (emergent, submerged, free-floating, and floating-leaved wetlands), and flow channel (horizontal and vertical). Several types of CWs can be merged to take use of the distinct advantages of the various systems (hybrid or mixed systems).

9.8.3.1 CW with horizontal subsurface flow

In CWs with horizontal subsurface flow (HSSF), wastewater is fed into the inlet and slowly moves through the porous medium beneath the bed in an estimated horizontal path till it approaches the outlet zone, where it is collected before being expelled via level control device at the outlet. Throughout this process, the wastewater will encounter a network of aerobic, anoxic, and anaerobic zones. The aerobic zones are located around roots and rhizomes, which add oxygen to the substrate. Both aerobic and anaerobic microbial activities, as well as sedimentation and filtering of particle organic matter, all contribute to the decomposition of organic matter in horizontal flow constructed wetlands (HF CWs). Due to severe loading and ongoing saturation of the filter bed, anoxic/anaerobic activities predominate, with aerobic processes limited to thin surface layers where diffusion of oxygen from the environment may occur and tiny zones next to rhizomes and roots (radial oxygen loss). Dissolved oxygen may also be transported by inflowing wastewater in systems with modest loads. The flocculation and settling of colloidal and super colloidal particles are one of the main removal/retention processes for SS in HF CWs. Gravity sedimentation, straining, physical capture, and adsorption on biomass film linked to gravel and root systems are additional efficient removal processes in HF systems. In HF CWS, nitrification and denitrification are the main methods of nitrogen removal. However, nitrification is restricted by the low level of oxygen in the filtration bed as a result of the filtration bed's continual submersion, hence, HF CWs are ineffective at removing ammonia. On the contrary, denitrification can occur in anoxic or anaerobic circumstances. Through sorption and precipitation, phosphorus can be eliminated. However, popular filtering materials like crushed rock or gravel do not have a large sorption capacity. The selection of materials with a superior P adsorption capacity, which depends on chemical and physical qualities, is required to improve phosphorus removal. These materials may be calcareous substances that might encourage the precipitation of Ca-phosphate or minerals having reactive Fe or Al hydroxide or oxide groups on their surfaces. Industrial waste and by-products, such as steel slags from electric arc and blast furnaces, fly ash, broken concrete, iron ore, and treated wood chips, have recently found value (Vymazal, 2005).

9.8.3.2 CWs with vertical subsurface flow

CWs with vertical flow (VSSF) consist of a flat bed of graded gravel covered in sand containing macrophytes. A big batch is intermittently supplied to VF CWs, saturating the top. After that, wastewater slowly seeps through the bed and is gathered at the base by a drainage system. The bed drains entirely without obstruction, allowing air to fill the bed. This type of dosage promotes efficient oxygen transport, which enhances the capacity for nitrification. Contrary to transfer of oxygen through the aerenchyma system of plants, airborne oxygen diffusion significantly increases the oxygenation of the filter bed. The fundamental function of vegetation in VF CWs is to support the bed's hydraulic conductivity (Vymazal, 2018).

9.8.3.3 CWs with free water surface

A typical FWS CW with sprouting macrophytes is a shallow isolated basin or series of basins with a liquid depth of 20–40 and 20–30 cm of rooting soil. A considerable portion of the surface is often covered with dense emergent vegetation, usually more than 50%. There may be naturally occurring species in addition to cultivated macrophytes. The litter provides the organic carbon required for denitrification, which can take place in anaerobic pockets inside this litter layer as plants are typically not harvested. FWS CWs are effective in removing organic materials owing to microbial decomposition and colloidal particle settling. By settling and filtering through the thick vegetation, suspended particles are successfully eliminated. Nitrification (in the water column) and denitrification (in the litter layer) are the main processes employed to remove nitrogen, as well as ammonia volatilization at higher pH levels because of algal photosynthesis. Due to the little water interaction with soil particles that adsorb and/or precipitate phosphorus, its retention is often poor. Because nutrients are returned to water during plant breakdown, plant intake only reflects temporary storage.

9.8.3.4 Hybrid CWs

Hybrid CWs with both horizontal and vertical flow both have distinct disadvantages (Cooper, 1999). BOD_5 and total suspended particles are effectively removed by horizontal flow-built wetlands, however, nitrification is not successfully removed due to a restricted oxygen transfer capacity. Since they have a significantly higher capacity for oxygen transfer and need less space to build, vertical flow-built wetland is of more importance. Yet, there are some drawbacks to vertical flow-built wetlands as well. They are less effective at removing sediments and can potentially produce clogging issues. Hybrid systems, a novel technology designed to address these issues, have been developed to lessen the issues associated with just horizontal and vertical flow.

9.8.4 Macrophyte used for CWs

There are three types of wetland plants that are used in CWs: free floating, submerged, and emergent. Researchers have reported different types of macrophytes used in CWs and their properties are presented in Table 9.1 (Kataki *et al.*, 2021).

9.8.4.1 Floating-leaved macrophytes

Plant species with roots in the substrate and leaves floating above are referred to as floating-leaved macrophytes. The typical members of this group are the waterlilies (*Nymphaea* spp.), the Indian lotus (*Nelumbo nucifera*), and the yellow waterlily (*Nuphar lutea*). Floating leafed macrophytes in CWs have a strong chance of removing suspended particles because leaves on the surface lessen the

Table 9.1 Concentrations of major pollutants removal reported in the literature for various industrial wastewater.

Location	Type of Macrophyte	Species of Macrophyte	Properties
India	Emergent, evergreen	*Cymbopogon citratus*	Antimicrobial characteristics, high COD, TSS, TN, and TP elimination.
India, Pakistan, Cameroon Nigeria	Emergent, submerged	*Pistia stratiotes*	Heavy metal accumulation, effective TN and ammonia-N removal, enhanced microbial development.
India, Colombia	Emergent perennial	*Colocasia esculenta*	Metal uptake.
India, Malaysia, Italy, Greece, Morocco, Portugal	Emergent	*Arundo donax*	High energy output, high methane generation, salt tolerance.
India, China, Thailand	Emergent, perennial	*Pennisetum purpureum*	Very high yield, low nutrient demand, high COD, TSS, TN, and TP removal.
India, China, Denmark, Vietnam	Emergent	*Canna indica*	High rate of loading, increased Fe plaque formation, high pollutant load tolerance, better TN, TP, and COD removal, as well as high capacity to absorb nutrients and high root activity in saline water.
India, Argentina, Brazil	Emergent, perennial	*Typha domingensis*	High productivity, high metal (Hg) accumulation and tolerance.
Malaysia	Perennial, evergreen	*Lepironia articulata*	High removal efficiency for SS, turbidity, available N, SS, BOD, and COD.
China	Emergent	*Acorus calamus*	Suggested for wastewater containing petroleum, good ability for bacterial enrichment, grows well in water with a high (N) load, extended duration of growth, high capacity for root aeration.
Thailand, China, Iran	Emergent	*Cyperus flabelliformis*	Excellent nutrient removal capability and rate of loading capacity.
China	Submerged, perennial	*Potamogeton crispus*	Increase the appropriate bacterial percentage (ammonia oxidizing bacteria), the ability for high oxygenation, and the efficiency of COD removal.
China	Emergent, perennial	*Zizania latifolia*	Low nutrient removal and intrusive.
China	Submerged	*Cladophora*	Removal of Mn.
China	Emergent, perennial	*Veronica serpyllifolia*	High rate of loading, good Zn, Pb, and Fe plaque formation.
China	Emergent, submerged	*Myriophyllum aquaticum*	Effective reduction of N, high discharge of organic exudates, metal tolerant.

Pakistan	Emergent, perennial	*Leptochloa fusca*	High growth rate and metal removal.
Pakistan, Vietnam	Emergent	*Brachiaria mutica*	High growth rate under high N stress make it good for COD, BOD, and Cr removal.
Thailand, China	Emergent	*Acorus calamus*	Increased O_2 release by roots and effective pollutant removal.
China, Thailand, Brazil	Emergent	*Pennisetum americanum*	High BOD, TN, and COD removal.
India, Thailand, South Africa	Emergent	*Vetiveria zizanioides*	Rapid growth and effective removal of ammonia, TN, TSS, COD, and chromium.
India, Sri Lanka, Argentina, China, Brazil	Free floating	*Eichhornia crassipes*	Excellent heavy metal accumulator (Cd, Cu, Al, productive, and highly invasive.
Throughout temperate and tropical regions	Perennial, floating	*Phragmites australis*	Highly invasive, metal tolerant, effective at removing N, P, BOD, and COD
USA	Emergent	*Scirpus pungens/validus*	Quick colonizer, drought-tolerant, efficient pollutant removal ability
USA	Submerged	*Najas guadalupensis*	Favorable for P uptake
Portugal	Emergent	*Sarcocornia fruticosa*	Salinity-tolerant and appropriate for highly salinized effluent, such as that from tanneries
Germany, Italy	Perennial, terrestrial	*Miscanthus* × *giganteus*	High biomass and energy yield, low nutrient requirement, low evapotranspiration and absorption of Fe, Cu, and Zn.
Czech Republic, New York	Perennial, emergent	*Phalaris arundinacea*	High biomass production, and effective removal of metals (Cr, Cd, Zn, Ni)

impact of wind affecting possible dispersion and water movement. The main methods for removing organics are microbial decomposition and particulate matter sedimentation. Although there is very little information available on these systems, it is probable that most of the activities take place in the water column aerobically since algae produce oxygen.

The removal of nitrogen from the water column can be done by plankton and periphytic communities clinging to the leaf peduncles and blades, which provide higher pH levels that allow for volatilization of nitrogen. The presence of algae within these systems is quite probable since the leaves of species with floating leaves frequently do not fully cover the water surface. While denitrification is unlikely to take place in the water column under aerobic circumstances, it may occur at the sediment–water interface if anoxic conditions exist. Algae may absorb phosphorus from the water column, however, this removal compartment is only temporary, as nutrients are soon washed out of decomposing algal tissue.

Roots and rhizomes of plants are capable of absorbing both nitrogen and phosphorus. According to literature, *Nuphar lutea* leaves that are floating and submerged may also absorb phosphorus. According to the season, this absorption through leaves, however, is 10–20 times less than root adsorption. The highest levels of phosphorus absorption by leaves are seen during summer.

9.8.4.2 Submerged macrophytes

Aquatic macrophytes that are submerged have all their photosynthetic tissue submerged. Nevertheless, submerged plants cannot be employed in wastewater that contains a lot of easily biodegradable organic matter since the microbial process that breaks down the organic matter would result in anoxic circumstances (Brix, 1997). Subsurface vegetation only thrives in oxygenated water. There is no concrete proof that the distribution of submerged plants is impacted by sudden reduction in oxygen content. But prolonged exposure to low oxygen levels can retard the development of submerged macrophytes (Shelef *et al*., 2013).

9.8.4.3 Emergent macrophytes

Aquatic plants with growing sections that emerge above the water's surface are known as emergent aquatic macrophytes. Aquatic macrophytes are believed to be especially fruitful, with their roots in sediments beneath water and their photosynthetic components in the air, making the greatest use of all three potential states (Westlake, 1963). Complicated development usually takes place above the water's surface and is inaccessible to many aquatic creatures, emergent macrophytes are generally thought to be less structurally complex. It is hypothesized that all emergent macrophytes provide homogenous habitat and perform a comparable role for many trophic levels due to the basic structural intricacy of these plants.

9.8.5 Microbiology in CWs

When organics and nitrogen are removed from wastewater in CW's, microorganisms are very important. The microbial assemblages have not been thoroughly investigated, despite playing crucial roles. Yet, the development of new, cutting-edge methodologies has made it possible to identify the microbial communities that are in charge of the biodegradation of particular contaminants. Bernardes *et al.* (2019) found an incredibly high variety of bacteria and archaea. The makeup of wastewater also affects the microbial community. In a system made up of a moving-bed biofilm reactor and a built wetland on top, Lai *et al.* (2021) studied microbial assemblages. The predominant genera detected in a created wetland, were *Gemnobacter* and *Parasegetibacter*, which are heterotrophic bacteria engaged in the breakdown of organic materials. The most common genera of denitrifiers included *Hydrogenophaga*, *Inhella*, *Methyloversatilis*, *Azo-spira*, and *Dechloromonas*, whereas *Hyphomicrobium* was associated with the nitrification process.

Many studies have established that the presence of substances can influence the existence of particular microorganisms. Organo-phosphate flame retardants (OPFRs) were shown to have a noticeable impact on the richness, variety, and nitrogen-related microorganisms in the rhizosphere (Liu *et al.*, 2020). Importantly, *Pseudomonas* and *Sphingobium* genera may serve as the necessary microorganisms for the biodegradation of OPFRs. Dibutyl phthalate, reduced the variety of the bacterial and fungal population. Liu *et al.* (2020) suggested that the addition of biochar boosted the abundance of *Gemnobacter* and denitrifiers like *Hydrogenophaga*.

The filtering material is another element influencing the bacterial diversity in built wetlands. When intertidal wetland sediments were added to VF CW, anaerobic decomposition primarily by the taxa Anaero-lineae, Desulfobacterales, and Desulfuromonadales improved the treatment efficacy. Also, it has been discovered that ethylene production from *Dehalococcoides* triggered by intertidal wetland sediment enhanced plant stress tolerance (Wang *et al.*, 2019). Lu *et al.* (2019) reported a significant change in the composition of the bacterial population during the studies on the arsenic removal and fluoride in a hybrid system made up of a sorption unit filled with pebbles and activated alumina and VF CW filled with coal cinder.

Currently, there are several studies conducted on the establishment of microbial assemblages in CWs, and it appears to be very difficult to pinpoint the single most crucial feature. The findings showed that there are numerous factors, including wastewater composition, the presence of specific compounds in the wastewater, the composition of the filtration material, the impact of different macrophyte species, the type of CW, oxygenation level, including artificial aeration, and seasonal variation. Nevertheless, almost all the research carried out so far have used artificial wastewater and have been conducted in laboratories or small-scale outdoor systems. Very little research has been conducted on fully created wetlands, and there is little information available regarding how microbial communities have changed over the years.

9.8.6 Design of CWs

The principal design parameters of CWs include the topography, soil permeability, substrate, hydraulic retention time, basin geometry, BOD_5 loading rate, hydraulic loading rate, vegetation, and so on.

One of the most crucial elements in the design of a wetland is topography (Lee *et al.*, 2009; Liu *et al.*, 2015). The best place to build a wetland is a low-level region where water flows naturally due to gravity. The landform concerns, such as shape, size, and orientation, are determined by the direction of the prevailing wind. While a gentle slope at the design site is preferred, artificial wetlands can be erected anyplace that satisfies the design requirements. With the approval of the undertaking authority, converted lands like agricultural sites could also be utilized for the development of wetland since they naturally hold all the designing requirements needed for wetland construction.

Another crucial parameter is the soil permeability and kind of substrate present. They determine the effectiveness of a CW. The accompanying soil permeability must be taken into account when choosing any site for the development of wetlands. For design purposes, a soil permeability of between 7 and 10 m/s is ideal. The wetlands' permeability can be maximized by adding a restrictive layer to the soil profile with reduced permeability since sandy soils are too permeable to sustain wetland plants. Small wastewater facilities may also be built on high-permeable soils by digging narrow trenches, but this requires that the ditches first be lined with clay. Small wastewater treatment facilities can also be built on highly permeable soils by digging shallow trenches first and filling them with clay or another synthetic liner.

The purpose of substrates in a wetland is to support the vegetation and to give room for all biochemical and chemical processes necessary for effective and smooth operation (Rana & Maiti, 2018). Substrates also serve as a place to store pollutants throughout the treatment process and to remove them. Natural substances including soil, sand, gravel, and broken boulders are frequently utilized as substrates for CWs. Organic compounds are another option. Natural substrate materials such as limestone, fly ash, slag, and coal cinder can be replaced with artificial substrates or industrial by-products. Natural substrates for subsurface flow CWs may be substituted in developing nations or regions with warm climates by lightweight aggregate, activated carbon, calcium silicate, or compost.

As it provides optimal permeability, hydraulic loading, and organic loading, all of which are crucial for the construction of wetlands. Coarse sand is chosen as the most ideal substrate.

One of the crucial design factors affecting how effectively pollutants and nutrients are removed from CWs is hydraulic retention time (HRT). Calculating nominal HRT, which is defined as the proportion of notional wetland volume to flow rate at outflow, is the simplest approach for estimating HRT (Kadlec & Wallace, 2008). The nominal HRT is predicated on 'plug-flow' reactors (PFR) as not all wetland volumes are included in the flow route. In fact, several researchers discovered that wetlands have three distinct hydraulic volumes or zones. The dynamic primary channel is the first zone; the temporary storage zone, is the second zone, where water and other elements are interchanged with the main flow channel; and the entirely isolated, 'dead' water is the third zone (Aleissa & Bakshi, 2021; Zahraeifard & Deng, 2011). Field tracer experiments and tracer test data are frequently used in conjunction with the moment technique to estimate HRT in conjunction to the empirical approach (Jadhav & Buchberger, 1995). The benefit of this method is that it enables the production of a residence time distribution (RTD), which in turn enables the calculation of a wetland's mean HRT. The main drawback of this strategy is that HRT is required during the design phase of a built wetland, whereas tracer tests can only be carried out in an existing wetland.

$$t = \frac{LWnd}{Q}$$

where L is the length of the CW in meters; W is the width of the CW in meters; Q is the average flowrate through the system in m^3/d; n is the fraction of cross-sectional area not occupied by plants and d is the depth of the basin in meters.

The required cross-sectional area is calculated using the following equation for subsurface flow:

$$A_c = \frac{Q}{K_s * S}$$

$K_s * S$ is the flow velocity and is to be limited to 6.8 m/d to reduce localized shearing of bacterial films.

The required width of the system is the function of the cross-sectional area and design depth and is calculated as

$$W = \frac{A_c}{d}$$

The BOD removal in CWs follows the first order, plug-flow kinetics (Vymazal, 2018) and hence can be computed as

$$C_t = C_0 e^{-kt}$$

Since t is a function of bed area (A), we can also write

$$A = \frac{Q * \left(ln\left(C_0 - C_t\right)\right)}{K_{BOD}}$$

where A is the bed area in m^2; Q is the average flow in m^3/day; C_0 is the inlet 5-day BOD_5 in mg/L; C_t is the outlet BOD_5 in mg/L; K_{BOD} is the BOD_5 reaction constant per day.

9.8.7 Use of CWs for the treatment of various industrial wastewater

In perspective of the urgent need to concentrate on the water–energy–environment via environmentally friendly and cost-effective treatment technologies, CW applications have recently been shown to have relatively high pollutant removal percentage and ecological advantages in both lab and field settings.

CWs may be used in a number of environments, from dry to tropical to cold; for water with a variety of contaminants in it; and for a broad range of pollutants.

Recently, treatment of industrial wastewater using CWs has received more attention. Treatment of effluents from various industries, including distilleries, cork-boiling operations, petro-chemical plants, coffee, potato, and seafood processors, as well as wastewater from dairies, slaughter houses, steel mills, coke-ovens, pulp and paper mills, oil fields, floriculture, textile, and agro-food effluents, using CW has been found to be satisfactory. Because of the different inherent underlying mechanisms, such as phytodegradation, rhizofiltration, microbial degradation, sorption, and so on, CWs could successfully remove the recalcitrant organic fraction of the wastewater (Jain *et al.*, 2020). Chang *et al.* (2022) worked on the treatment of high metal-bearing and acidic wastewater, also known as 'acid mine drainage' and found that the biochar substrate had the greatest proportional concentration of sulfate-reducing bacteria (SRB), followed by walnut shells. The biotic acid mine drainage therapy in the CW was made possible by the collaboration of organic-degrading bacteria, primarily *Cellulomonas*, *Clostridium*, and *Bacteroides*, and SRB groups. For CW systems addressing acid mine drainage, a combination of organic solid refuse and biochar is a better filler option; however, more research is needed to determine its capability under various conditions (Chang *et al.*, 2022).

Industries have different discharge of various pollutants in their effluents depending on the material inflow and outflow, the adopted unit operations, treatment efficiency, and so on. Table 9.2 gives a brief overview for some of the pollutants being discharged in the effluents by different industries in their waste water.

CWs treatment strategies are utilized effectively to treat a variety of wastewaters with varied organic or inorganic loadings. Many countries around the world, including China, India, Australia, Finland, United States, and so on use CW's technology on a large scale. In recent years, the use of CWs has been expanding to include additional types of wastewaters from industrial, agricultural, and agro-industrial sources (Feng *et al.*, 2020; Gomes *et al.*, 2018; Saeed *et al.*, 2018; Tunçsiper *et al.*, 2015). However, the efficiency of treatment may vary at times with respect to the type of wastewater and type of plant species used in CW system. Table 9.3 gives the basic outline of the removal efficiency observed along with the treatment mechanism employed for the CW system.

Table 9.2 Concentrations of major pollutants removal reported in the literature for various industrial wastewater.

Type of Industrial Wastewater	TSS (mg/L)	TKN (mg/L)	COD (mg/L)	BOD$_5$ (mg/L)	Phenol (mg/L)	TP (mg/L)	Reference(s)
Pulp and paper	100–23 300	11–600	500–40 000	100–13 300	–	0.02–36	Vymazal (2014)
Distillery	1000–17 000	1000–17 000	4000–212 000	6000–65 000	35–10 000	740–2500	Chandra *et al.* (2008), Mohana *et al.* (2009)
Tannery effluents	1500–42 500	–	2000–23 000	800–4000	–	8–40	Espinoza-Quiñones *et al.* (2009), Mannucci *et al.* (2010)
Sugar mill	350	53	3500–10 000	4000–7000	–	4.8	Güven *et al.* (2009)
Soft drink	140–5000	54	1400–33 000	770	–	2.5	Oktay *et al.* (2007)
Pharmaceuticals	2500	–	5000–12 000	1300–6400	–	–	Vymazal (2014)
Textile	25–24 500	–	220–31 300	75–6300	–	–	dos Santos *et al.* (2007), Sharma *et al.* (2007)

Table 9.3 Efficiency of CW system treating different sources wastewater including species of macrophyte and treatment system adopted.

Type of Wastewater	Plant Species	Type of CW	Pollutant Removal Efficiency	Reference
Activated sludge effluent	*Phragmites australius*	Horizontal subsurface flow	BOD: 89%, TSS:81%, FC: 99%, TC: 97%,	Ahmed *et al.* (2008)
Domestic	*Phragmites carca* *Typha latifolia*	Horizontal subsurface flow	N: 58–63%, BOD: 67–90% Higher N was removed by *P. carca*	Juwarkar *et al.* (1995)
Municipal	*Canna, Phragmites,* and *Cyperus* sp.	Horizontal subsurface flow Vertical subsurface flow	BOD: 93%, TSS: 92%, COD: 91%, BOD: 94%, TSS: 94%, COD: 93%,	Abou-Elela *et al.* (2013)
	Phragmites australis	Hybrid	COD:91%, BOD5: 93%, TSS: 95%, TN: 67% and TP:62%	Elfanssi *et al.* (2018)
	Phragmites australis and Cyperus papyrus	Vertical subsurface flow	RE of *C. papyrus* higher (COD:70%, BOD: 81%, TP: 50%, ammonia-N: 70%, FC: 95%, TC: 98%,	García-Ávila *et al.* (2019)
Paper and pulp	*Typha domingensis, Phragmites mauritianus, Cyperus immensus, and Cyperus papyrus*	Subsurface flow	N: 49–75% in planted CW and 42–49% in unplanted CW; phenol: 60–77% (higher at lower HRT); P: 4–38% in the unplanted CW and 30–60% in planted CW	Abira *et al.* (2005)
Distillery	*Typha latifolia, Phragmites karka*	Horizontal	BOD: 84%, COD: 64%, TP: 79%, TN: 59%	Billore *et al.* (2001)
Tannery	*Phragmites* sp.	Horizontal subsurface flow	COD: 82%, NH_4^+-N: 96%	Ramírez *et al.* (2019)
Textile	*Brachiaria mutica*	Vertical	Color: 74%, TN: 84%, BOD: 72%, COD: 81%, TSS: 32%, TP: 79% Cd: 72%, Cr: 97%, Ni: 88%, Fe: 89%	Hussain *et al.* (2018)

9.9 CONCLUSION

As an alternative to current chemical-intensive treatment systems consuming surplus amounts of energy and resources, CW is a sustainable and economically advantageous technology. In virtue to being a potential net energy producer with remarkably low operational costs, CW is also capable of providing a range of ecosystem services and lowering its carbon impact. The development of several applications has made it clear that more work has to be conducted to increase the CW system's efficiency through a broader set of design criteria, including component change or upgrade. This review summarizes some relatively unexplored issues relating to improving vital wetland elements (macrophytes, substrates, and biofilm), which would improve system efficiency for removing various contaminants and the potential for expanding the technology's application to effluent emanating from various industries.

The goal of this study is to explain how the interface structure and synergetic effects of these components may be functionalized and developed logically to aid advance CW efficiency. Additionally, the main difficulties, possibilities, and options for future growth of these elements are highlighted. It is well known that the growth of rhizosphere manipulation/management methods would enable the interaction of macrophyte–biofilm–substrate, which in turn would enhance overall efficiency and implementations of the technology to a wide range of wastewater, by appropriately designing and tying in every individual component.

Owing to the future of the CW's there can be various aspects on which further thought can be put into for macrophyte utilization of genetically modified plants, using hyperaccumulators, phyto mining. For the influent wastewater that has already been given a pre-treatment can be used. Substrate modifications can also be done as in using substrate from organic carbon source, utilization of hybrid substrate that can target multiple pollutants and focus on reusability and recyclability of substrate. Biofilm development can be augmented furthermore and designer microbes developed in the laboratory can also be utilized.

REFERENCES

Abira M. A., van Bruggen J. J. A. and Denny P. (2005). Potential of a tropical subsurface constructed wetland to remove phenol from pre-treated pulp and papermill wastewater. *Water Science and Technology*, **51**(9), 173–176, https://doi.org/10.2166/wst.2005.0312

Abou-Elela S. I., Golinielli G., Abou-Taleb E. M. and Hellal M. S. (2013). Municipal wastewater treatment in horizontal and vertical flows constructed wetlands. *Ecological Engineering*, **61**, 460–468, https://doi.org/10.1016/j.ecoleng.2013.10.010

Ahmed S., Popov V. and Trevedi R. C. (2008). Constructed wetland as tertiary treatment for municipal wastewater. *Proceedings of Institution of Civil Engineers: Waste and Resource Management*, **161**(2), 77–84, https://doi.org/10.1680/warm.2008.161.2.77

Aleissa Y. M. and Bakshi B. R. (2021). Constructed wetlands as unit operations in chemical process design: benefits and simulation. *Computers and Chemical Engineering*, **153**, 107454, https://doi.org/10.1016/j.compchemeng.2021.107454

Bernardes F. S., Herrera P. G., Chiquito G. M., Morales M. F., Castro A. P. and Paulo P. L. (2019). Relationship between microbial community and environmental conditions in a constructed wetland system treating greywater. *Ecological Engineering*, **139**, 105581, https://doi.org/10.1016/j.ecoleng.2019.105581

Billore S. K., Singh N., Ram H. K., Sharma J. K., Singh V. P., Nelson R. M. and Dass P. (2001). Treatment of a molasses based distillery effluent in a constructed wetland in central India. *Water Science and Technology*, **44**(11–12), 441–448, https://doi.org/10.2166/wst.2001.0864

Brix H. (1997). Do macrophytes play a role in constructed treatment wetlands? *Water Science and Technology*, **35**(5), 11–17, https://doi.org/10.1016/S0273-1223(97)00047-4

Campbell R. and Greaves M. P. (1990). Anatomy and community structure of the rhizosphere. In: The rhizosphere, Wiley, Lynch, J.M. (ed.), Chichester, United Kingdom, pp. 11–34.

Chandra R., Yadav S., Bharagava R. N. and Murthy R. C. (2008). Bacterial pretreatment enhances removal of heavy metals during treatment of post-methanated distillery effluent by *Typha angustata* L. *Journal of Environmental Management*, **88**(4), 1016–1024, https://doi.org/10.1016/j.jenvman.2007.05.001

Chang J., Deng S., Li X., Li Y., Chen J. and Duan C. (2022). Effective treatment of acid mine drainage by constructed wetland column: coupling walnut shell and its biochar product as the substrates. *Journal of Water Process Engineering*, **49**(August), 103116, https://doi.org/10.1016/j.jwpe.2022.103116

Cooper P. (1999). A review of the design and performance of vertical-flow and hybrid reed bed treatment systems. *Water Science and Technology*, **40**(3), 1–9, https://doi.org/10.1016/S0273-1223(99)00414-X

Donnelly P.K. and Fletcher J. S. (1994). Potential use of mycorrhizal fungi as bioremediation agents. In: Bioremediation Through Rhizosphere Technology, Anderson, T.A. (ed.), pp. 93–99. American Chemical Society. Symposium Series 563. ACS Chicago, Illinois, USA, https://doi.org/10.1021/bk-1994-0563.ch008

dos Santos A. B., Cervantes F. J. and van Lier J. B. (2007). Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. *Bioresource Technology*, **98**(12), 2369–2385, https://doi.org/10.1016/j.biortech.2006.11.013

Elfanssi S., Ouazzani N., Latrach L., Hejjaj A. and Mandi L. (2018). Phytoremediation of domestic wastewater using a hybrid constructed wetland in mountainous rural area. *International Journal of Phytoremediation*, **20**(1), 75–87, https://doi.org/10.1080/15226514.2017.1337067

Espinoza-Quiñones F. R., Fornari M. M. T., Módenes A. N., Palácio S. M., da Silva F. G., Szymanski N., Kroumov A. D. and Trigueros D. E. G. (2009). Pollutant removal from tannery effluent by electrocoagulation. *Chemical Engineering Journal*, **151**(1–3), 59–65, https://doi.org/10.1016/j.cej.2009.01.043

Feng L., Wang R., Jia L. and Wu H. (2020). Can biochar application improve nitrogen removal in constructed wetlands for treating anaerobically-digested swine wastewater? *Chemical Engineering Journal*, **379**, 122273, https://doi.org/10.1016/j.cej.2019.122273

García-Ávila F., Patiño-Chávez J., Zhinín-Chimbo F., Donoso-Moscoso S., Flores del Pino L. and Avilés-Añazco A. (2019). Performance of *Phragmites australis* and *Cyperus papyrus* in the treatment of municipal wastewater by vertical flow subsurface constructed wetlands. *International Soil and Water Conservation Research*, **7**(3), 286–296, https://doi.org/10.1016/j.iswcr.2019.04.001

Gomes A. C., Silva L., Albuquerque A., Simões R. and Stefanakis A. I. (2018). Investigation of lab-scale horizontal subsurface flow constructed wetlands treating industrial cork boiling wastewater. *Chemosphere*, **207**, 430–439, https://doi.org/10.1016/j.chemosphere.2018.05.123

Güven G., Perendeci A. and Tanyolaç A. (2009). Electrochemical treatment of simulated beet sugar factory wastewater. *Chemical Engineering Journal*, **151**(1–3), 149–159, https://doi.org/10.1016/j.cej.2009.02.008

Hussain Z., Arslan M., Malik M. H., Mohsin M., Iqbal S. and Afzal M. (2018). Integrated perspectives on the use of bacterial endophytes in horizontal flow constructed wetlands for the treatment of liquid textile effluent: phytoremediation advances in the field. *Journal of Environmental Management*, **224**(May), 387–395, https://doi.org/10.1016/j.jenvman.2018.07.057

Jadhav R. S. and Buchberger S. G. (1995). Effects of vegetation on flow through free water surface wetlands. *Ecological Engineering*, **5**(4), 481–496, https://doi.org/10.1016/0925-8574(95)00039-9

Jain M., Majumder A., Ghosal P. S. and Gupta A. K. (2020). A review on treatment of petroleum refinery and petrochemical plant wastewater: a special emphasis on constructed wetlands. *Journal of Environmental Management*, **272**(May), 111057, https://doi.org/10.1016/j.jenvman.2020.111057

Juwarkar A. S., Oke B., Juwarkar A. and Patnaik S. M. (1995). Domestic wastewater treatment through constructed wetland in India. *Water Science and Technology*, **32**(3), 291–294, https://doi.org/10.1016/0273-1223(95)00637-0

Kadlec R. H. and Wallace S. (2008). Treatment Wetlands, https://doi.org/10.1201/9781420012514

Kataki S., Chatterjee S., Vairale M. G., Dwivedi S. K. and Gupta D. K. (2021). Constructed wetland, an eco-technology for wastewater treatment: a review on types of wastewater treated and components of the technology (macrophyte, biolfilm and substrate). *Journal of Environmental Management*, **283**, 111986, https://doi.org/10.1016/j.jenvman.2021.111986

Lai C., Sun Y., Guo Y., Cai Q. and Yang P. (2021). A novel integrated bio-reactor of moving bed and constructed wetland (MBCW) for domestic wastewater treatment and its microbial community diversity. *Environmental Technology (United Kingdom)*, **42**(17), 2653–2668, https://doi.org/10.1080/09593330.2019.1709904

Lee C. G., Fletcher T. D. and Sun G. (2009). Nitrogen removal in constructed wetland systems. *Engineering in Life Sciences*, **9**(1), 11–22, https://doi.org/10.1002/elsc.200800049

Liu R., Zhao Y., Doherty L., Hu Y. and Hao X. (2015). A review of incorporation of constructed wetland with other treatment processes. *Chemical Engineering Journal*, **279**, 220–230, https://doi.org/10.1016/j.cej.2015.05.023

Liu T., Lu S., Wang R., Xu S., Qin P. and Gao Y. (2020). Behavior of selected organophosphate flame retardants (OPFRs) and their influence on rhizospheric microorganisms after short-term exposure in integrated vertical-flow constructed wetlands (IVCWs). *Science of the Total Environment*, **710**, 136403, https://doi.org/10.1016/j.scitotenv.2019.136403

Lu H., Li J., Liu X., Yu Z. and Liu R. (2019). Removal of fluoride and arsenic by a hybrid constructed wetland system. *Chemistry and Biodiversity*, **16**(7), 1–8, https://doi.org/10.1002/cbdv.201900078

Mannucci A., Munz G., Mori G. and Lubello C. (2010). Anaerobic treatment of vegetable tannery wastewaters: a review. *Desalination*, **264**(1–2), 1–8, https://doi.org/10.1016/j.desal.2010.07.021

Mohana S., Acharya B. K. and Madamwar D. (2009). Distillery spent wash: treatment technologies and potential applications. *Journal of Hazardous Materials*, **163**(1), 12–25, https://doi.org/10.1016/j.jhazmat.2008.06.079

Oktay S., Iskender G., Germirli Babuna F., Kutluay G. and Orhon D. (2007). Improving the wastewater management for a beverage industry with in-plant control. *Desalination*, **211**(1–3), 138–143, https://doi.org/10.1016/j.desal.2006.02.088

Ramírez S., Torrealba G., Lameda-Cuicas E., Molina-Quintero L., Stefanakis A. I. and Pire-Sierra M. C. (2019). Investigation of pilot-scale constructed wetlands treating simulated pre-treated tannery wastewater under tropical climate. *Chemosphere*, **234**, 496–504, https://doi.org/10.1016/j.chemosphere.2019.06.081

Rana V. and Maiti S. K. (2018). Municipal wastewater treatment potential and metal accumulation strategies of *Colocasia esculenta* (L.) Schott and *Typha latifolia* L. in a constructed wetland. *Environmental Monitoring and Assessment*, **190**(6), 328, https://doi.org/10.1007/s10661-018-6705-4

Saeed T., Muntaha S., Rashid M., Sun G. and Hasnat A. (2018). Industrial wastewater treatment in constructed wetlands packed with construction materials and agricultural by-products. *Journal of Cleaner Production*, **189**, 442–453, https://doi.org/10.1016/j.jclepro.2018.04.115

Schnoor J. L. and Huang W. (1993). Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. *Critical Reviews in Environmental Science and Technology*, **23**(1), 41–77, https://doi.org/10.1080/10643389309388441

Sharma K. P., Sharma S., Sharma S., Singh P. K., Kumar S., Grover R. and Sharma P. K. (2007). A comparative study on characterization of textile wastewaters (untreated and treated) toxicity by chemical and biological tests. *Chemosphere*, **69**(1), 48–54, https://doi.org/10.1016/j.chemosphere.2007.04.086

Shelef O., Gross A. and Rachmilevitch S. (2013). Role of plants in a constructed wetland: current and new perspectives. *Water (Switzerland)*, **5**(2), 405–419, https://doi.org/10.3390/w5020405

Tunçsiper B., Drizo A. and Twohig E. (2015). Constructed wetlands as a potential management practice for cold climate dairy effluent treatment – VT, USA. *Catena*, **135**, 184–192, https://doi.org/10.1016/j.catena.2015.07.028

Vymazal J. (2005). Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. *Ecological Engineering*, **25**(5), 478–490, https://doi.org/10.1016/j.ecoleng.2005.07.010

Vymazal J. (2014). Constructed wetlands for treatment of industrial wastewaters: a review. *Ecological Engineering*, **73**, 724–751, https://doi.org/10.1016/j.ecoleng.2014.09.034

Vymazal J. (2018). Constructed Wetlands for Wastewater Treatment. Encyclopedia of Ecology (2nd Edition), **45**, 14–21, https://doi.org/10.1016/B978-0-12-409548-9.11238-2

Wang Q., Cao Z., Liu Q., Zhang J., Hu Y., Zhang J., Xu W., Kong Q., Yuan X. and Chen Q. F. (2019). Enhancement of COD removal in constructed wetlands treating saline wastewater: intertidal wetland sediment as a novel inoculation. *Journal of Environmental Management*, **249**(August), 109398, https://doi.org/10.1016/j.jenvman.2019.109398

Westlake D. F. (1963). Comparisons of plants productivity. *Biological Reviews*, **38**, 385–425, https://doi.org/10.1111/j.1469-185X.1963.tb00788.x

Zahraeifard V. and Deng Z. (2011). Hydraulic residence time computation for constructed wetland design. *Ecological Engineering*, **37**(12), 2087–2091, https://doi.org/10.1016/j.ecoleng.2011.08.011

doi: 10.2166/9781789063783_0197

Chapter 10

Myconanoremediation of various environmental toxicants: challenges and future perspectives

*N. Prabhu[1], A. Archana[1], G. Someshwari[1], G. Priyanka[1], S. Livithra[1], S. Chozhavendhan[1] and T. Gajendran[2,]**

[1]Department of Biotechnology, Vivekanandha College of Engineering for Women (Autonomous), Tiruchengode 637-207, Namakkal, Tamilnadu, India
[2]Department of Biotechnology, Bharathidasan Institute of Technology, Anna University, Tiruchirappalli 620024, Tamilnadu, India
**Corresponding author: gajendran01@gmail.com*

ABSTRACT

The application of nanotechnology in the area of environment has been studied extensively. With the rise in environmental challenges, one of biotechnology's primary concerns is the remediation of the environment. This technique can be called bioremediation and it works to decontaminate the environment by using microorganisms like fungi or their enzymes to break down hazardous compounds into non-hazardous ones. The combination of various particle and organism types, including fungi along with nanotechnology, can offer ecologically responsible alternatives for bioremediation. Nanoparticles are one of the varieties of nanomaterials and have found extensive use in a wide range of applications. Iron oxide nanoparticles were considered a good candidate for nanoparticles that is appropriate for usage in biosystems because of their chemical stability and low toxicity. As a result, components like bioactive nanoparticles, nanocomposites, and nanocatalysts have been used more in the creation of procedures and techniques for cleaning up an environment contaminated with chemical waste.

Keywords: nanosensor, nanoremediation, fungi, nanoencapsulation, nanocatalyst

10.1 INTRODUCTION

Environmental pollution and natural contamination are key issues in society. Innovations are reconnoitered steadily for the remediation of pollutants in the air, water, and soil (Masciangoli *et al.*, 2003). Particulate matter, herbicides, weight metals, pesticides, manures, oil slicks, harmful gases, sewage effluents, and natural mixtures are some of the instances of the many concerning impurities (Vaseashta *et al.*, 2007). As the altered form of environmental contamination can be trying because of the difficulty of the combination of various mixtures, high instability, and low reactivity, late investigations have zeroed in on the utilization of nanomaterial and the advancement of new natural remediation innovation.

The chapter is from the book *Clean Technologies Towards a Sustainable Future: Physicochemical, Biochemical and Biotechnological Approaches*, Pradeep Verma, Maulin P. Shah (Editors).

Numerous studies and applications of nanotechnology are being made in the field of the environment. Together with other fields of knowledge, this 'new' scientific field can significantly advance environmental research and application. The advancement and significant growth of nanotechnology will enhance human well-being and contribute to environmental preservation (Kango *et al.*, 2013). The basic sciences, engineering, and their corresponding applications have been revolutionized by this science, which has developed into a multidisciplinary discipline (Lodhia *et al.*, 2010). The decontamination of environments is one of biotechnology's primary concerns in light of the growing environmental issues. This process is known as bioremediation and this process uses microorganisms and their enzymes to break down toxic substances into non-toxic ones to decontaminate the environment.

Nanoparticles are created due to their normal systems for the detoxification of metallic particles through biosystems (Vijay Pradhap Singh *et al.*, 2021). In any case, such cycles are frequently not achievable because of the cost of production, harmful muck synthesis, and the requirement for a persistent contribution of synthetic compounds (Han *et al.*, 2006). In this circumstance, metal-poisoning bioremediations have arisen as an innovation that welcomes nature. A few microorganisms are powerful that eliminate metal particles through biosorption instruments (Volesky, 2022). As a result, they can adapt to grow in abnormal pH, temperature, and nutrient availability conditions and at high heavy metal fixation levels (Anand *et al.*, 2006). Additionally, important to reduction are the infectious microbial structures. A practical strategy for both the biosynthesis of nanoparticles and contemporary bioremediation is bioreduction, one of the crucial cycles of biosynthesis (Malik, 2004; Srivastava *et al.*, 2013).

10.2 SYNTHESIS OF MYCONANOPARTICLES

Each extracellular active organism produces metal nanoparticles. There are numerous studies on fungi that can produce nanoparticles both intracellularly and extracellularly. In intracellular active organisms, the fungal biomass reacts with metals intracellularly and the desired nanoparticles are produced. Whereas in extracellular synthesis the enzyme in the cell wall of the microbes reduces the metal ions and aggregates to produce the nanoparticles. Therefore, extracellular synthesis is mostly preferred (Yadav *et al.*, 2015). When the fungi were exposed to metal ions during extracellular nanoparticle production, the result has been unused enzymes and the generation of very stable myconanoparticles (Kashyap *et al.*, 2013). The fungus species was made to treat extracellularly and the gold nanoparticles have been produced (Du *et al.*, 2011). The extracellular synthesis started after the resolution of $AuCl_4$ was incubated with fungal biomass for 8 h, whereas the golden nanoparticles were obtained when $AuCl_4$ ions reacted with the fungal cell deposit over a period of time. A living thing's biosynthesis may be a time-limited problem that depends on in vivo cell synthesis because there are two fundamentally different extracellular syntheses, namely fast synthesis and slow synthesis (Moghaddam, 2010). Apart from the two processes, an additional process is added to produce the uncontaminated nanoparticles. Hence, the extracellular production process is significantly more favorable than the intracellular method (Kuber *et al.*, 2006).

The extracellular and intracellular fungal nanoparticle synthases have been reported by various researchers (Table 10.1). Fatima *et al.* (2016) stated that the 50 nm silver nanoparticle produced from *Aspergillus flavus* has significant antibacterial activity against pathogenic bacteria and fungi. Myconanoparticles are said to be microbiological agents on agricultural grounds. Ingle *et al.* (2009) reported that *Fusarium solani* is a unique biohazard that has been used in the fusion of silver nanoparticles in a separate investigation.

10.3 BIOSYNTHESIS OF NANOPARTICLES USING FILAMENTOUS FUNGI

Fungi are heterotrophic, eukaryotic cells that could not produce chlorophyll but can produce spores (Veglio & Beolchini, 1997). The fungus is capable of absorbing and accumulating the metals through

Table 10.1. List of fungal synthesis of nanoparticles.

Fungal Species	Nano Particles	Synthesis	Reference
Mucorhils	Silver	Intracellular	Aziz *et al.* (2016)
Aspergills KF934407)	Silver	Extracellular	Fatima *et al.* (2016)
Coriolusr	Silver	Intracellular Extracellular	Sanghi and Verma (2009)
Fusarium oxysporm	Platinum	Extracellular	Syed and Ahmad (2012)
Aspergillus terreus	Zinc oxide	Extracellular	Baskar *et al.* (2013)
Fusarium solani	Silver	Extracellular	Ingle *et al.* (2009)
Saccharomyces cerevisiae	Cds	Extracellular	Prasad and Jha (2010)
Alternaria alternata	Silver	Extracellular	Sarkar *et al.* (2011)
Aspergillus clavatus	Silver	Extracellular	Verma *et al.* (2010)
Aspergillus flavus	Silver	Intracellular	Vala *et al.* (2014)
Aspergillus fumigatus	Silver	Extracellular	Ranjbar-Navazi *et al.* (2010)
Aspergillus fumigatus	Silver	Extracellular	Bhainsa and D'souza (2006)
Aspergillus niger	Silver	Extracellular	Gade *et al.* (2008)
Aspergillus niger	Silver	Extracellular	Kathiresan *et al.* (2010)
Aspergillus tamarii	Silver	Extracellular	Kumar *et al.* (2012)
Aspergillus terreus	Silver	Extracellular	Li *et al.* (2011)
Aspergillus species	Zinc	Extracellular	Pavani *et al.* (2012)
Cladosporium cladosporioides	Silver	Extracellular	Balaji *et al.* (2009)
Coriolus versicolor	Cadmium sulfide	Extracellular	Sanghi and Verma (2009)
Fusarium acuminatum	Silver	Extracellular	Ingle *et al.* (2008)
Fusarium semitectum	Silver	Extracellular	Basavaraja *et al.* (2008)
Fusarium solani	Silver	Extracellular	Ingle *et al.* (2009)
Fusarium oxysporum	Silver	Extracellular	Ahmad *et al.* (2003)
Fusarium oxysporum	Zirconia	Extracellular	Bansal *et al.* (2004)
Fusarium oxysporum	Silica/titanium	Extracellular	Bansal *et al.* (2005)
Fusarium oxysporum	Magnetite	Extracellular	Bharde *et al.* (2006)
Fusarium oxysporum	Gold	Extracellular	Mukherjee *et al.* (2002)
Fusarium oxysporum f.sp. *lycopersici*	Platinum	Intra-/extracellular	Riddin *et al.* (2006)
Hormoconis resinae	Gold	Extracellular	Varshney *et al.* (2009)
Helminthosporium tetramera	Silver	Extracellular	Shelar and Chavan (2014)
Mucor hiemalis	Silver	Extracellular	Aziz *et al.* (2016)
Neurospora crassa	Gold, silver	Extracellular	Castro-Longoria *et al.* (2011)
Nigrospora oryzae	Gold	Extracellular	Kar *et al.* (2014)
Penicillium brevicompactum	Gold	Extracellular	Mishra *et al.* (2011)
Penicillium citrinum	Silver	Extracellular	Honary *et al.* (2013)
Penicillium citrinum	Copper oxide	Extracellular	Honary *et al.* (2012)
Penicillium fellutanum	Silver	Extracellular	Kathiresan *et al.* (2009)
Penicillium nalgiovense	Silver	Extracellular	Maliszewska *et al.* (2014)
Penicillium purpurogenum NPMF	Silver	Extracellular	Nayak *et al.* (2011)
Penicillium glabrum	Silver	Extracellular	Nanda and Majeed (2014)

(*Continued*)

Table 10.1. List of fungal synthesis of nanoparticles. (*Continued*)

Fungal Species	Nano Particles	Synthesis	Reference
Phanerochaete chrysosporium	Silver	Extracellular	Vigneshwaran *et al.* (2006)
Phoma glomerata	Silver	Extracellular	Birla *et al.* (2009)
Phoma gardenia	Silver	Extracellular	Rai *et al.* (2014)
Phoma sp.3.2883	Silver	Extracellular	Chen *et al.* (2003)
Pleurotus sajor-caju	Silver	Extracellular	Nithya and Ragunathan (2009)
Thermomonospora sp.	Gold	Extracellular	Ahmad *et al.* (2003)
Trichoderma asperellum	Silver	Extracellular	Mukherjee *et al.* (2008)
Trichoderma viride	Silver	Extracellular	Fayaz *et al.* (2010)
Trichothecium sp.	Gold	Intra-/extracellular	Ahmad *et al.* (2005)
Verticillium sp.	Magnetite	Extracellular	Bharde *et al.* (2006)
Yeast cells	Cadmium telluride	Extracellular	Bao *et al.* (2010)

its metal-binding functional group present in it via a biological metabolism-based mechanism (Sastry *et al.*, 2003). The characteristic of microbes as potent secretors of oxidative enzymes being utilized in metallic nanoparticle synthesis and an additional evaluation of distinct microorganisms makes the fungi seen as a promising tool for large-scale nanoparticle manufacturing (Kitching *et al.*, 2015; Prasad, 2016, 2017). This technique uses fungi to synthesize clean and economical fungal nanoparticles produced either in the laboratory or on a commercial scale (Moghaddam *et al.*, 2015). Through the use of biomimetic mineralization and lowering enzymes either intracellularly or extracellularly, fungi can produce metal nanoparticles and nanostructures (Ahmad *et al.*, 2003).

Due to its operational flexibility and affordability, using fungal dead biomass in the design of nanomaterials is particularly advantageous (Salvadori *et al.*, 2013). The filamentous fungus *Verticillium* sp. mediates the biosynthesis of silver nanoparticles. It has been noted by one of the earliest reviews on the synthesis of nanoparticles by a fungus that was illustrated by Mukherjee *et al.* (2001). *Rhizopus oryzae* has produced other noble metallic nanoparticles as nano gold-bio coupled within the side of the floor cell, with the length of 10 nm fungal lines isolated from wastewater present in a copper mine located in the Amazon region of Brazil as *Hypocrealixii* and *Trichoderma*. Steel or copper nanoparticles are not produced by yeasts (Salvadori *et al.*, 2013, 2014a). So, many fungi revealed a plethora of potential metal nano adsorbents for bioremediation.

10.4 FACTORS THAT AFFECT SYNTHESIS OF MYCO NANOPARTICLE

The factors such as temperature, biomass, concentration, substrate utilization time, pH, and the presence of a specific catalyst all have an impact on the biosynthesis of fungus nanoparticles. These are widely acknowledged as the primary factors that are influencing the shape and size of the nanoparticles (Kashyap *et al.*, 2013). Khan *et al.* (2016) investigated different parameters such as pH, quantity of fungal biomass, temperature, and concentration of nitrate, which could be improved to produce silver nanoparticles produced by *Aspergillus niger*. It has been stated that an increase in the above-mentioned factors can improve both the yield and synthesis of silver nanoparticles. Birla *et al.* (2013) looked into ways to improve the social and environmental conditions that were involved in the synthesis of the nanoparticles from *Fusarium oxysporum* and the produced nanoparticle was silver.

The pH is considered as one of the important factors that help in the improvement of fungal nanoparticles production. The properties and size of nanoparticles that have been synthesized are strongly influenced by the pH (Armendariz *et al.*, 2004; Gardea-Torresdey *et al.*, 1999). The nanoparticles that are synthesized from the species *Aspergillus terreus* and their efficacy against *E. coli* have been discussed by Priyadarshini *et al.* (2014) and the type of nanoparticle produced is gold. The nanoparticles have a size range between 10 and 19 nm. Fourier transform infrared spectroscopy (FTIR) has been used for the characterization of the nanoparticles that were synthesized. The regulation of biological activity and the transport of ions were greatly influenced by temperature (Dhillon *et al.*, 2012). Temperatures less than or near 100°C are required for the environmentally friendly method of synthesizing nanoparticles. Fayaz *et al.* (2009) investigated the effect of temperature on the production of plant-based nanoparticles. They discovered that raising the temperature of the reaction can cause a reduction in the size of the nanoparticles but an increase in monodispersity. Another crucial thing influencing the advent of fungal nanoparticles is the incubation period. The response medium's simple composition in large part impacts the type of nanoparticle that is produced and consequently, their quality (Darroudi *et al.*, 2011). Due to combining the debris, the nanoparticle can be stored for an extended period of time, and the quality can be modified by making changes in the incubation time (Baer, 2011).

For the biosynthesis of nanoparticles, there are extensive strategies. Mistreatment of the upstream and downstream processes is regularly combined. Physical strategies, including diffusion, thermal breakdown, irradiation, and so on, are used in the upstream method. In the downstream method, nanoparticles have created the usage of organic strategies which include organic entities and chemical strategies like a chemical discount, seeded growth, chemical technological know-how synthesis, and polyol synthesis (Tikariha *et al.*, 2012). Etching and machining tactics are used in the upstream method, where huge substances slowly decrease to nano-sized substances (Lengke *et al.*, 2011). Fungal nanoparticle synthesis has probably finished the usage of a downstream strategy. The majority of mycosynthesis reactions that bring about nanoparticles resemble backside-up processes due to the fact they encompass substrate oxidation or discount that ends in combined structures. The downstream method has more benefits over different processes due to the fact of a better probability of the usage of structures in nanoscale such as nanotubes, nanorods, nanowires, nanocubes, nanosheets, and so forth with better chemical composition homogeneity. This is regularly carried out because of the method, which is guided especially via lower chemist-unfastened power that moves the synthesized nanomaterials or nanostructures toward the nation of thermodynamic equilibrium (Kashyap *et al.*, 2013).

10.5 FUNGAL DEAD BIOMASS IN THE BIOREMEDIATION PROCESS

Microscopic fungi-like yeast and fungi that are single cellular can grow facultatively anaerobically and are frequently spherical or oval. The last electron acceptor allows yeast to utilize oxygen or a natural substance. This is a priceless trait that enables the fungi to survive and thrive in a variety of situations. It was one of the first attempts to employ organic materials for this purpose, producing CdS nanoparticles intracellularly using *Schizosaccharomyces pombe* and *Candida glabrata* (Dameron *et al.*, 1989). Silver NPs were created using yeast pressure MKY3, while gold nanoparticles were created using the humid marine yeast *Yarrowia lipolytica* NCIM 3589 (Agnihotri *et al.*, 2009). Kowshik *et al.* (2003) extracted Au and Ag's nanoparticles using *Candida guilliermondii* and *Saccharomyces cerevisiae* (Lim *et al.*, 2011; Mishra *et al.*, 2011). As part of the bioremediation process, the yeast develops the ability to synthesize intracellular metal copper nanoparticles with a length of 10.5 nm and round shaped, simultaneously for the metal ions in wastewater (Salvadori *et al.*, 2014b).

There have been few studies on the formation of nanoparticles by contagious dead biomass, such as the growth of fungi. *Hypocrealixii* can deliver extracellular metallic copper nanoparticles as well as intra and extracellular NiO_2 nanoparticles (Salvadori *et al.*, 2015). Extracellular metallic copper nanoparticles were produced by *Trichoderma koningiopsis*. *Aspergillus aculeatus* could produce extracellular nickel oxide nanoparticles in the form of film structures. *Rhodotorula mucilaginosa* could approve intracellular, metallic copper nanoparticles, and appealing Ni/NiO_2 center shell nanostructured nanoparticles in film structures (Salvadori *et al.*, 2016). Because the metal ions cannot biodegrade, substance or real treatment is largely responsible for removing them from contaminated soil and water. The readily available treatment methods are precipitation, particle exchange, electrolysis, and converse membrane partition.

Today, progressively more research is being done on the bioremediation process and the synthesis of metallic nanoparticles (Gad, 2010). The primary method commonly used for each nanoparticle production and bioremediation using fungal waste biomass includes a process known as biosorption that involves floor-level adherence of ions that are metallic to the wall of the molecular surface (Bishnoi, 2005; Dhankhar & Guriyan, 2011). The chitin, chitosan, glucan, lipid, and phospholipid walls of the fungal biomass are composed of COH groups, amino organization phosphates, lipids, melanin, sulfates, and hydroxides. The great advantage of waste biomass is attributed to its superior ability to bind metals compared to living cells, mathematical modulation of the metallic elimination reactors, lack of need for media and supplements present in solution feed, reusability of the biomass regenerated, and low toxicity (Kapoor & Viraraghavan, 1996; Kogej & Pavko, 2001; Merrin *et al.*, 1998; Salvadori *et al.*, 2013).

Physicochemical factors such as concentration of biomass, pH, temperature, agitation rate, contact time, and metallic ion concentration, on biosorption must be highlighted to improve a green nanoparticle biosynthesis process. The amount of biosorbents is a crucial factor in determining the system's sorbents equilibrium (Hanif *et al.*, 2007; Sari *et al.*, 2007). The pH of the solution indicates how well the organizations are ionized at the molecular floor of the biomass wall (Ozer & Ozer, 2003). The extracellular nanoparticles within the molecular wall of the fungi *Trichoderma koningiopsis* impregnated with copper were confirmed by the transmission electron microscope (TEM) as having a mean diameter of 87.5 nm, with a majority of them being round shaped, and few aggregates of NPs having a mean diameter of 328.27 nm. The bottom of the copper nanoparticle was covered with a capping fabric made of proteins, as revealed by the energy-dispersive and x-ray photoelectron spectra.

10.5.1 Microorganisms used in bioremediation

Microorganisms play a vital part in the dietary process and are a dominant part of life's organic stability. Bioremediation is the process of removing contaminated substances with the service of multicellular organisms such as yeast, fungi, and algae. Microbes can expand in subzero temperatures and maximum conditions of heat in the presence of hazardous compounds or streams of waste. The changeability of microbes and properties that are machinable make them fit for remediation. The activity of microorganisms is determined based on the amount of carbon. In exceptional environments, the monopoly of microorganisms can also be used for bioremediation. *Achromobacter, Arthrobacter, Alcaligenes, Bacillus, Corynebacterium, Pseudomonas, Flavobacterium, Mycobacterium, Nitrosomonas, Xanthobacter*, and other microorganisms are among these (Jaiswal, 2016). Aerobic microorganisms have degradative properties to degrade complex compounds along with *Pseudomonas, Acinetobacter, Sphingomonas, Nocardia, Flavobacterium, Rhodococcus,* and *Mycobacterium*. The above-mentioned microorganisms are used to degrade pesticides, hydrocarbons, alkanes, and polyaromatic compounds. Many microorganisms use carbon from pollutants as a sole representation of strength.

Cardio microorganisms are not often used as anaerobic microorganisms. A growing hobby in cardio microorganisms used for bioremediation of fragmented compounds that are chlorinated, polychlorinated biphenyls, and de-chlorination belong to the solvents trichloroethylene and chloroform, breakdown pollutants to reduce them further.

10.6 SOIL AND GROUNDWATER BIOREMEDIATION WITH METAL NANOPARTICLES

An important natural resource that significantly helps the ecosystem is the soil. Agriculture is a major part of the economy in many countries. The area is polluted by industrial effluents, metals from miners, and home wastes as a result of anthropogenic activities. Excavation and landfilling with incineration have been used to remediate soil, but this process takes a long time and uses items to create toxic gases as well as involves months to complete (Theng & Yuan, 2008). The Green era method of bioremediation currently reduces environmental pollution and the utilization of electricity consumption (Chozhavendhan *et al.*, 2021). Pollutants are continuously released by households and businesses, mixing with the surrounding soil. Their greatest attributes are agricultural damage, chemical harm, and landfill leakage. Ex-situ soil cleaning, pump-and-deal operations, chemical oxidation, in-situ thermal treatment, reactive boundary use, and iron treatment are all examples of traditional remediation technology (Hodson, 2010). The cost of soil and groundwater cleanup is frequently quite high, and conventional methods either fail or take a very long time to be effective. Pump-and-deal techniques, according to Bezbaruah *et al.* (2009), require several years of preparation than 1–2 years of actual use.

The containment of the groundwater is indirectly impacted by methods aimed at reducing soil contamination and vice versa. Metallic nanoparticles are being used to complete small paintings on topsoil remediation. Globally, natural or metallic substances that are carcinogenic can contaminate soil and groundwater. Halogenated substances or herbicides are also frequently found in groundwater resources. Chemical pesticides and metals in wastewater can also be purified using nanoparticles. Bootharaju and Pradeep (2010) discussed that the relationship between Hg^{2+} ions and silver nanoparticles is identified with the unification of mercury into the silver nanoparticle. As a way to create nanoparticle alloys, silver nanoparticles can decrease other metals that are apparent. For instance, the development of Pd–Ag and Pt–Ag nanoparticles using the electroplating method serves as an illustration of the development of alloy nanoparticles. Through the use of nanomaterials and colorimetric techniques, several heavy metals, including lead, arsenic, and mercury have been identified. Ono and Togashi (2004) stated that the exhaustion of steel ions mixture and nucleotides in which the Hg^{2+} facilitated the creation of thymine–thymine base pairs.

Using nanomaterials to eliminate insecticides play a favorable solution in significant surroundings because pesticides are currently heavily used in agricultural operations as organophosphorus groups, leaving toxic residues in potable water. Numerous techniques have been improved with nanotechnology in recent decades to reduce the impact of insecticides on the environment. The adsorption of organophosphorus insecticides was discovered by Das *et al.* (2009) using gold nanoparticles produced by the fungus *Rhizopus oryzae*.

10.6.1 Application in environmental areas

10.6.1.1 Heavy metals removal by mycoremediation

Heavy metals are a major problem for the ecosystem because they are frequently released into the earth and groundwater (Gupta *et al.*, 2010). The finding of metals, effective treatment, and heavy metal removal from the water bodies are the more pressing environmental problems. Due to their increased discharge, acute toxicity, non-biodegradable, harm to the environment, and propensity for bioaccumulation, these metals have been a major worry for decades (Feng *et al.*, 2010). These factors could be caused by a variety of activities, including agricultural and manufacturing activities and mineral extraction (Xu *et al.*, 2012). Prominent heavy metals and lead contaminants cause neurological effects on the central nervous system, the brain, and kidneys (Southichak *et al.*, 2006; Xu *et al.*, 2012). The abundant presence of a beneficial group of fungi has an upper hand in binding heavy metals and the elimination system (Cheng *et al.*, 2015).

10.6.1.2 Textile industries effluent treatment by myconanoparticles

The textile industry's effluent water causes major contamination of the environment because of the intake of huge quantities of water consumed in complex processes discarding the difficult ones. The effluent water from the textile industries contains a dye and its by-product which causes deleterious effects like carcer (Saharan *et al.*, 2014). Around 15% of the dye used for dyeing is directly released as effluents into the water and makes them unusable (Mollea *et al.*, 2005).

10.6.1.3 Phenol removal by mycoremediation

The phenol substance, which is harmful even in low quantities, can be ingested, touched, or inhaled, is another significant environmental pollution issue (Huang *et al.*, 2015). Acute phenol exposure disrupts the central nervous system, resulting in coma and collapse (Nair *et al.*, 2008). This contaminant is typically found in contaminated rivers, lakes, and industrial effluents. This harmful pollutant is removed from these environments using microbes and nanoparticles (Martnkova *et al.*, 2009). Large concentrations of this contaminant have been discovered in effluent from coal gasification facilities, phenol polymer manufacturing facilities, and petroleum refineries.

Magnetic nanoparticles are being employed frequently in the bioremediation process to create distinct systems that are removed using a peripheral system of magnets. A biological material possesses diamagnetic properties and can enable the efficient and selective separation of materials with magnetic modifications, this capability is crucial for biological applications.

Huang *et al.* (2015) revealed removal of phenol from effluents under the influence of Fe_3O_4 nanoparticles and Pc fungus (BKMF-1767, *P. chrysosporium*). The experiment was carried out at various concentrations of Fe_3O_4 nanoparticles, phenol concentrations, and the oxalate generation process. The binding of two important ligninolytic enzymes, MnP and LiP, released by Pc (BKMF-1767), with the phenol degradation was recorded.

10.6.1.4 Treatment of radioactive pollutants

Li *et al.* (2015) investigated *Aspergillus* with Fe_3O_4 nanoparticles as a successful application in nanobiocomposites to eliminate radioactive uranium oxide ions from uranium $(UO_2)^{2+}$ polluted water. The fungus-derived iron oxide bio-nanocomposite had a better capacity for adsorbing radioactive $(UO_2)^{2+}$ ions, with a 55% increase in comparison to the fungus and a 170% increase in comparison to the Fe_3O_4 nanoparticles. The biological system can create a magnetic adsorbent with the magnetic nanoparticles attached to the fungus and allow for the separation of the fungus from water using a magnet (Li *et al.*, 2015).

Ding *et al.* (2015) carried out a similar experiment with nanocomposites that are bio-based like fungus like *Penicillium* sp. and particles of nano-size Fe_3O_4 (VI) for the elimination of the radionuclides such as U, Th (IV), and Sr (II). This technique was low-cost and during *Penicillium* sp. growth, nano- Fe_3O_4 particles are trapped on the core of the pellet mycelia. The new bio-nanocomposites of fungus- Fe_3O_4 are more economical and environmentally friendly than the other mentioned templates because they may expand evenly on the fungal wall without aggregation. According to their studies, the fungus–Fe_3O_4 has a higher adsorption capacity than *Penicillium* sp., nano- Fe_3O_4, and other adsorbents. Sr (II), U (VI), and Th (IV) had the highest sorption capacities of the fungus–Fe_3O_4 at pH's of 5.0 and 3.0, respectively.

As a result of their capacity to alter or degrade substances, as well as their role as decomposing organisms and recyclers, fungi are extensively studied. However, when combined with magnetic nanoparticles, they create novel systems that might provide exceptional processes that are often absent when simply fungus or nanoparticles are employed. These nanobiocomposites have degraded from the polluted environment using a magnetic separation technique, which makes them potentially effective in the remediation of habitats contaminated with various pollutants, as we have already seen in multiple situations. For usage in remediation contexts, inexpensive technology, quick, and large-scale approaches are crucial.

10.6.1.5 Other pollution removal

Xenobiotic compounds are persistent organic pollutants that are manmade chemicals released from various industrial and agricultural activities. The release of xenobiotic compounds creates an impact on the environment and the ecosystem through the outbreak of adverse diseases (Karthigadevi *et al.*, 2021). Activated mono-adsorbents interact with contaminants over other things at various adsorption sites to absorb the contaminants by flexible chemistry (Kurwadkar *et al.*, 2019).

10.7 CONCLUSION

The amazing potential of using fungal dead biomass as a remediation method in the synthesis of nanoparticles in contaminated areas using hazardous metals left over from industry. Biomass is utilized (biosorption based on bioremediation) through sustainable management and reduced cost of operation, which may be employed both on small and bioremediation processes expands, contributing to sustainability in various industries like galvanoplasty industries, foundry, and mining industries. This attractive commercial waste-cleaning method of fungal dead biomass biosorption can produce metallic nanoparticles in an economical and environmentally friendly way, opening up new markets for unique herbal nanotechnologies. Promising dazzling advancements have been shown alongside the effective application of nano remediation in the feature of the current review.

REFERENCES

Agnihotri M., Joshi S., Kumar A. R., Zinjarde S. and Kulkarni S. (2009). Biosynthesis of gold nanoparticles by the tropical marine yeast *Yarrowia lipolytica* NCIM 3589. *Materials Letters*, **63**, 1231–1234, https://doi.org/10.1016/j.matlet.2009.02.042

Ahmad A., Senapati S., Khan M. I., Kumar R. and Sastry M. (2005). Extra−/intracellular biosynthesis of gold nanoparticles by an alkalotolerant fungus, Trichothecium sp. *J Biomed Nanotechnol*, **1**, 47–53.

Ahmad A., Senapati S., Khan M. I., Kumar R., Ramani R., Srinivas V. and Sastry M. (2003). Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete *Rhodococcus species*. *Nanotechnology*, **14**, 824–828, https://doi.org/10.1088/0957-4484/14/7/323

Anand P., Isar J., Saran S. and Saxena R. K. (2006). Bioaccumulation of copper by Trichoderma viride. *Bioresour Technol*, **97**, 1018–1025.

Armendariz V., Herrera I. and Peralta-Videa J. R. (2004). Size controlled gold nanoparticle formation by Avena sativa biomass: use of plants in nanobiotechnology. *J Nanopart Res*, **6**(4), 377–382.

Aziz N., Pandey R., Barman I. and Prasad R. (2016). Leveraging the attributes of *Mucor hiemalis*-derived silver nanoparticles for a synergistic broad-spectrum antimicrobial platform. *Frontiers in Microbiology*, **7**, 1984, https://doi.org/10.3389/fmicb.2016.01984

Baer D. R. (2011). Surface characterization of nanoparticles: critical needs and significant challenges. *J Surf Anal*, **17**(3), 163–169.

Balaji D. S., Basavaraja S., Deshpande R., Mahesh D. B., Prabhakar B. K. and Venkataraman A. (2009). Extracellular biosynthesis of functionalized silver nanoparticles by strains of Cladosporium cladosporioides fungus. *Colloid Surf B*, **68**, 88–92.

Bansal V., Rautaray D., Ahmad A. and Sastry M. (2004). Biosynthesis of zirconia nanoparticles using the fungus Fusarium oxysporum. *J Mater Chem*, **14**, 3303–3305.

Bansal V., Rautaray D., Bharde A., Ahire K., Sanyal A., Ahmad A. and Sastry M. (2005). Fungus-mediated biosynthesis of silica and titania particles. *J Mater Chem*, **15**, 2583–2589.

Bao H., Hao N., Yang Y. and Zhao D. (2010). Biosynthesis of biocompatible cadmium telluride quantum dots using yeast cells. *Nano Res*, **3**, 481–489.

Basavaraja S., Balaji S. D., Lagashetty A., Rajasab A. H. and Venkataraman A. (2008). Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. *Mat Res Bullet*, **43**, 1164–1170.

Baskar G., Chandhuru J., Fahad K. S. and Praveen A. S. (2013). Mycological synthesis, characterization and antifungal activity of zinc oxide nanoparticles. *Asian Journal of Pharmacy*, **3**(4), 142–146.

Bezbaruah A. N., Krajangpan S., Chisholm B. J., Khan E. and Bermudez J. J. E. (2009). Entrapment of iron nanoparticles in calcium alginate beads for groundwater remediation applications. *Journal of Hazardous Materials*, **166**, 1339–1343, https://doi.org/10.1016/j.jhazmat.2008.12.054

Bedekar P. A., Saratale R., Saratale G. D. and Govindwar S. (2014). Oxidative stress response in dye degrading bacterium Lysinibacillus sp. RGS exposed to Reactive Orange 16, degradation of RO16 and evaluation of toxicity. *Environ Sci Pollut Res*, **21**(18), 11075–11085.

Bhainsa K. C. and D'souza S. F. (2006). Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. *Colloid Surf B*, **47**, 160–164.

Bharde A., Rautaray D., Bansal V., Ahmad A., Sarkar I., Yusuf S. M. and Sastry M. (2006). Extracellular biosynthesis of magnetite using fungi. *Small*, **2**, 135–141.

Birla S. S., Tiwari V. V., Gade A. K., Ingle A. P., Yadav A. P. and Rai M. K. (2009). Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. *Lett Appl Microbiol*, **48**, 173–179.

Bishnoi N. R. G. (2005). Fungus – an alternative for bioremediation of heavy metals containing wastewater: a review. *Journal of Scientific & Industrial Research*, **64**, 93–100.

Bootharaju M.S. and Pradeep T. (2010). Uptake of Toxic Metal Ions from Water by Naked and Monolayer Protected Silver Nanoparticles: An X-Ray Photoelectron Spectroscopic Investigation. *The Journal of Physical Chemistry C*, **114**, 8328–8336. https://doi.org/10.1021/jp101988h

Castro-Longoria E., Vilchis-Nestor A. R. and Avalos-Borja M. (2011). Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus Neurospora crassa. *Colloid Surf B*, **83**, 42–48.

Cheng W., Ding C., Sun Y. and Wang X. (2015). Fabrication of fungus/attapulgite composites and their removal of U (VI) from aqueous solution. *Chem Eng J*, **269**, 1–8.

Chen J. C., Lin Z. H. and Ma X. X. (2003). Evidence of the production of silver nanoparticles via pretreatment of Phoma sp. 3.2883 with silver nitrate. *Lett Appl Microbiol*, **37**, 105–108.

Chozhavendhan S., Rajamehala M., Karthigadevi G., Kumar R. P., Bharathiraja B. and Jayakumar M. (2021). Application of nanotechnology for the sustainable development of algal biofuel industries. In Nanomaterials, (pp. 401–410). Academic Press.

Dameron C. T., Reese R. N., Mehra R. K., Kortan A. R., Carroll P. J., Steigerwald M. L. *et al.* (1989). Biosynthesis of cadmium sulfide quantum semiconductor crystallites. *Nature*, **338**, 596–597, https://doi.org/10.1038/338596a0

Darroudi M., Ahmad M. B., Zamiri R., Zak A. K., Abdullah A. H. and Ibrahim N. A. (2011). Time-dependent effect in green synthesis of silver nanoparticles. *Int J Nanomedicine*, **6**(1), 677–681.

Das S. K., Das A. R. and Guha A. K. (2009). Gold nanoparticles: microbial synthesis and application in water hygiene management. *Langmuir*, **25**, 8192–8199.

Dhankhar R. and Guriyan R. B. (2011). Bacterial biosorbents for detoxification of rapid extra-/intracellular biosynthesis of gold nanoparticles by the fungus *Penicillium* sp. *Journal of Nanoparticle Research*, **13**(3), 921–930, https://doi.org/10.1007/s11051-010-0165-2

Dhillon G. S., Brar S. K., Kaur S. and Verma M. (2012). Green approach for nanoparticle biosynthesis by fungi: current trends and applications. *Crit Rev Biotechnol*, **32**, 49–73.

Ding C., Cheng W., Sun Y. and Wang X. (2015). Novel fungus-Fe_3O_4 bio-nanocomposites as high performance adsorbents for the removal of radionuclides. *J Hazard Mater*, **295**, 127–137.

Du L., Xian L. and Feng J.-X. (2011). Rapid extra-/intracellular biosynthesis of gold nanoparticles by the fungus Penicillium sp. *J Nanopart Res*, **13**(3), 921–930.

Fatima F., Verma S. R., Pathak N. and Bajpai P. (2016). Extracellular mycosynthesis of silver nanoparticles and their microbicidal activity. *Journal of Global Antimicrobial Resistance*, **7**, 88–92, https://doi.org/10.1016/j.jgar.2016.07.013

Fayaz M., Tiwary C. S., Kalaichelvan P. T. and Venkatesan R. (2010). Blue orange light emission from biogenic synthesized silver nanoparticles using Trichoderma viride. *Colloid Surf B*, **75**, 175–178.

Feng Y., Gong J. L., Zeng G. M., Niu Q. Y., Zhang H. Y., Niu C. G., Deng J. H. and Ming Y. (2010). Adsorption of Cd (II) and Zn (II) from aqueous solutions using magnetic hydroxyapatite nanoparticles as adsorbents. *Chem Eng J*, **162**(2), 487–494.

Gad G. M. (2010). Metals, minerals, and microbes: geomicrobiology and bioremediation. *Microbiology*, **156**, 609–643, https://doi.org/10.1099/mic.0.037143-0

Gade A. K., Bonde P., Ingle A. P., Marcato P. D., Duran N. and Rai M. K. (2008). Exploitation of Aspergillus niger for synthesis of silver nanoparticles. *J Biobase Mat Bioenerg*, **2**, 243–247.

Gardea-Torresdey J. L., Tiemann K. J., Gamez G., Dokken K. and Pingitore N. E. (1999). Recovery of gold (III) by alfalfa biomass and binding characterization using X-ray microfluorescence. *Adv Environ*, **3**(1), 83–93.

Gupta V. K., Rastogi A. and Nayak A. (2010). Adsorption studies on the removal of hexavalent chromium from aqueous solution using a low cost fertilizer industry waste material. *J Colloid Interface Sci*, **342**(1), 135–141.

Hanif M. A., Nadeem R., Bhatti H. N., Ahmad M. R. and Ansari T. M. (2007). Nickel biosorption by *Cassia fistula* (golden shower) biomass. *Journal of Hazardous Materials*, **139**, 345–355, https://doi.org/10.1016/j.jhazmat.2006.06.040

Han R., Zhang J., Zou W., Xiao H., Shi J. and Liu H. (2006). Biosorption of copper (II) and lead (II) from aqueous solution by chaff in a fixed-bed column. *J Hazard Mater*, **133**, 262–268.

Hodson M. E. (2010). The need for sustainable soil remediation. *Elements*, **6**, 363–336, https://doi.org/10.2113/gselements.6.6.363

Honary S., Barabadi H., Gharaei-Fathabad E. and Naghibi F. (2013). Green synthesis of silver nanoparticles induced by the fungus Penicillium citrinum. *Trop J Pharm Res*, **12**, 7–11.

Honary S., Barabadi H. and Gharaei-Fathabad N. F. (2012). Green synthesis of copper oxide nanoparticles using Penicillium aurantiogriseum, Penicillium citrinum and Penicillium waksmanii. *Dig J Nanomater Bios*, **7**, 999–1005.

Huang D. L., Wang C., Xu P., Zeng G. M., Lu B. A., Li N. J., Huang C., Lai C., Zhao M. H., Xu J. J. and Luo X. Y. (2015). A coupled photocatalytic-biological process for phenol degradation in the Phanerochaete chrysosporium-oxalate-Fe_3O_4 system. *Int Biodeterior Biodegrad*, **97**, 115–123.

Ingle A., Rai M., Gade A. and Bawaskar M. (2009). *Fusarium solani*: a novel biological agent for the extracellular synthesis of silver nanoparticles. *Journal of Nanoparticle Research*, **11**, 2079–2085, https://doi.org/10.1007/s11051-008-9573-y

Ingle A., Gade A., Pierrat S., Sonnichsen C. and Rai M. (2008). Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. *Curr Nanosci*, **4**, 141–144.

Kar P. K., Murmu S., Saha S., Tandon V. and Acharya K. (2014). Anthelmintic Efficacy of Gold Nanoparticles Derived from a Phytopathogenic Fungus, *Nigrospora oryzae*. *PLoS ONE*, **9**, e84693, https://doi.org/10.1371/journal.pone.0084693

Kashyap P. L., Kumar S., Srivastava A. K. and Sharma A. K. (2013). Myconanotechnology in agriculture: a perspective. *World Journal of Microbiology and Biotechnology*, **29**(2), 191–207, https://doi.org/10.1007/s11274-012-1171-6

Kapoor A. and Viraraghavan T. (1996). Biosorption of heavy metals on fungal biomass. Proceeding, 4th CSCE Environmental Engineering Specialty Conference (Edmonton, Alberta), pp. 333–343.

Kitching M., Ramani M. and Marsili E. (2015). Fungal biosynthesis of gold nanoparticles: mechanism and scale up. *Microbial Biotechnology*, **8**, 904–917, https://doi.org/10.1111/1751-7915.12151

Kogej A. and Pavko A. (2001). Laboratory experiments of lead biosorption by self-immobilized *Rhizopus nigricans* pellets in the batch stirred tank reactor and the packed dead column. *Chemical and Biochemical Engineering*, **15**, 75–79.

Kowshik M., Ashtaputre S., Kulkani S. K. and Parknikar K. M. M. (2003). Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. *Nanotechnology*, **14**, 95–100.

Kuber C., Bhainsa S. F. and Souza D. (2006). Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. *Colloids and Surfaces B: Biointerfaces*, **47**, 160–164, https://doi.org/10.1016/j.colsurfb.2005.11.026

Kango S., Kalia S., Celli A., Njuguna J., Habibi Y. and Kumar R. (2013). Surface modification of inorganic nanoparticles for development of organic–inorganic nanocomposites-a review. *Prog Polym Sci*, **38**(8), 1232–126.

Karthigadevi G., Manikandan S., Karmegam N., Subbaiya R., Chozhavendhan S., Ravindran B. and Awasthi M. K. (2021). Chemico-nanotreatment methods for the removal of persistent organic pollutants and xenobiotics in water–A review. *Bioresource Technology*, **324**, 124678.

Kathiresan K., Alikunhi N. M., Pathmanaban S., Nabikhan A. and Kandasamy S. (2010). Analysis of antimicrobial silver nanoparticles synthesized by coastal strains of Escherichia coli and Aspergillus niger. *Can J Microbiol*, **56**, 1050–1059.

Kathiresan K., Manivannan S., Nabeel M. A. and Dhivya B. (2009). Studies on silver nanoparticles synthesized by a marine fungus, Penicillium fellutanum isolated from coastal mangrove sediment. *Colloid Surf B*, **71**, 133–137.

Khan A., Rashid R., Murtaza G. and Zahra A. (2014). Gold nanoparticles: synthesis and applications in drug delivery. *Trop J Pharm Res*, **13**(7), 1169–1177.

Kumar R. R., Priyadharsani K. P. and Thamaraiselvi K. (2012). Mycogenic synthesis of silver nanoparticles by the Japanese environmental isolate Aspergillus tamarii. *J Nanopart Res*, **14**, 1–7.

Kurwadkar S., Hoang T. V., Malwade K., Kanel S. R., Harper W. F. and Struckhoff G. (2019). Application of carbon nanotubes for removal of emerging contaminants of concern in engineered water and wastewater treatment systems. *Nanotechnology for Environmental Engineering*, **4**, 1–16.

Lengke M. F., Sanpawanitchakit C. and Southam G. (2011). Biosynthesis of gold nanoparticles: a review. In: Rai M. K., Duran N. (eds) Metal nanoparticles in microbiology. Springer, New York, pp. 37–74.

Lim H. A., Mishra A. and Yun S. I. (2011). Effect of pH on the extra cellular synthesis of gold and silver nanoparticles by Saccharomyces cerevisae. *J Nanosci Nanotechnol*, **11**, 518–522.

Li G., He D., Qian Y., Guan B., Gao S., Cui Y. and Wang L. (2011). Fungus-mediated green synthesis of silver nanoparticles using Aspergillus terreus. *Int J Mol Sci*, **13**, 466–476.

Li L., Xu M., Chubik M., Chubik M., Gromov A., Wei G. and Han W. (2015). Entrapment of radioactive uranium from wastewater by using fungus-Fe_3O_4 bio-nanocomposites. *RSC Adv*, **5**(52), 41611–41616.

Lodhia J., Mandarano G., Ferris N. J., Eu P. and Cowell S. F. (2010). Development and use of iron oxide nanoparticles (part 1): synthesis of iron oxide nanoparticles for MRI. *Biomed Imaging Inter J*, **6**(2), e12.

Malik A. (2004). Metal bioremediation through growing cells. *Environ Int*, **30**, 261–278.

Maliszewska I., Juraszek A. and Bielska K. (2014). Green synthesis and characterization of silver nanoparticles using ascomycota fungi Penicillium nalgiovense AJ12. *J Clust Sci*, **25**, 989–1004.

Martínkova L., Uhnakova B. and Patek M. (2009). Biodegradation potential of the genus Rhodococcus. *Environ Int*, **35**(1), 162–177.

Masciangioli T. and Zhang W. X. (2003). Peer reviewed: environmental technologies at the nanoscale. *Environ. Sci. Technol.*, 2003, **37**(5), 102A–108A.

Merrin J. S., Sheela R., Saswathi N., Prakasham R. S. and Ramakrishna S. V. (1998). Biosorption of chromium (VI) using *Rhizopus arrhizus*. *Indian Journal of Experimental Biology*, **36**, 1052–1055.

Mollea C., Bosco F. and Ruggieri B. (2005). Fungal biodegradation of naphthalene: microcosmos studies. *Chemosphere*, **60**(5), 636–643.

Moghaddam A. B., Namvar F., Moniri M., Tahir P. M., Azizi S. and Mohamad R. (2015). Nanoparticles biosynthesized by fungi and yeast: a review of their preparation, properties, and medical applications. *Molecules*, **20**, 16540–16565, https://doi.org/10.3390/molecules200916540

Moghaddam K. M. (2010). An introduction to microbial metal nanoparticle preparation methods. *Young Investig*, **19**, 1–7.

Mukherjee P., Senapati S., Mandal D., Ahmad A., Khan M. I., Kumar R. and Sastry M. (2002). Extracellular synthesis of gold nanoparticles by the fungus Fusarium oxysporum. *Chem Biol Chem*, **3**, 461–463.

Mukherjee R., Paul T., Soren J. P., Halder S. K., Mondal K. C., Pati B. R. *et al.* (2019). Acidophilic α-amylase production from Aspergillus Niger RBP7 using potato peel as substrate: a waste to value added approach. *Waste Biomass Valori*, **10**(4), 851–863.

Mukherjee P., Ahmad A., Mandal D., Senapati S., Sainkar S. R., Khan M. I., Parishcha R., Ajaykumar P. V., Alam M., Kumar R. and Sastry M. (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nano Letters*, **1**, 515–519, https://doi.org/10.1021/nl0155274

Mukherjee, Roy M., Mandal B. P., Dey G. K., Mukherjee P. K., Ghatak J. and Kale S. P. (2008). Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus T. asperellum. *Nanotechnology*, **19**(7), 075103.

Mishra A., Tripathy S. K. and Yun S. I. (2011). Biosynthesis of gold and silver nanoparticles from *Candida guilliermondii* and their antimicrobial effect against pathogenic bacteria. *Journal of Nanoscience and Nanotechnology*, **11**, 243–248, https://doi.org/10.1166/jnn.2011.3265

Nair C. I., Jayachandran K. and Shashidhar S. (2008). Biodegradation of phenol. *Afr J Biotechnol*, **7**(25), 4951–4958.

Nanda A. and Majeed S. (2014). Enhanced antibacterial efficacy of biosynthesized AgNPs from Penicillium glabrum (MTCC1985) pooled with different drugs. *Int J Pharm Tech Res*, **6**, 217–223.

Nayak R. R., Pradhan N., Behera D., Pradhan K. M., Mishra S., Sukla L. B. and Mishra B. K. (2011). Green synthesis of silver nanoparticle by Penicillium purpurogenum NPMF: the process and optimization. *J Nanopart Res*, **13**, 3129–3137.

Nithya R. and Ragunathan R. (2009). Synthesis of silver nanoparticle using Pleurotus sajor caju and its antimicrobial study. *Digest J Nanomater Biostructur*, **4**, 623–629.

Ono A. and Togashi H. (2004). Highly selective oligonucleotide-based sensor for mercury(II) in aqueous solutions. *Angew Chem Int*, Ed **43**, 4300–4302.

Ozer A. and Ozer D. (2003). Comparative study of the biosorption of Pb (II), Ni (II) and Cr (VI) ions onto *S. cerevisiae*: determination of biosorption hats. *Journal of Hazardous Materials*, **100**, 219–229, https://doi.org/10.1016/S0304-3894(03)00109-2

Pavani K. V., Kumar N. S. and Sangameswaran B. B. (2012). Synthesis of lead nanoparticles by Aspergillus species. *Pol J Microbiol*, **61**, 61–63.

Prasad K. and Jha A. K. (2010). Biosynthesis of CdS nanoparticles: an improved green and rapid procedure. *Journal of Colloid and Interface Science*, **342**(1), 68–72, https://doi.org/10.1016/j.jcis.2009.10.003

Prasad R. (2016). Advances and Applications Through Fungal Nanobiotechnology. Springer International Publishing, Springer Cham (ISBN: 978-3-319-42989-2), https://link.springer.com/book/10.1007/978-3-319-68424-6

Prasad R. (2017). Fungal nanotechnology: applications in agriculture, industry, and medicine. (978-3-319-68957-9)

Priyadarshini E., Pradhan N., Sukla L. B. and Panda P. K. (2014). Controlled synthesis of gold nanoparticles using Aspergillus terreus IF0 and its antibacterial potential against Gram negative pathogenic bacteria. *J Nanotechnol*, **2014**, 1–9. http://dx.doi.org/10.1155/2014/653198

Saharan P., Chaudhary G. R., Mehta S. K. and Umar A. (2014). Removal of water contaminants by iron oxide nanomaterials. *J Nanosci Nanotechnol*, **14**(1), 627–643.

Salvadori M. R., Lepre L. F., Ando R. A., Nascimento C. A. O. and Corrêa B. (2013). Biosynthesis and uptake of copper nanoparticles by dead biomass of *Hypocrea lixii* isolated from the metal mine in the Brazilian Amazon region. *PLoS One*, **8**, e80519, https://doi.org/10.1371/journal.pone.0080519

Salvadori M. R., Ando R. A., Nascimento C. A. O. and Corrêa B. (2015). Extra and intracellular synthesis of nickel oxide nanoparticles mediated by dead fungal biomass. *PLoS One*, **5**, 1–15.

Sarkar J., Chattopadhyay D., Patra S., Deo S. S., Sinha S., Ghosh M. and Acharya K. (2011). Alternaria alternata mediated synthesis of protein capped silver nanoparticles and their genotoxic activity. *Dig J Nanomat Biostruct*, **6**, 563–573.

Sastry M., Ahmad A., Khan M. I. and Kumar R. (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Curr Sci*, **85**(2), 162–170.

Southichak B., Nakano K., Noura M., Chiba N. and Nushimura O. (2006). Phragmites austral: a novel biosorbent for the removal of heavy metals from aqueous solution. *Water Res*, **40**(12), 2295–2302.

Salvadori M. R., Ando R. A., do Nascimento C. A. O. and Corrêa B. (2014a). Bioremediation from wastewater and extracellular synthesis of copper nanoparticles by the fungus *Trichoderma koningiopsis*. *Journal of Environmental Science and Health, Part A*, **49**, 1286–1295, https://doi.org/10.1080/10934529.2014.910067

Salvadori M. R., Ando R. A., do Nascimento C. A. O. and Corrêa B. (2014b). Intracellular biosynthesis and removal of copper nanoparticles by dead biomass of yeast isolated from the wastewater of a mine in the Brazilian Amazonia. *PLoS One*, **9**, e87968, https://doi.org/10.1371/journal.pone.0087968

Salvadori M. R., Ando R. A., Muraca D., Knobel M., Nascimento C. A. O. and Corrêa B. (2016). Magnetic nanoparticles of Ni/NiO nanostructured in film form synthesized by dead organic matrix of yeast. *RSC Advances*, **6**, 60683–60692, https://doi.org/10.1039/C6RA07274G

Sari A., Tuzen M., Uluuozlu O. D. and Soylak M. (2007). Biosorption of Pb (II) and Ni (II) from aqueous solution by lichen (*Cladonia furcata*) biomass. *Biochemical Engineering Journal*, **37**, 151–158, https://doi.org/10.1016/j.bej.2007.04.007

Shelar G. B. and Chavan A. M. (2014). Extracellular biological synthesis, characterization and stability of gold nanoparticles using the fungus *Helminthosporium tetramera*. *International Journal of Pure & Applied Bioscience*, **2**(3), 281–285.

Srivastava S. K., Yamada R., Ogino C. and Condo A. (2013). Biogenic synthesis and characterization of gold nanoparticles by *Escherichia coli* K12 and its heterogeneous catalysis in degradation of 4-nitrophenol. *Nanoscale Research Letters*, **8**, 70, https://doi.org/10.1186/1556-276X-8-70

Syed A. and Ahmad A. (2012). Extracellular biosynthesis of platinum nanoparticles using the fungus *F. oxysporum*. *Colloids and Surfaces B: Biointerfaces*, **97**, 27–31, https://doi.org/10.1016/j.colsurfb.2012.03.026

Sanghi R. and Verma P. A. (2009). A facile green extracellular biosynthesis of CdS nanoparticles by immobilized fungus. *Chemical Engineering Journal*, **155**, 886–891, https://doi.org/10.1016/j.cej.2009.08.006

Rai M., Ingle A. P., Gade A. and Duran N. (2014). Synthesis of silver nanoparticles by Phoma gardenia and in vitro evaluation of their efficacy against human disease-causing bacteria and fungi. *IET Nanobiotechnol*, **9**, 71–75.

Ranjbar-Navazi Z., Pazouki M. and Halek F. S. (2010). Investigation of culture conditions for biosynthesis of silver nanoparticles using Aspergillus fumigatus. *Iran J Biotechnol*, **8**, 56–61.

Riddin T. L., Gericke M. and Whiteley C. G. (2006). Analysis of the inter-and extracellular formation of platinum nanoparticles by Fusarium oxysporum f. sp. lycopersici using response surface methodology. *Nanotechnology*, **17**, 3482

Theng B. K. and Yuan G. (2008). Nanoparticles in the soil environment. *Elements*, **4**, 395–399, https://doi.org/10.2113/gselements.4.6.395

Tikariha S., Singh S. S., Banerjee S. and Vidyarthi A. S. (2012). Biosynthesis of gold nanoparticles, scope and application: a review. *Int J Pharm Sci Res*, **3**(6), 1603–1615.

Vala A. K., Shah S. and Patel R. (2014). Biogenesis of silver nanoparticles by marine derived fungus Aspergillus flavus from Bhavnagar coast, Gulf of Khambhat, India. *J Mar Biol Oceanogr*, **3**, 1–3.

Varshney R., Mishra A. N., Bhadauria S. and Gaur M. S. (2009). A novel microbial route to synthesize silver nanoparticles using fungus Hormoconis resinae. *Digest J Nanomater Biostruct*, **4**, 349–355.

Vaseashta A., Vaclavikova M., Vaseashta S., Gallios G., Roy P. and Pummakarnchana O. (2007). Nanostructures in environmental pollution detection, monitoring, and remediation. *Science and Technology of Advanced Materials*, **8**, 47–59, https://doi.org/10.1016/j.stam.2006.11.003

Verma V. C., Kharwar R. N. and Gange A. C. (2010). Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus Aspergillus clavatus. *Nanomedicine*, **5**, 33–40.

Veglio F. and Beolchini F. (1997). Removal of metals by biosorption: a review. *Hydrometallurgy*, **44**, 301–316, https://doi.org/10.1016/S0304-386X(96)00059-X

Vigneshwaran N., Kathe A. A., Varadarajan P. V., Nachane R. P. and Balasubramanya R. H. (2006). Biomimetics of silver nanoparticles by white rot fungus, Phaenerochaete chrysosporium. *Colloid Surf B*, **53**, 55–59.

Volesky B. (2022). Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy*, **59**, 203–216, https://doi.org/10.1016/S0304-386X(00)00160-2

Xu P., Zeng G. M., Huang D. L., Lai C., Zhao M. H., Wei Z., Li N. J., Huang C. and Xie G. X. (2012). Adsorption of Pb (II) by iron oxide nanoparticles immobilized Phanerochaete chrysosporium: equilibrium, kinetic, thermodynamic and mechanisms analysis. *Chem Eng J*, **203**, 423–431.

doi: 10.2166/9781789063783_0211

Chapter 11

Rhizoremediation of organic emerging soil contaminants: green technology

Prasann Kumar[1]***, *Duppala Hema Latha*[1] *and Joginder Singh*[2]

[1]Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara 144411, Punjab, India
[2]Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144 411, Punjab, India
**Corresponding author: prasann0659@gmail.com*

ABSTRACT

For a very long time there has been interest in creating in situ techniques for remediation of natural contaminants. Rhizoremediation, a specific type of phytoremediation that includes the two plants and their related rhizosphere microorganisms, can take place naturally or can be incited by intentionally presenting specific microorganisms. These microorganisms degrade contaminants and can also promote plant development under stress conditions. Currently, improvement is related to the rapid pace of urbanization and steady increase in population, which must be considered. These cycles lead to soil contamination, which is a result of dumping hazardous wastes into the environment. Any type of hazardous component such as radiation and sound or different resources like heavy metals or particulate substance can be termed a contaminant. Among several contaminants, natural contaminants attract more attention as they are highly water repellent and are highly toxic. Owing to these properties, the contaminants are capable of being embedded in the established environment for longer periods of time, which further increases their risk of causing damage to our environment. This study is focussed on the rhizoremediation process that can be used to clean up natural toxins from the soil.

Keywords: heavy metals, rhizosphere, plants, microorganism, sustainability, no poverty, zero hunger

11.1 INTRODUCTION

It is important to note that natural contaminants have a tremendously large range, and include: hydrocarbons, various solvents, oil-based products, pesticides, and herbicides (Oleszczuk, 2006; MacKinnon & Duncan, 2013; Moore *et al.*, 2006). Meager amounts of these contaminants are able to change the properties of soil by altering the microbial population as well as the metabolic changes that are executed with it. In spite of the fact that there are conventional methods to degrade these pollutants, the advancements are exorbitant, which further deters their emanating release (Alkorta & Garbisu, 2001). In order to eliminate natural toxins from contaminated soil, bioremediation not only provides an excellent solution but also is eco-friendly and practical; however, it has certain limitations which

The chapter is from the book *Clean Technologies Towards a Sustainable Future: Physicochemical, Biochemical and Biotechnological Approaches*, Pradeep Verma, Maulin P. Shah (Editors).

must be taken into account (Glazer & Nikaido, 1995). As an alternative to bioremediation, a part of it is called rhizoremediation, which is used to handle the limitations of bioremediation. In rhizoremediation, plants remove contaminants from soil with the assistance of organisms that live in the rhizospheres in order to remediate soil contamination. (Oberai & Khanna, 2018). By providing a carbon source through their root exudates, plants serve as an ecosystem similar to a carbon source for all organisms (Cannon & Bowles, 1962). Microorganisms are responsible for the debasement process, and the buildups are taken up by plants via water as media. Seeds and microorganisms exist in the soil, and also environmental toxins are observed in soil. In 2001, United Nations Environment Program (UNEP) made a distinction among modern synthetics, pesticides, and side effects of the soil, which are classified into three categories that include numerous natural toxins in the soil. Polychlorinated biphenyls (PCBs) have been included in the class of modern synthetic substances, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans have been placed under the result class. Natural contaminants are found in the soil in a variety of forms, but polycyclic aromatic hydrocarbons (PAHs) and PCBs attract a great deal of attention due to the unpredictable nature of their presence and the fact that they can be found in soil in high concentrations. There are two types of sand: those that repel water and those that accumulate water at an exceptionally slow pace (Fabietti *et al.*, 2010; Gupta *et al.*, 2018; Ma *et al.*, 2009; Zhang *et al.*, 2007). In the environment, PAHs can be introduced by burning natural materials, which results in a build-up of PAHs. The PAHs that are released due to ignition can either be caused by natural situations or by manmade intervention, whereas PCBs, on the other hand, can only be caused by human activity and are exclusively anthropogenic in nature. (Cachada *et al.*, 2012). As a result of an increase in population, the use of fertilizers and pesticides has also increased causing contamination of soil. Initially, the use of composts and pesticides proved beneficial but later showed adverse effects on the soil. (Merrington *et al.*, 2002). Globally, more than two million tons of chemical pesticides are being used on an annual basis. Atapattu and Kodituwakku (2009) report that South Asia uses a large amount of pesticides, with 14% of the country's land located in rural areas and the use of synthetically synthesized pesticides at a high rate. Prevalence of weeds in the countryside can cause a loss of anywhere between 37% and 79% in rural areas; in order to counteract this colossal loss, ranchers apply herbicides and weedicides in order to eliminate weeds (Behera & Singh, 1999). A majority of farmers rely on insecticides to avoid the adverse effects of weeds, bugs, and also ants in fields (Ross, 2005). Several studies have shown that these blended synthetics are less used in developed nations, however, they are still used at high rates in developing nations such as India (Eqani *et al.*, 2011; Syed & Malik, 2011; Tariq *et al.*, 2007). Pesticides and insecticides are extensively used in India for controlling vermin and insects that are harmful to the environment. As part of its efforts to achieve this goal, India began developing Benzene hexachloride (BHC) and dichloro diphenyl trichloroethane (DDT) in 1952, and the development of these synthetic compounds has continued uninterrupted since that time. According to Gupta (2004), it has been noted that India alone delivered over 5000 metric tons of pesticides in 1958, including BHC and DDT, which were two of the most prominent pesticides that were shipped out of the country. There were an estimated 145 pesticides added to the market during mid-1990s, which resulted in almost 85 000 metric tons of waste. As individuals become increasingly aware of what they are using and consuming, they are causing a decrease in the use of artificially orchestrated pesticides; ranchers are now moving toward greener alternatives like plant-derived products and other options (such as biopesticides), which are causing a decline in the use of artificially orchestrated pesticides. Currently, India produces colossal amounts of pesticides that places it in the highest position in Asia and in the twelfth position globally when it comes to pesticide production. It has been estimated that India produces 90 000 tons of pesticides annually, which is a staggering amount when looking at the global scenario. Pesticides are produced in India as well as other products that are exported to different countries (Pozo *et al.*, 2011). As it relates to toxins, there are different classifications that include synthetic compounds that are more likely to remain in the environment after they have been applied as well as those that tend to bioaccumulate and spread for an extremely long distance. The potential of bioaccumulation and spreading of industrial and natural contaminants is also known as persistent organic pollutants (POPs) (Buccini, 2003). According to some scientists, POPs have been found elsewhere in other places as well, where pesticides have not been used. It might be due to the fact that they have a dispersing property, which they share with two natural pollutants found in soil and their sources, rainfall and wind (Barra *et al.*, 2005; Zhang *et al.*, 2008). Vos *et al.* (2000) reported that a significant portion of the mixtures within the POP category are capable of altering the ability of chemicals by upsetting the subatomic structure of the compounds in order to alter their properties. The purpose of conducting such an examination is to make individuals more aware of the severity of the effects that these synthetic compounds have on the environment as a whole as a result of the investigation. Approximately 17 synthetic substances that fall under the POP classification have been identified by the UNEP as pesticides, modern synthetic chemicals, and hazardous substances that may cause adverse health effects to humans. In the UNEP's fourth meeting, which was held in 2009, a few more synthetic substances were added to the POP list in every classification. In its subsequent meeting in 2011, endosulfan and its connected isomer were also added to the list of POP chemicals. Despite the fact that the use of these synthetic compounds has been banned some time ago due to their harmful effects on the environment and on human health, they are still widely used in some countries throughout the world, namely in countries in South Asia, despite these restrictions. The use of DDT is routinely used in both India and Pakistan to control mosquitoes, as evidenced by several studies (Alamdar *et al.*, 2014; Chakraborty *et al.*, 2010; Eqani *et al.*, 2011; Syed & Malik, 2011; Syed *et al.*, 2013, 2014; Zhang *et al.*, 2008). Since POPs are capable of irreversible dissipation, they might have the capability of polluting immaculate regions of the earth, such as the South Pole and the North Pole, if they are not controlled. South Asian nations, according to Minh *et al.* (2006), lack adequate facilities to deal with metropolitan waste; in an effort to dispose of waste, these nations rely on open waste disposal sites that are inadequately equipped to handle the waste. As a result of this study, Syed and Malik (2011) have focused on a few areas of Pakistan where processing plants and storage facilities that contained pesticides like DDT have been closed. As a result of this study, it was demonstrated that spill over from these places can lead to contamination of surrounding soil and water bodies. It has been found that similar types of studies have been conducted by various researchers instructing individuals to consider the possible health risks for people living in impacted regions (Agusa *et al.*, 2003).

11.2 DESTINY OF NATURAL CONTAMINATIONS IN SOIL

In case natural contamination enters the biological system and becomes part of it, the development of this contamination within the biological system is determined by the synthetic nature of the contamination; on the whole, if the substance is biodegradable, it will eventually become mineralized with the passage of time; however, if it is not biodegradable, then it will likely persist in the environment. When everything works out over time, it is possible to calculate the convergence of contaminations stored in the system by estimating how similar the groups of dregs are. Bhattacharya *et al.* (2003) as well as Guzzella *et al.* (2005) have demonstrated that these compartments play important roles in the accumulation of these toxins. It is important to note that there are different types of dregs (natural, unnatural, and inorganic) that come from a variety of different sources and are composed of different elements. The mechanisms that are responsible for incorporating bioaccumulative and polycyclic aromatic hydrocarbons into the residual as well as vaporization, surface overflow, and drainage are those that are responsible for incorporating them into the residual (Sarkar *et al.*, 2008). As a result of the fact that POPs are capable of degrading slowly, it is easy to detect POPs by focusing on silt since they have such a low degrading potential in comparison with other pollutants (El Nemr *et al.*, 2013).

It has been reported that out of the various natural synthetic substances that are present in the soil in the Indian scenario, pesticides are the most widely known. They are used in rural areas to protect crops from pests, as well as in civil areas to prevent diseases by controlling bugs that may lead to disease. As a result, it should be possible to observe high levels of contaminants in a few places within a few radii of home-grown and modern trash (Pozo *et al.*, 2011) that are located on the edges of those release points. Ali *et al.* (2014) studied residues found in river Gomti, India, where the author observed that hexachlorocyclohexane (HCH) and DDT concentrations were very high (BDL-81.2 mg/g). The east and west shorelines of India were also examined by the researcher, where concentrations of HCHs and DDTs were detected as well (BDL 109 mg/g). The same can be said about Pandey *et al.* (2011), who focused on the residues from river Ganga and parts of different waterways associated with it in order to observe the progression of the centralization of POPs through different seasons, as well as in different waterways. There was a study in which OCP centralization was recorded during a storm, before a rainstorm (158e308 ng/g), and after a rainstorm (307e844 ng/g) seasons. It was concluded that the significant cause of DDT contamination in the silt was the endured and utilized soils from farming fields. The study by Malik *et al.* (2009) explains that the ill-conceived use of DDT may also have contributed to its extreme taint in riverine regions. Bhattacharya *et al.* (2003) observed elevated levels of DDTs, HCHs, and endosulfan sulfate in the air before the storm season. As a result, the centralization of the above was tracked and arranged as follows: 1. HCH, 2. N-endosulfan sulfate, 3. DDTs, 4. a-endosulfan. This shows that DDT is degraded through a dehydrochlorination process, which may be due to decay of nonliving or living organisms. Elevated degree of pesticides in certain regions is due to the after effect from horticultural lands, domestic wastes, and hazardous disposables. Khwaja *et al.* (2006) studied samples of soil, additional ingredients, mortar tests of plant walls, and harmed sacks due to ban of DDT production in Pakistan. Disposed of packs were found to have the most elevated convergence of DDT (2822e2841 mg/g). According to Khwaja *et al.*, 91% of the soil samples collected after one year had been profoundly contaminated with DDT, with the majority of the soil samples having DDT deposits over the permissible limit of 0.05 mg/g in soil, which is the limit at which DDT can be used in soil. The study by Jan *et al.* (2009) conducted in the same region found a similar type of contamination around the plant. The study found that 500 m of the area surrounding the plant were still contaminated with DDT deposits. Based on their research, the authors found that the distance and grouping of DDT metabolites, such as p', p'-DDT, from the fixed assembling unit had diminished with the depth and distance from the fixed assembly unit of the DDT metabolites. Yadav *et al.* (1981) discovered that the soil around a DDT-combining industry in Delhi had a high concentration of DDT, and that night crawlers were contaminated with DDT. The results of this study provided evidence that a region that is close to DDT-combining factories has an elevated concentration of DDT in its soil.

11.3 RHIZOREMEDIATION: A TRADITIONAL METHODOLOGY

Rhizoremediation is a method of removing soil impurities by utilizing microorganisms from the rhizosphere to degrade them and therefore degrade soil impurities. A rhizodegradation process would also be called an organism-assisted phytoremediation innovation, or an organism-assisted bioremediation process. It is widely accepted that the rhizosphere is one of the most dynamic areas around roots (i.e., length of 2 mm) impacted due to plant action (Brink, 2016; Dzantor, 2007; Liu *et al.*, 2014). In a majority of cases, it is the rhizospheric microorganisms that are the principal supporters, while the plants provide a carbon source that is essential for the rhizospheric microorganisms to degrade contaminants. Hence, microorganisms can provide plants with supplements that will help to improve their development, protect the plants from microbes, degrade impurities, as well as diminish the degree of stress chemicals produced by the plants (Macek *et al.*, 2000; Qixing *et al.*, 2011). It is important to note that study on rhizospheric microbial contamination has been focused on ensuring the safety of flora from agrochemicals (Hoagland *et al.*, 1994; Jacobsen, 1997; Zablotowicz *et al.*, 1994). According to the available evidence, there are only a few cases that can be attributed to degrading natural mixtures, like PAHs (Radwan *et al.*, 1995), trichloroethylene (Walton & Anderson, 1990), and PCBs (Brazil *et al.*, 1995), to the degradation of natural contaminants. In order to reach an accurate diagnosis, it was important to dissect the nitty gritty of these examinations and the arrangement of the microorganisms. In addition, there are no data available regarding the expansion, endurance, and movement of the microbial population within the rhizosphere. As a result of different investigations, it was found that leguminous plants are best for rhizoremediation (Kuiper *et al.*, 2001; Qiu *et al.*, 1994; Shann & Boyle, 1994). The variables that impact the outcome of rhizoremediation are the natural connections with different living beings, the essential and optional digestion, endurance, and the foundation of the system. It has been shown that the development of bacteria in the rhizosphere is largely influenced by root exudation, and this is considered to be the most important impetus for the development of rhizoremediation (Martin *et al.*, 2014; Phillips *et al.*, 2012). A significant part of plant root exudates are mixtures of sugars, amino acids, and natural acids that are present in the oozes (Vancura & Hovadik, 1965). Despite these mixtures as an add up to the root cells, adhesive discharge through cells of roots, the rot of entire roots, or the hunger of cells of roots, additional supplements can also be derived (Lugtenberg & de Weger, 1992; Lynch & Whipps, 1990; Rovira, 1956). In order to isolate root exudates according to the process of their creation, they can be divided into four distinct groups, auxiliary floral metabolites, lysates (from senescent tissues and roots), detached exudations, and adhesives (tip of root and epidermis). A study has demonstrated that carbohydrate components with a low subatomic weight (LMW), auxiliary metabolites, AAs, and natural anions which are soluble in water like components of oozes of roots, while natural polymers, on the other, have been considered insoluble components (Martin *et al.*, 2014; Walker *et al.*, 2003a, 2003b). At the time of their metabolic cycle, soil organisms use these mixtures of carbon and energy as a carbon and energy source to fuel their metabolic activity (Chaudhry *et al.*, 2005). It is believed that root exudates should be responsible for causing a change in the microbial local environment of tainted soils in two unique ways: first, by altering the way organisms articulate the catabolic quality of their cells, and second, by choosing the particular strains of these organisms (Baudoin *et al.*, 2003; Benizri *et al.*, 2002; Butler *et al.*, 2003; Hartmann *et al.*, 2009; Siciliano *et al.*, 2003). Biological processes such as the biodegradation of toxins like TPH are achieved by the connections between organisms and soil air circulation that are provided by plant pulls (Martin *et al.*, 2014) (Table 11.1).

Despite the controversies surrounding the role of root exudates in determining the composition of the microbiome of the rhizosphere in soils polluted with nitro-fragrance mixtures with petroleum (Siciliano *et al.*, 2003; Thijs *et al.*, 2016), there is no doubt about their significance. Miya and Firestone (2001) discovered after collecting the root exudates of the slim oat that this root exudate was able to cope with the most extreme populations of PHC-corrupting organisms, as well as help to improve the degradation

Table 11.1 In the process of rhizoremediation of pollutants, plant interactions with microbes are important.

Particulates from Organic Sources	Plants	Microbes	References
Polychlorinated biphenyl	Beetroot	*P. fluorescenes*	Brazil *et al.* (1995)
Polyacrylic aromatic hydrocarbons	Alfa	NA	Nichols *et al.* (1997)
2,4-D	Wheat	*P. putida*	Kingsley *et al.* (1994)
Total petroleum hydrocarbons	Rye	*Rhodococcus*	Ingrid *et al.* (2016)
Pyrene	*Salix*	*P. putida*	Khan *et al.* (2014)
Fixed nitrogen	Reed	*Nitrospira* sp.	El Haleem *et al.* (2000)
3-Methylbenzoate	*Zea mays*	*P. putida*	Ronchel and Ramos (2001)
2,4-D	Barley	*B. cepacian*	Jacobsen (1997)
Trichloroethylene	Wheat	*P. fluorescens*	Yee *et al.* (1998)
Polyacrylic aromatic hydrocarbons	Wheat	*Rhizophagus*	Ingrid *et al.* (2016)
Naphthalene	Grass	*P. putida*	Kuiper *et al.* (2001)
Polycyclic aromatic hydrocarbons	*Poplus*	*Micrococcus* sp.	Bisht *et al.* (2010, 2014)
Mefenoxam	*Z. augustifolia*	*P. fluorescenes*	Pai *et al.* (2001)
1,4-dioxane	*Poplar*	*Actinomyces*	Kelley *et al.* (2001)

of phenanthrene. As a result, it exposed that combination of engineered oozes from roots and mineral supplements improved the bio-deprivation fastness of 3–5 ring polycyclic aromatic hydrocarbons (Joner *et al.*, 2002). A study by Yoshitomi and Shann (2001) affirmed that the microbial mineralization of pyrene in corn roots (*Zea mays* L.) was caused by sterile exudates produced by the roots. Regardless, for the purpose of determining whether any specific mixture of carboxylates, sugars, auxiliary metabolites, and amino acids plays a role in the exudate of the root, no reasonable investigations were conducted in order to determine the role of any particular mixture in the exudate of the root. It is also important to note that from time to time, it has also been discovered that root exudates also have a suppressive effect on the debasement of PCCs to some extent. As outlined by Phillips *et al.* (2012), it has been found that root exudates have an adverse effect on the mineralization fastness of n-hexadecane, naphthalene, and phenanthrene in horse feed (*Medicago sativa* L.) and wild rye (*Elymus angustus* Trin.) when exposed to the root exudates. Root exudate intensities of individual plants can be exceptionally diverse, depending on factors such as the growth phase of the plants, the availability of supplements, the amount of sunlight, the pH level of the soil, and the degree of climate (Gransee & Wittenmayer, 2000; Hodge *et al.*, 1997; Hütsch *et al.*, 2002; Leigh *et al.*, 2002; Neumann, 2007; Rovira, 1959).

Moreover, it has also been observed that organic boundaries, such as mycorrhizal parasites, can additionally affect the rate at which the roots exude; in particular, there are several examples of plants that show a slowdown in root exudation as a result of these parasites (Marschner *et al.*, 1997; Ryan *et al.*, 2012). It is observed that there is a fluctuation in the convergence of exudates at different parts of the root. As it has been reported, there are many exudates that are delivered to the end of the roots, as well as to the horizontal stretch of the roots at the tip of the roots (Marschner *et al.*, 2011; McDougall & Rovira, 1970; Neumann, 2007). Ryegrass (*Lolium perenne* L.) is one of the plants that contain a significant amount of PHC degrading microorganisms that are present close to the root surface (within 3 mm of the root surface), which results in the most intense deprivation of PHC at that site (Corgie *et al.*, 2004).

11.4 A COMPONENT OF THE RHIZOREMEDIATION PROCESS FOR THE REMOVAL OF NATURAL CONTAMINANTS

Debasements of bacteria for natural impurities at the rhizospheric area happen by two vigorous and anaerobic cycles in regular soils (Boopathy, 2004). Root oozes increment corruption of natural toxins, for example TPH and PHC, through four different routes, causing direct degradation of the rhizosphere by chemicals obtained from the plant, thereby increasing the bioavailability of impurities, metabolic interaction, and feelings as a consequence of the energy/supplement stream (Martin *et al.*, 2014). According to the exploration of the rhizoremediation of natural foreign substances, the accompanying significant systems are assumed to be engaged with this cycle. There is no doubt that compounds of microbial origin are fundamentally facilitating natural pollution corruption pathways; nevertheless, plant exudates contain various extracellular proteins that are capable of accelerating the degradation of a number of polyaromatic hydrocarbons within a short period of time, including peroxidases and laccases (Martin *et al.*, 2014). It has also been suggested that mycorrhizal parasites may play a role in the treatment of PHC particles by delivering extracellular compounds to the cells (Gramss *et al.*, 1999). It is generally accepted that the number of xenobiotics absorbed by a plant from a given environmental condition is directly dependent on the logarithm of the compound's octanol–water segment coefficient (K_{ow}) for that particular plant. Briggs *et al.* (1982) utilized the primary movement relationship as the happening stream focus factor (TSCF) as a mechanism for examining the relationship between the uptake of an active compound and its log K_{ow} esteem in a soilless framework, as a connection between the uptake and its log K_{ow} esteem. At a log K_{ow} value of 2.1, the TSCF for natural mixtures reaches a limit of 0.8 at a log K_{ow} value of 0.8. Compositions with a log K_{ow} value greater than 2.1 are more hydrophobic and are tightly bound to the lipid film of the root, while mixtures with a log K_{ow} value lower than 2.1 are not capable of traveling through the lipid film of the root epidermis. Most natural contaminations are hydrophobic, which means that they are insoluble in soil, and therefore cannot be degraded effectively by microorganisms because they are not readily available to them. By increasing the solubility of toxins, the root exudates were able to enhance their bioavailability, which in turn allowed microorganisms to find them more readily available to attack.

11.5 BIOSURFACTANTS DEVELOPMENT

As far as soil bioremediation is concerned, the bioavailability of the contaminant presents a significant challenge. The hydrophobic way of natural toxins, those are ineffectively disintegrated in the polar atoms like H_2O, and large numbers of these foreign substances turn into big soil particles. These shortages of bioavailability normally diminish evacuation productivity (Johnsen *et al.*, 2005). With the assistance of various techniques, for example, the discharge of biosurfactants, development of bio films, and creation of extracellular polymeric substances (EPS), microorganisms increment the availability of water-repellent mixtures (i.e., PAHs). Biosurfactants are hydrophilic and hydrophobic particles in nature that structure micelles with natural pollutants; accordingly, bioavailability increments and upgrades the rhizoremediation cycle. It is due to the hydrophobic nature of the central area of micelles that the water-repellent pollutants in the micelles are diluted, resulting in an expansion in the movement of the compound from the solid stage to the water stage, which makes them more accessible to bacterial degradation, as they become more water repellent. As biosurfactants, glycolipids are important biomolecules that are produced by microorganisms. Rhamnolipids, which are the main constituents of glycolipids, have been shown to increase the speed at which toxins are absorbed (Cui *et al.*, 2008; Mulligan, 2005; Providenti *et al.*, 1995; Shreve *et al.*, 1995; Zhang & Miller, 1994). Kuiper *et al.* (2004) observed that two lipopeptide biosurfactants secreted by *Pseudomonas putida* at the PAH contaminated areas expanded the emulsion development with toluene due to the disengagement of these putisolvins from plant roots. In terms of bioremediation, the pursuit of rhizobacteria is of great importance as it assists in improving the bioavailability of pollutants, and as such is of great importance. There are a large number of microorganisms that follow a positive chemotaxis pattern toward toxins, making them a good candidate for further research (Parales & Haddock, 2004). Therefore, due to the common activity of chemotaxis and biosurfactants in contaminated soils, they help to promote the development of microbial populations and to promote bacteria proliferation, thereby encouraging more zones to be cleared.

11.6 NATURAL CORROSIVE CREATION

Different rhizospheric microorganisms secrete various natural acids, for example, gluconic corrosive, phytic corrosive, and so on, which bring down the pH of the soil, in this manner upgrading the dissolvability of impurities and expanding their debasement (Oberai & Khanna, 2018). In recent times, it has been demonstrated that certain LMW aliphatic carboxylates, for example, oxalate and citrate, increase the PHCs in soil by causing a deviation from the soil grid (An *et al.*, 2010, 2011; Gao *et al.*, 2010). The system of desorption, on the other hand, is quite insignificant, and it is speculated that delivered soil natural components might cause the liberation of sorbed natural impurities or because of a competition for adsorption spots among carboxylates and certain PHCs, the adsorption of toxins could be diminished (An *et al.*, 2010; Reilley *et al.*, 1996).

11.7 UNDERLYING RELATIONSHIP AND CO-METABOLISM

Different auxiliary metabolites discharged from the roots of plants, for example, flavonoids, showed a comparable primary likeness to natural contaminations, specifically to sweet-smelling hydrocarbons (Bais *et al.*, 2008; Singer, 2006). According to this underlying relationship, the hazard of contaminations is improved by impacting appropriate enzymatic pathways, notwithstanding expansion in metabolic processes (Fletcher & Hegde, 1995). As per Kanaly and Bartha (1999), the mineralization of benzo-a-pyrene took place in the presence of other essential substrata by metabolic methods.

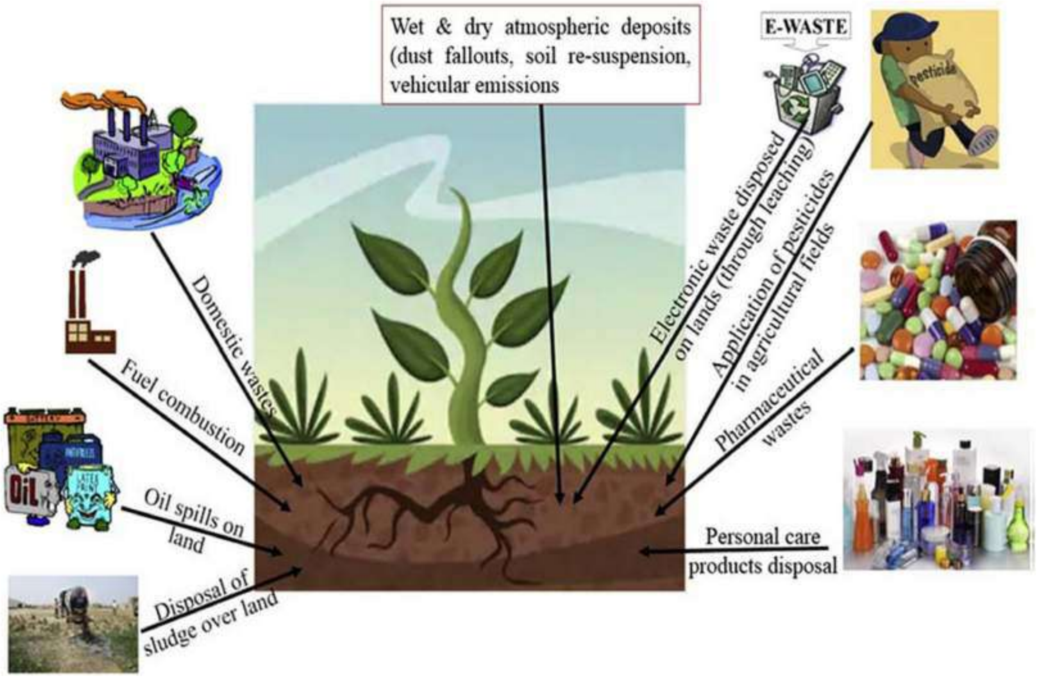


Figure 11.1 Wet and dry atmospheric depositions.

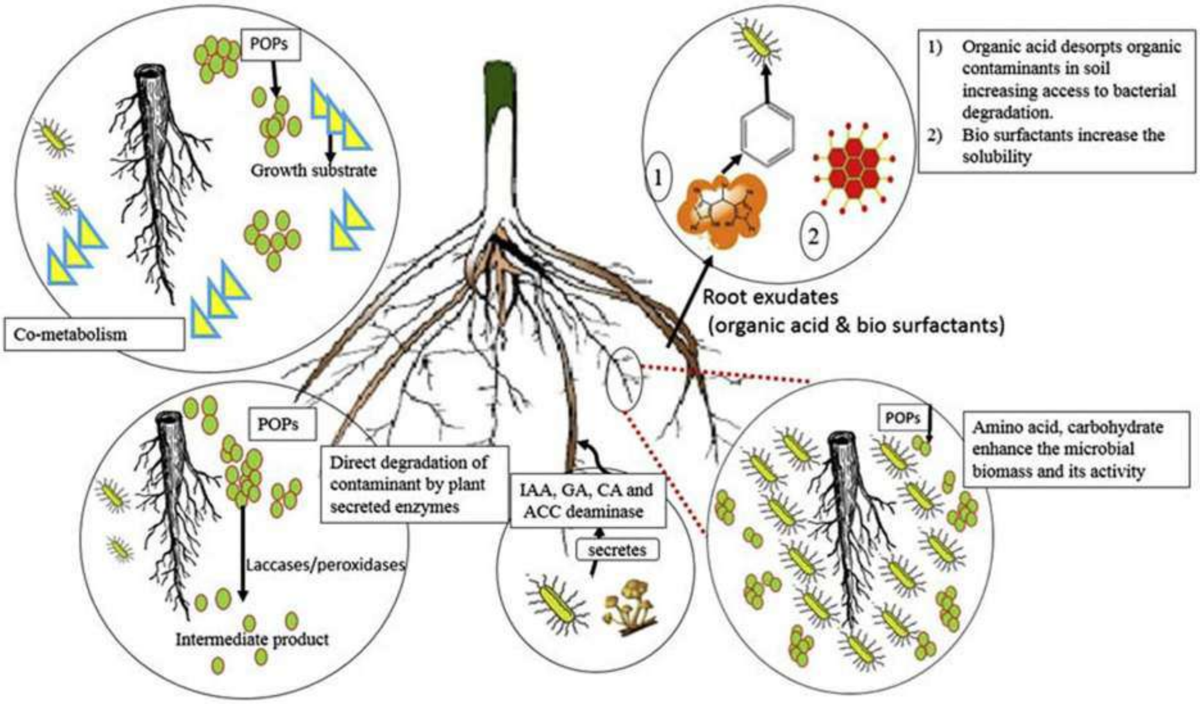


Figure 11.2 Interaction among plants and microbes at the time of biodegradation of organic contaminants.

11.8 LIVELINESS AND SUPPLEMENT STREAM

Root oozes of plants convey a consistent measure of supplements and energy to the microbes to upgrade the corruption of natural contamination (Anderson *et al.*, 1993; Kuiper *et al.*, 2004). Up to 10–40% of photosynthetic results of flora might return to the soil, which relates to approximately 15–60 kg nitrogen/ha/yr and 800–4500 kg carbon/ha/yr (Gerhardt *et al.*, 2009; Grayston *et al.*, 1997; Hütsch *et al.*, 2002; Lynch & Whipps, 1990; Singh *et al.*, 2004). Nitrogen and carbon are conveyed by oozes of roots to rhizospheric microorganisms like hydrophobic, high atomic weight (HMW) natural polymers or, as a solvent, LMW natural particles. The HMW natural polymers partake as a line of adhesive, shedding cells, and different types of root trash (Bais *et al.*, 2008). Since HMW natural polymers are generally nonsoluble and complexes naturally, it consequently has biodegradation rates to some degree (Kalbitz *et al.*, 2003; Kuzyakov, 2002). LMW exudates have higher solvency in water, and as a result of this explanation, microorganisms rapidly take up these mixtures. The principal parts of LMW root oozes are AAs, natural corrosive anions, and carbs. These parts are considered the fundamental wellspring of supplements and energy to the rhizosphere on the grounds that these are effectively decomposable (Van Hees *et al.*, 2005). Quick reaction is displayed regarding their breadth (inside of) by microbe networks when LMW root oozes are included in soil; notwithstanding, perception for quality articulation deviations might be required a few times (Darrah, 1991; Jones & Murphy, 2007). Da Silva *et al.* (2006) found the oozes delivered by roots of *Morus* (*Morus rubra* L.) that affected the development of PAH debasers in an equal way to that of all-out microbes. Nevertheless, there is a possibility of some catabolic restraint because of the presence of some additional more labile carbon bases in PHC-contaminated soils, as well as root oozes (Cases & de Lorenzo, 2005; Singer, 2006). Yuste *et al.* (1998) uncovered that *Pseudomonas* species contained high levels of carbon bases. As further concentration was initiated, it was found that when root concentrates and oozes were added to polluted soils, *P. putida* showed a decline in its ability to degrade phenanthrene (Rentz *et al.*, 2004).

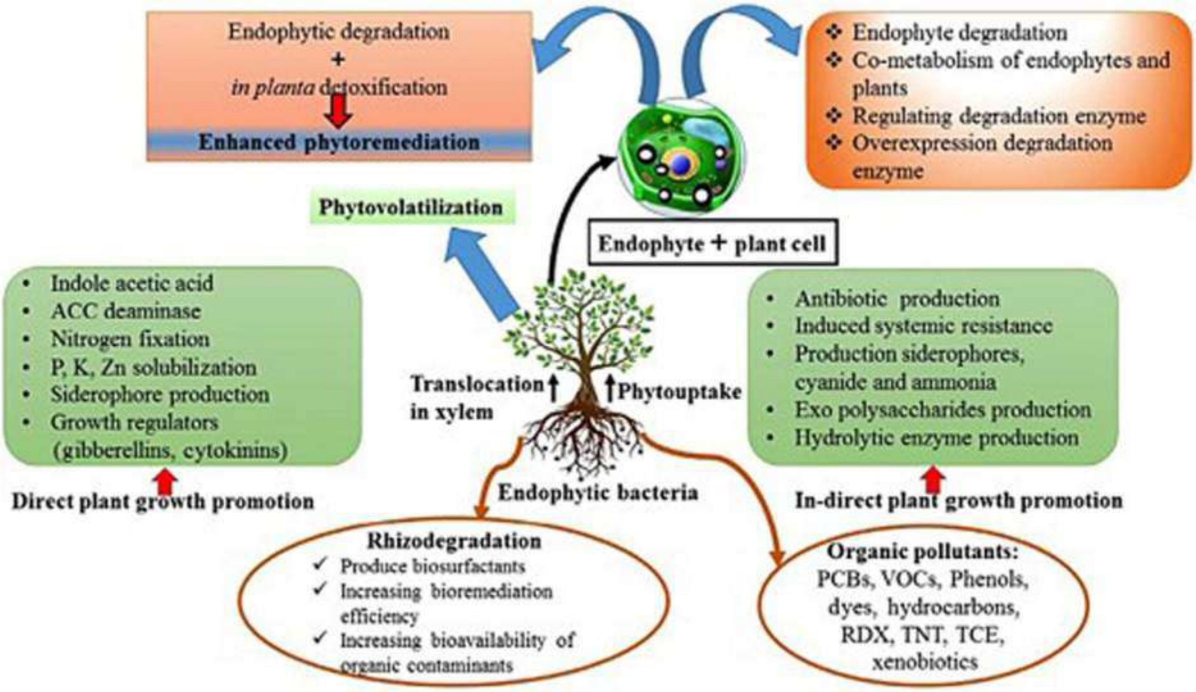


Figure 11.3 Rhizoremediation – a green technology.

11.9 RHIZOREMEDIATION: FACTORS INFLUENCING ITS EFFECTIVENESS

A number of factors influences the course of rhizoremediation, for example, natural variables, such as the soil temperature, pH, and soil organic matter, which are factors related to rhizoremediation. Other than this, different factors, for example, plant species and microbial populace associated with the strategy, additionally influence the cycle; bioavailability of the contaminations is another significant angle in the technique. These components eventually impact the contamination's appearance toward microbial variety.

11.10 ECOLOGICAL VARIABLES AND ITS POSSIBLE IMPACT

11.10.1 Temperature

Bandowe *et al.* (2014) expressed that it fills in as an urgent job in the redress of PAH by increasing its bioavailability as its dissolvability will, in general, ascent along the increment of temperature. The enzymatic exercises interceded due to the bacterial populace get accelerated along the climb of temperature to the ideal level, that is, Liang *et al.* (2003) led a trial in which they used to gage how much oxygen is required for the action of organisms during the most common way of treating the soil. At the time of this cycle, the convergence expanding oxygen seems a lot higher at 43°C rather than 22°C, 29°C, or 36°C. Similarly, Chung *et al.* (2007) found that temperature also affects the ability of bacterial cells and soil elements to adsorb and desorb toxins by influencing their efficiency of adsorption and desorption.

11.10.2 pH

In general, the majority of microorganism proteins released for the biodegradation of mixtures are reliant on pH levels that range from 6.5 to 7.5, which is about the same as the pH inside the cell. As described by Siddique *et al.* (2002), *Pandoraea* sp., which was isolated from an enhanced culture by Okeke *et al.* (2002), degrades HCH isomers at a pH range of 4–9 (Siddique *et al.*, 2002). The biodegradation of bio pesticides in the soil has been found to be at a low pH as opposed to nonpartisan and basic soils, as indicated by Singh *et al.* (2006) in their study. There is a strong correlation between pH and the remedy of PAH, since various kinds of microorganisms have different ideal pH levels for their development, thereby enabling them to perform their enzymatic action. As a result of contamination, such as PAHs, it is possible to alter the pH of the surrounding soil of the local microbiological zone, which in turn influences the R-O response and the solubility of toxins in the rhizosphere (Brito *et al.*, 2015). Several of the microbes show potential to degrade PAHs in situ, as described by Bamforth and Singleton (2005), yet they will be eminently obstructed if there is a significant change in pH, which is why change in pH is recommended in places where there is a significant amount of soil contamination.

11.10.3 Soil organic matter

Natural substances in the soil bring about supplement hotspot of rhizospheric microbial bio-organisms, and subsequently assist them in multiplication and enhance adsorption and desorption components of toxins.

11.10.4 Floral species

Flora has the ability to eliminate or debase toxins. For example, organics in soil and microorganisms exist in relationship with these flora add additional work to their assets. A few microorganisms advance the development of plants by their PGP movement; consequently, plants discharge a few metabolites, which help organisms engendering and evacuation proficiency of toxins. Other than this, flora discharge root oozes, which act as a C source to the microorganisms populace and assist them in accomplishing more populace in the rhizo zone; what's more, root oozes might have biosurfactants that upgrade the bio availability of the pollutants in rhizo zoned soil (Vergani *et al.*, 2017). Flora can influence natural toxins and particle matter levels in the air; McLachlan (1999) referred to it as 'backwoods channel impact'; yet it can bind to soil particles firmly with low solvency in water, which turn the accessibility difficult for degradation (Terzaghi *et al.*, 2018).

11.10.5 Movement of microorganisms

The movement of organisms in the rhizosphere can be impacted due to variables, sorting conditions, and species utilized, yet the existence of impurities can change the capability of microorganisms which normally takes place in the rhizosphere. There is a pressure state created by these toxins on the microbial local area, which drives the microbial local area to embrace and foster new characteristics against the pollutants in order to cope with these toxins. There is no doubt that species that are not a part of these polluted locales are capable of handling pollutants to a greater degree, and so these species can be used for bioremediation of impurities at sites where there is high contamination. However, they could replace the existing organisms at that site, and it would be unwise to ignore their opposition (Momose *et al.*, 2008). Aside from that, the microorganisms also possess resistance against toxins such as PAHs, or they can also mirror the capacity for degrading, so we can additionally be able to create the valuable opportunity for separating those qualities and using them in alternate ways of bioremediation (Mahmoudi *et al.*, 2011). Li *et al.* (2015) proved the beneficial properties of debasing arbitrates of PAHs from *Arthrobacter* SA02.

11.11 FACTORS INFLUENCING RHIZOREMEDIATION: ACCESSIBILITY OF TOXINS

11.11.1 Accessibility of toxins

The bio-accessibility of the impurities that are impacted by their dissolvability, focus on soil contamination, adsorption, and construction. Surfactants that are generally used improve the accessibility of contamination. For instance, sodium dodecyl benzenesulfonate was additional, D9 unsaturated fat desaturase focus was supported, which prompts enhanced ease of cell film by raising the unsaturated fat level, consequently strengthening the vehicle of impurity atom across the layer (Li *et al.*, 2015). Surfactants increment the corruption of PAHs in microbes by regulating certain qualities, that is, 1H2Nase and RHDase and guidelines of the cell film (Kuiper *et al.*, 2004; Ronchel & Ramos 2001).

11.12 CONCLUSION AND FUTURE POINTS OF VIEW

Natural substances that are contaminated pose a danger to the health of humans, as well as negatively affect our current circumstances, so it is urgent that a proficient arrangement be put in place to address this issue. In past examinations, a few plants like *M. sativa*, *Hordeum vulgare*, *Triticum aestivum*, and *Phragmites australis*, accounted for the effective expulsion of natural toxins from the soil (Kuiper *et al.*, 2004). For the rhizoremediation process, a rhizosphere microbial strain ought to be immunized with the plant seeds by covering them with microorganisms, so organisms can develop in relationship with the underlying foundations of that plant effectively and speed up remediation of contamination. This sort of vaccination of microorganisms will assist the organisms to spread along the underground part and increment the possibility of spreading in the soil. Kuiper *et al.* (2001) have screened a plant–organism connection, which showed the capability to debase naphthalene and the ability to give soil seeds some resistance to the presence of high concentrations of this toxin when sown. At the point when the seeds of grasses were covered with this bacterial strain and afterwards developed, it was recorded that the local bacterial area had the option to develop and spread further in soil with the germination and growth of the plant. Currently, *Pseudomonas* with recombinant DNA innovation has the ability to endure toxins in the rhizosphere, which was impractical for the parent bacterial strain. Despite the fact that there are many studies indicating rhizoremediation as a feasible method for determining removal of natural contaminants from soil, further research studies, newer plants, and microbial strains are required for the implementation of this method on a larger scale. As a general rule, we can conclude that rhizoremediation has demonstrated to be a successful approach for removing natural toxins from soil. In spite of this, most of the ongoing research is being carried out on a laboratory scale, and more efforts will be made in the near future to enhance this method to a field setting. Recent decades have seen a tremendous emphasis on rhizoremediation technologies, which are cost-effective and eco-friendly, as well as the utilization of plant-related microbes to decontaminate and eradicate engineered natural mixtures from ecological soils. There is a possibility that this innovation might provide an effective, financial, and reasonable green remediation innovation for the reduction of natural toxins from the soil in the event that is viewed in a serious way.

ACKNOWLEDGEMENT

The authors wish to express their sincere appreciation to Lovely Professional University's Department of Agronomy for the constant support and encouragement provided throughout the research process.

AUTHOR CONTRIBUTIONS

As well as contributing to the outline, the author is responsible for leading the writing process and editing the manuscript after it has been drafted. Because of extensive literature searches, D.H.L., P.K., and J.S. contributed to the drafting of the sections as well as the construction of figures and tables. In addition to providing professional advice, P.K. and J.S. helped revise the final version of the document. The final version of the chapter was read and approved by all authors before it was submitted for publication.

CONFLICTS OF INTEREST

None.

REFERENCES

Agusa T., Kunito T., Nakashima E., Minh T. B., Tanabe S., Subramanian A. and Viet P. H. (2003). Preliminary studies on trace element contamination in dumping sites of municipal wastes in India and Vietnam. In: Journal de Physique IV (Proceedings). EDP Sciences, France, Vol. **107**, pp. 21–24, https://doi.org/10.1051/jp4:20030233

Alamdar A., Syed J. H., Malik R. N., Katsoyiannis A., Liu J., Li J., Zhang G. and Jones K. C. (2014). Organochlorine pesticides in surface soils from obsolete pesticide dumping ground in Hyderabad City, Pakistan: contamination levels and their potential for air-soil exchange. *The Science of the Total Environment*, **470**, 733–741, https://doi.org/10.1016/j.scitotenv.2013.09.053

Ali U., Syed J. H., Malik R. N., Katsoyiannis A., Li J., Zhang G. and Jones K. C. (2014). Organochlorine pesticides (OCPs) in South Asian region: a review. *The Science of the Total Environment*, **476**, 705–717, https://doi.org/10.1016/j.scitotenv.2013.12.107

Alkorta I. and Garbisu C. (2001). Phytoremediation of organic contaminants in soils. *Bioresource Technology*, **79**(3), 273–276, https://doi.org/10.1016/S0960-8524(01)00016-5

An C., Huang G., Yu H., Wei J., Chen W. and Li G. (2010). Effect of short-chain organic acids and pH on the behaviors of pyrene in soilewater system. *Chemosphere*, **81**(11), 1423–1429, https://doi.org/10.1016/j.chemosphere.2010.09.012

An C. J., Huang G. H., Wei J. and Yu H. (2011). Effect of short-chain organic acids on the enhanced desorption of phenanthrene by rhamnolipid biosurfactant in soil water environment. *Water Research*, **45**(17), 5501–5510, https://doi.org/10.1016/j.watres.2011.08.011

Anderson T. A., Guthrie E. A. and Walton B. T. (1993). Bioremediation in the rhizosphere. *Environment Science Technology*, **27**(13), 2630–2636, https://doi.org/10.1021/es00049a001

Atapattu S. S. and Kodituwakku D. C. (2009). Agriculture in South Asia and its implications on downstream health and sustainability: a review. *Agricultural Water Management*, **96**(3), 361–373, https://doi.org/10.1016/j.agwat.2008.09.028

Bais H. P., Broeckling C. D. and Vivanco J. M. (2008). Root exudates modulate plant and microbe interactions in the rhizosphere. In: Secondary Metabolites in Soil Ecology, P. Karlovsky (ed.), Springer, Berlin, Heidelberg, pp. 241–252.

Bamforth S. M. and Singleton I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology and Biotechnology*, **80**(7), 723–736, https://doi.org/10.1002/jctb.1276

Bandowe B. A. M., Bigalke M., Boamah L., Nyarko E., Saalia F. K. and Wilcke W. (2014). Polycyclic aromatic compounds (PAHs and oxygenated PAHs) and trace metals in fish species from Ghana (West Africa): bioaccumulation and health risk assessment. *Environment International*, **65**, 35–146.

Barra R., Popp P., Quiroz R., Bauer C., Cid H. and von Tümpling W. (2005). Persistent toxic substances in soils and waters along an altitudinal gradient in the Laja River Basin, Central Southern Chile. *Chemosphere*, **58**(7), 905–915, https://doi.org/10.1016/j.chemosphere.2004.09.050

Baudoin E., Benizri E. and Guckert A. (2003). Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biology and Biochemistry*, **35**, 1183–1192, https://doi.org/10.1016/S0038-0717(03)00179-2

Behera B. and Singh G. S. (1999). Studies on weed management in monsoon season crop of tomato. *Indian Journal of Weed Science*, **31**(1 and 2), 67–70.

Benizri E., Dedourge O., Dibattista-Leboeuf C., Piutti S., Nguyen C. and Guckert A. (2002). Effect of maize rhizodeposits on soil microbial community structure. *Applied Soil Ecology*, **21**, 261–265, https://doi.org/10.1016/S0929-1393(02)00094-X

Bhattacharya B., Sarkar S. K. and Mukherjee N. (2003). Organochlorine pesticide residues in sediments of a tropical mangrove estuary, India: implications for monitoring. *Environment International*, **29**(5), 587–592, https://doi.org/10.1016/S0160-4120(03)00016-3

Bisht S., Pandey P., Sood A., Sharma S. and Bisht N. S. (2010). Biodegradation of naphthalene and anthracene by chemotactically active rhizobacteria of *Populus deltoides*. *Brazilian Journal of Microbiology*, **41**(4), 922–930, https://doi.org/10.1590/S1517-83822010000400011

Bisht S., Pandey P., Kaur G., Aggarwal H., Sood A., Sharma S., Kumar V. and Bisht N. S. (2014). Utilization of endophytic strain *Bacillus* sp. SBER3 for biodegradation of polyaromatic hydrocarbons (PAH) in soil model system. *European Journal of Soil Biology*, **60**, 67–76, https://doi.org/10.1016/j.ejsobi.2013.10.009

Boopathy R. (2004). Anaerobic biodegradation of no. 2 diesel fuel in soil: a soil column study. *Bioresource Technology*, **94**(2), 143–151, https://doi.org/10.1016/j.biortech.2003.12.006

Brazil G. M., Kenefick L., Callanan M., Haro A., De Lorenzo V., Dowling D. N. and O'gara F. (1995). Construction of a rhizosphere pseudomonad with potential to degrade polychlorinated biphenyls and detection of bph gene expression in the rhizosphere. *Applied and Environmental Microbiology*, **61**(5), 1946–1952, https://doi.org/10.1128/aem.61.5.1946-1952.1995

Briggs G. G., Bromilow R. H. and Evans A. A. (1982). Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pesticide Science*, **13**(5), 495–504, https://doi.org/10.1002/ps.2780130506

Brink S. C. (2016). Unlocking the secrets of the rhizosphere. *Trends in Plant Science*, **21**(3), 169–170, https://doi.org/10.1016/j.tplants.2016.01.020

Brito E. M., De la Cruz Barrón M., Caretta C. A., Goñi-Urriza M., Andrade L. H., Cuevas-Rodríguez G., Malm O., Torres J. P., Simon M. and Guyoneaud R. (2015). Impact of hydrocarbons, PCBs and heavy metals on bacterial communities in Lerma River, Salamanca, Mexico: investigation of hydrocarbon degradation potential. *The Science of the Total Environment*, **521–522**, 1–10, https://doi.org/10.1016/j.scitotenv.2015.02.098

Buccini J. (2003). The development of a global treaty on persistent organic pollutants (POPs). In: Persistent Org Pollut, H. Fiedler (ed.), Springer, Berlin, Heidelberg, pp. 13–30.

Butler J. L., Williams M. A., Bottomley P. J. and Myrold D. D. (2003). Microbial community dynamics associated with rhizosphere carbon flow. *Applied and Environmental Microbiology*, **69**(11), 6793–6800, https://doi.org/10.1128/AEM.69.11.6793-6800.2003

Cachada A., Pato P., Rocha-Santos T., da Silva E. F. and Duarte A. C. (2012). Levels, sources and potential human health risks of organic pollutants in urban soils. *The Science of the Total Environment*, **430**, 184–192, https://doi.org/10.1016/j.scitotenv.2012.04.075

Cannon H. L. and Bowles J. M. (1962). Contamination of vegetation by tetraethyl lead. *Science*, **137**(3532), 765–766, https://doi.org/10.1126/science.137.3532.765

Cases I. and de Lorenzo V. (2005). Genetically modified organisms for the environment: stories of success and failure and what we have learned from them. *International Microbiology*, **8**(3), 213–222.

Chakraborty P., Zhang G., Li J., Xu Y., Liu X., Tanabe S. and Jones K. C. (2010). Selected organochlorine pesticides in the atmosphere of major Indian cities: levels, regional versus local variations, and sources. *Environmental Science and Technology*, **44**(21), 8038–8043, https://doi.org/10.1021/es102029t

Chaudhry Q., Blom-Zandstra M., Gupta S. K. and Joner E. (2005). Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. *Environmental Science and Pollution Research International*, **12**, 34–48, https://doi.org/10.1065/espr2004.08.213

Chung M. K., Tsui M. T., Cheung K. C., Tam N. F. and Wong M. H. (2007). Removal of aqueous phenanthrene by brown seaweed *Sargassum hemiphyllum*: sorption-kinetic and equilibrium studies. *Separation and Purification Technology*, **54**(3), 355–362, https://doi.org/10.1016/j.seppur.2006.10.008

Corgie S. C., Beguiristain T. and Leyval C. (2004). Spatial distribution of bacterial communities and phenanthrene degradation in the rhizosphere of *Lolium perenne* L. *Applied and Environmental Microbiology*, **70**(6), 3552–3557, https://doi.org/10.1128/AEM.70.6.3552-3557.2004

Cui C. Z., Zeng C., Wan X., Chen D., Zhang J. Y. and Shen P. (2008). Effect of rhamnolipids on degradation of anthracene by two newly isolated strains, *Sphingomonas* sp. 12A and *Pseudomonas* sp. 12B. *Journal of Microbiology and Biotechnology*, **18**(1), 63–66.

Da Silva M. L., Kamath R. and Alvarez P. J. (2006). Effect of simulated rhizodeposition on the relative abundance of polynuclear aromatic hydrocarbon catabolic genes in a contaminated soil. *Environmental Toxicology and Chemistry*, **25**(2), 386–391, https://doi.org/10.1897/05-321R.1

Darrah P. R. (1991). Models of the rhizosphere. *Plant and Soil*, **133**(2), 187–199, https://doi.org/10.1007/BF00009191

Dzantor E. K. (2007). Phytoremediation: the state of rhizosphere 'engineering' for accelerated rhizodegradation of xenobiotic contaminants. *Journal of Chemical Technology and Biotechnology*, **82**(3), 228–232, https://doi.org/10.1002/jctb.1662

El Haleem D. A., Von Wintzingerode F., Moter A., Moawad H. and Göbel U. B. (2000). Phylogenetic analysis of rhizosphere-associated b-subclass proteobacterial ammonia oxidizers in a municipal wastewater treatment plant based on rhizoremediation technology. *Letters in Applied Microbiology*, **31**(1), 34–38, https://doi.org/10.1046/j.1472-765x.2000.00760.x

El Nemr A., Moneer A. A., Khaled A. and El-Sikaily A. (2013). Levels, distribution, and risk assessment of organochlorines in surficial sediments of the Red Sea coast, Egypt. *Environmental Monitoring and Assessment*, **185**(6), 4835–4853, https://doi.org/10.1007/s10661-012-2907-3

Eqani S. A. M. A. S., Malik R. N. and Mohammad A. (2011). The level and distribution of selected organochlorine pesticides in sediments from river Chenab, Pakistan. *Environmental Geochemistry and Health*, **33**(1), 33–47, https://doi.org/10.1007/s10653-010-9312-z

Fabietti G., Biasioli M., Barberis R. and Ajmone-Marsan F. (2010). Soil contamination by organic and inorganic pollutants at the regional scale: the case of Piedmont, Italy. *Journal of Soils and Sediments*, **10**(2), 290–300, https://doi.org/10.1007/s11368-009-0114-9

Fletcher J. S. and Hegde R. S. (1995). Release of phenols by perennial plant roots and their potential importance in bioremediation. *Chemosphere*, **31**(4), 3009–3016, https://doi.org/10.1016/0045-6535(95)00161-Z

Gao Y., Ren L., Ling W., Kang F., Zhu X. and Sun B. (2010). Effects of low-molecular-weight organic acids on sorption and desorption of phenanthrene in soils. *Soil Science Society of America Journal*, **74**(1), 51–59, https://doi.org/10.2136/sssaj2009.0105

Gerhardt K. E., Huang X. D., Glick B. R. and Greenberg B. M. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Science*, **176**(1), 20–30, https://doi.org/10.1016/j.plantsci.2008.09.014

Glazer A. N. and Nikaido H. (1995). Biomass. In: Microbial Biotechnology: Fundamentals of Applied Microbiology, A. N. Glazer (ed.), Freeman and Co, New York, p. 662.

Gramss G., Kirsche B., Voigt K., Gunther T. and Fritsche W. (1999). Conversion rates of five polycyclic aromatic hydrocarbons in liquid cultures of fifty-eight fungi and the concomitant production of oxidative enzymes. *Mycological Research*, **103**, 1009–1018, https://doi.org/10.1017/S0953756298008144

Gransee A. and Wittenmayer L. (2000). Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. *Journal of Plant Nutrition and Soil Science*, **163**, 381–385, https://doi.org/10.1002/1522-2624(200008)163:4<381::AID-JPLN381>3.0.CO;2-7

Grayston S. J., Vaughan D. and Jones D. (1997). Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology*, **5**(1), 29–56, https://doi.org/10.1016/S0929-1393(96)00126-6

Gupta P. K. (2004). Pesticide exposure – Indian scene. *Toxicology*, **198**(1–3), 83–90, https://doi.org/10.1016/j.tox.2004.01.021

Gupta A., Singh M., Pandey K. D. and Kumar A. (2018). Biodegradation of xenobiotics using cyanobacteria. *Journal of Scientific Research ISSN*, **62**, 35–40.

Guzzella L., Roscioli C., Vigano L., Saha M., Sarkar S. K. and Bhattacharya A. (2005). Evaluation of the concentration of HCH, DDT, HCB, PCB and PAH in the sediments along the lower stretch of Hugli estuary, West Bengal, northeast India. *Environment International*, **31**(4), 523–534, https://doi.org/10.1016/j.envint.2004.10.014

Hartmann A., Schmid M., Tuinen D. and Berg G. (2009). Plant-driven selection of microbes. *Plant and Soil*, **321**, 235–257, https://doi.org/10.1007/s11104-008-9814-y

Hoagland R. E., Zablotowicz R. M. and Locke M. A. (1994). Propanil metabolism by rhizosphere microflora. In: Bioremediation Through Rhizosphere Technology, T. A. Anderson and J. R. Coats (eds.), American Chemical Society, Washington, DC, pp. 160–183.

Hodge A., Paterson E., Thornton B., Millard P. and Killham K. (1997). Effects of photon flux density on carbon partitioning and rhizosphere carbon flow of *Lolium perenne*. *Journal of Experimental Botany*, **48**, 1797–1805, https://doi.org/10.1093/jxb/48.10.1797

Hütsch B. W., Augustin J. and Merbach W. (2002). Plant rhizodeposition: an important source for carbon turnover in soils. *Journal of Soil Science and Plant Nutrition*, **165**(4), 397–407, https://doi.org/10.1002/1522-2624(200208)165:4<397::AID-JPLN397>3.0.CO;2-C

Ingrid L., Sahraoui A. L. H., Frédéric L., Yolande D. and Joël F. (2016). Arbuscular mycorrhizal wheat inoculation promotes alkane and polycyclic aromatic hydrocarbon biodegradation: microcosm experiment on aged-contaminated soil. *Environmental Pollution*, **213**, 549–560, https://doi.org/10.1016/j.envpol.2016.02.056

Jacobsen C. S. (1997). Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading *Burkholderia* (*Pseudomonas*) *cepacia* DBO1 (pRO101) in 2, 4-D contaminated soil. *Plant and Soil*, **189**(1), 139–144, https://doi.org/10.1023/A:1004296615446

Jan M. R., Shah J., Khawaja M. A. and Gul K. (2009). DDT residue in soil and water in and around abandoned DDT manufacturing factory. *Environmental Monitoring and Assessment*, **155**(1–4), 31–38, https://doi.org/10.1007/s10661-008-0415-2

Johnsen A. R., Wick L. Y. and Harms H. (2005). Principles of microbial PAH-degradation in soil. *Environmental Pollution*, **133**(1), 71–84, https://doi.org/10.1016/j.envpol.2004.04.015

Joner E. J., Corgié S. C., Amellal N. and Leyval C. (2002). Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. *Soil Biology and Biochemistry*, **34**, 859–864, https://doi.org/10.1016/S0038-0717(02)00018-4

Jones D. L. and Murphy D. V. (2007). Microbial response time to sugar and amino acid additions to soil. *Soil Biology and Biochemistry*, **39**(8), 2178–2182, https://doi.org/10.1016/j.soilbio.2007.03.017

Kalbitz K., Schmerwitz J., Schwesig D. and Matzner E. (2003). Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma*, **113**(3–4), 273–291, https://doi.org/10.1016/S0016-7061(02)00365-8

Kanaly R. A. and Bartha R. (1999). Cometabolic mineralization of benzo [a] pyrene caused by hydrocarbon additions to soil. *Environmental Toxicology and Chemistry*, **18**(10), 2186–2190.

Kelley S. L., Aitchison E. W., Deshpande M., Schnoor J. L. and Alvarez P. J. (2001). Biodegradation of 1, 4-dioxane in planted and unplanted soil: effect of bioaugmentation with *Amycolata* sp. CB1190. *Water Research*, **35**(16), 3791–3800, https://doi.org/10.1016/S0043-1354(01)00129-4

Khan Z., Roman D., Kintz T., delas Alas M., Yap R. and Doty S. (2014). Degradation, phytoprotection and phytoremediation of phenanthrene by endophyte *Pseudomonas putida*, PD1. *Environmental Science and Technology*, **48**(20), 12221–12228, https://doi.org/10.1021/es503880t

Kingsley M. T., Fredrickson J. K., Metting F. B. and Seidler R. J. (1994). Environmental Restoration Using Plant-Microbe Bioaugmentation. Bioremediation of Chlorinated and Polyaromatic Hydrocarbon Compounds. Lewis Publishers, Boca Raton, Florida, pp. 287–292.

Kuiper I., Bloemberg G. V. and Lugtenberg B. J. (2001). Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading bacteria. *Molecular Plant-Microbe Interactions*, **14**(10), 1197–1205, https://doi.org/10.1094/MPMI.2001.14.10.1197

Kuiper I., Lagendijk E. L., Bloemberg G. V. and Lugtenberg B. J. (2004). Rhizoremediation: a beneficial plant-microbe interaction. *Molecular Plant-Microbe Interactions*, **17**(1), 6–15, https://doi.org/10.1094/MPMI.2004.17.1.6

Kuzyakov Y. (2002). Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science*, **165**(4), 382–396, https://doi.org/10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-#

Leigh M. B., Fletcher J. S., Fu X. and Schmitz F. J. (2002). Root turnover: an important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. *Environmental Science and Technology*, **36**, 1579–1583, https://doi.org/10.1021/es015702i

Li F., Zhu L., Wang L. and Zhan Y. (2015). Gene expression of an *Arthrobacter* in surfactant-enhanced biodegradation of a hydrophobic organic compound. *Environmental Science and Technology*, **49**(6), 3698–3704, https://doi.org/10.1021/es504673j

Liang C., Das K. C. and McClendon R. W. (2003). The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. *Bioresource Technology*, **86**(2), 131–137, https://doi.org/10.1016/S0960-8524(02)00153-0

Liu R., Xiao N., Wei S., Zhao L. and An J. (2014). Rhizosphere effects of PAH-contaminated soil phytoremediation using a special plant named Fire Phoenix. *The Science of the Total Environment*, **473**, 350–358, https://doi.org/10.1016/j.scitotenv.2013.12.027

Löffler F. E., Ritalahti K. M. and Zinder S. H. (2013). Dehalococcoides and reductive dechlorination of chlorinated solvents. In: Bioaugmentation for Groundwater Remediation, H. F. Stroo, A. Leeson, and C. H. Ward (eds.), Springer, New York, NY, pp. 39–88.

Lugtenberg B. J. J. and de Weger L. A. (1992). Plant root colonization by *Pseudomonas* spp. In: Pseudomonas: Molecular Biology and Biotechnology, E. Galli, S. Silver and B. Witholt (eds.), American Society for Microbiology, Washington, DC, pp. 13–19.

Lynch J. M. and Whipps J. M. (1990). Substrate flow in the rhizosphere. *Plant and Soil*, **129**(1), 1–10, https://doi.org/10.1007/BF00011685

Ma W. L., Li Y. F., Sun D. Z. and Qi H. (2009). Polycyclic aromatic hydrocarbons and polychlorinated biphenyls in topsoils of Harbin, China. *Archives of Environmental Contamination and Toxicology*, **57**(4), 670, https://doi.org/10.1007/s00244-009-9314-y

Macek T., Mackova M. and Kás J. (2000). Exploitation of plants for the removal of organics in environmental remediation. *Biotechnology Advances*, **18**(1), 23–34, https://doi.org/10.1016/S0734-9750(99)00034-8

MacKinnon G. and Duncan H. J. (2013). Phytotoxicity of branched cyclohexanes found in the volatile fraction of diesel fuel on germination of selected grass species. *Chemosphere*, **90**(3), 952–957, https://doi.org/10.1016/j.chemosphere.2012.06.038

Mahmoudi N., Slater G. F. and Fulthorpe R. R. (2011). Comparison of commercial DNA extraction kits for isolation and purification of bacterial and eukaryotic DNA from PAH-contaminated soils. *Canadian Journal of Microbiology*, **57**(8), 623–628, https://doi.org/10.1139/w11-049

Malik A., Ojha P. and Singh K. P. (2009). Levels and distribution of persistent organochlorine pesticide residues in water and sediments of Gomti River (India) tributary of the Ganges River. *Environmental Monitoring and Assessment*, **148**(1–4), 421–435, https://doi.org/10.1007/s10661-008-0172-2

Marschner P., Crowley D. and Higashi R. (1997). Root exudation and physiological status of a root-colonizing fluorescent pseudomonad in mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L.). *Plant and Soil*, **189**, 11–20, https://doi.org/10.1023/A:1004266907442

Marschner P., Crowley D. and Rengel Z. (2011). Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis-model and research methods. *Soil Biology and Biochemistry*, **43**(5), 883–894, https://doi.org/10.1016/j.soilbio.2011.01.005

Martin B. C., George S. J., Price C. A., Ryan M. H. and Tibbett M. (2014). The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: current knowledge and future directions. *The Science of the Total Environment*, **472**, 642–653, https://doi.org/10.1016/j.scitotenv.2013.11.050

McDougall B. M. and Rovira A. D. (1970). Sites of exudation of ^{14}C-labelled compounds from wheat roots. *New Phytologist*, **69**, 999–1003, https://doi.org/10.1111/j.1469-8137.1970.tb02479.x

McLachlan M. S. (1999). Framework for the interpretation of measurements of SOCs in plants. *Environmental Science and Technology*, **33**(11), 1799–1804, https://doi.org/10.1021/es980831t

Merrington G., Nfa L. W., Parkinson R., Redman M. and Winder L. (2002). Agricultural Pollution: Environmental Problems and Practical Solutions. CRC Press, London.

Minh N. H., Minh T. B., Kajiwara N., Kunisue T., Subramanian A., Iwata H., Tana T. S., Baburajendran R., Karuppiah S., Viet P. H. and Tuyen B. C. (2006). Contamination by persistent organic pollutants in dumping sites of Asian developing countries: implication of emerging pollution sources. *Archives of Environmental Contamination and Toxicology*, **50**(4), 474–481, https://doi.org/10.1007/s00244-005-1087-3

Miya R. K. and Firestone M. K. (2001). Enhanced phenanthrene biodegradation in soil by slender oat root exudates and root debris. *Journal of Environmental Quality*, **30**(6), 1911–1918, https://doi.org/10.2134/jeq2001.1911

Momose Y., Hirayama K. and Itoh K. (2008). Competition for proline between indigenous *Escherichia coli* and *E. coli* O157: H7 in gnotobiotic mice associated with infant intestinal microbiota and its contribution to the colonization resistance against E. coli O157: H7. *Antonie van Leeuwenhoek*, **94**(2), 165–171, https://doi.org/10.1007/s10482-008-9222-6

Moore F. P., Barac T., Borremans B., Oeyen L., Vangronsveld J., Van Der Lelie D., Campbell C. D. and Moore E. R. (2006). Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. *Systematic and Applied Microbiology*, **29**(7), 539–556, https://doi.org/10.1016/j.syapm.2005.11.012

Mulligan C. N. (2005). Environmental applications for biosurfactants. *Environmental Pollution*, **133**(2), 183–198, https://doi.org/10.1016/j.envpol.2004.06.009

Neumann G. (2007). Root exudates and nutrient cycling. In: Nutrient Cycling in Terrestrial Ecosystems, P. Marschner and Z. Rengel (eds.), Springer, Berlin, pp. 123–157.

Nichols T. D., Wolf D. C., Rogers H. B., Beyrouty C. A. and Reynolds C. M. (1997). Rhizosphere microbial populations in contaminated soils. *Water, Air, and Soil Pollution*, **95**(1–4), 165–178.

Oberai M. and Khanna V. (2018). Rhizoremediation plant microbe interactions in the removal of pollutants. *International Journal of Current Microbiology and Applied Science*, **7**(1), 2280–2287, https://doi.org/10.20546/ijcmas.2018.701.276

Okeke B. C., Siddique T., Arbestain M. C. and Frankenberger W. T. (2002). Biodegradation of g-hexachlorocyclohexane and a-hexachlorocyclohexane in water and soil slurry by *Pandoraea* sp. *Journal of Agricultural and Food Chemistry*, **50**, 2548–2555, https://doi.org/10.1021/jf011422a

Oleszczuk P. (2006). Persistence of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge-amended soil. *Chemosphere*, **65**(9), 1616–1626, https://doi.org/10.1016/j.chemosphere.2006.03.007

Pai S. G., Riley M. B. and Camper N. D. (2001). Microbial degradation of mefenoxam in rhizosphere of *Zinnia angustifolia*. *Chemosphere*, **44**(4), 577–582, https://doi.org/10.1016/S0045-6535(00)00368-4

Pandey P., Khillare P. S. and Kumar K. (2011). Assessment of organochlorine pesticide residues in the surface sediments of river Yamuna in Delhi, India. *Journal of Environmental Protection*, **2**(05), 511, https://doi.org/10.4236/jep.2011.25059

Parales R. E. and Haddock J. D. (2004). Biocatalytic degradation of pollutants. *Current Opinion in Biotechnology*, **15**(4), 374–379, https://doi.org/10.1016/j.copbio.2004.06.003

Phillips L. A., Greer C. W., Farrell R. E. and Germida J. J. (2012). Plant root exudates impact the hydrocarbon degradation potential of a weathered-hydrocarbon contaminated soil. *Applied Soil Ecology*, **52**, 56–64, https://doi.org/10.1016/j.apsoil.2011.10.009

Pozo K., Harner T., Lee S. C., Sinha R. K., Sengupta B., Loewen M., Geethalakshmi V., Kannan K. and Volpi V. (2011). Assessing seasonal and spatial trends of persistent organic pollutants (POPs) in Indian agricultural regions using PUF disk passive air samplers. *Environmental Pollution*, **159**(2), 646–653, https://doi.org/10.1016/j.envpol.2010.09.025

Providenti M. A., Flemming C. A., Lee H. and Trevors J. T. (1995). Effect of addition of rhamnolipid biosurfactants or rhamnolipid-producing *Pseudomonas aeruginosa* on phenanthrene mineralization in soil slurries. *FEMS Microbiology Ecology*, **17**(1), 15–26, https://doi.org/10.1111/j.1574-6941.1995.tb00123.x

Qiu X., Shah S. I., Kendall E. W., Sorensen D. L., Sims R. C. and Engelke M. C. (1994). Grass-enhanced bioremediation for clay soils contaminated with polynuclear aromatic hydrocarbons. In: Bioremediation Through Rhizosphere Technology, T. A. Anderson and J. R. Coats (eds.), American Chemical Society, Washington, DC, pp. 142–157.

Qixing Z., Zhang C., Zhineng Z. and Weitao L. (2011). Ecological remediation of hydrocarbon contaminated soils with weed plant. *Molecular Ecology Resources*, **2**(2), 97–105.

Radwan S., Sorkhoh N. and Ei-Nemr I. (1995). Oil biodegradation around roots. *Nature*, **376**, 302, https://doi.org/10.1038/376302a0

Reilley K. A., Banks M. K. and Schwab A. P. (1996). Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. *Journal of Environmental Quality*, **25**(2), 212–219, https://doi.org/10.2134/jeq1996.00472425002500020002x

Rentz J. A., Alvarez P. J. and Schnoor J. L. (2004). Repression of *Pseudomonas putida* phenanthrene-degrading activity by plant root extracts and exudates. *Environmental Microbiology*, **6**(6), 574–583, https://doi.org/10.1111/j.1462-2920.2004.00589.x

Ronchel M. C. and Ramos J. L. (2001). Dual system to reinforce biological containment of recombinant bacteria designed for rhizoremediation. *Applied and Environmental Microbiology*, **67**(6), 2649–2656, https://doi.org/10.1128/AEM.67.6.2649-2656.2001

Ross G. (2005). Risks and benefits of DDT. *The Lancet*, **366**(9499), 1771–1772, https://doi.org/10.1016/S0140-6736(05)67722-7

Rovira A. D. (1956). Plant root excretions in relation to the rhizosphere effect. *Plant and Soil*, **7**, 209–217, https://doi.org/10.1007/BF01666119

Rovira A. D. (1959). Root excretions in relation to the rhizosphere effect. *Plant and Soil*, **11**, 53–64, https://doi.org/10.1007/BF01394753

Ryan M. H., Tibbett M., Edmonds-Tibbett T., Suriyagoda L. D., Lambers H., Cawthray G. R. and Pang J. (2012). Carbon trading for phosphorus gain: the balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition. *Plant, Cell and Environment*, **35**, 2170–2180, https://doi.org/10.1111/j.1365-3040.2012.02547.x

Sarkar S. K., Bhattacharya B. D., Bhattacharya A., Chatterjee M., Alam A., Satpathy K. K. and Jonathan M. P. (2008). Occurrence, distribution and possible sources of organochlorine pesticide residues in tropical coastal environment of India: an overview. *Environment International*, **34**(7), 1062–1071, https://doi.org/10.1016/j.envint.2008.02.010

Shann J. R. and Boyle J. J. (1994). Influence of plant species on in situ rhizosphere degradation. In: Bioremediation Through Rhizosphere Technology, T. A. Anderson and J. R. Coats (eds.), American Chemical Society, Washington, DC, pp. 70–81.

Shreve G. S., Inguva S. and Gunnam S. (1995). Rhamnolipid biosurfactant enhancement of hexadecane biodegradation by *Pseudomonas aeruginosa*. *Molecular Marine Biology and Biotechnology*, **4**(4), 331–337.

Siciliano S. D., Germida J. J., Banks K. and Greer C. W. (2003). Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial. *Applied and Environmental Microbiology*, **69**(1), 483–489, https://doi.org/10.1128/AEM.69.1.483-489.2003

Siddique T., Okeke B. C., Arshad M. and Frankenberger W. T. Jr. 2002. Temperature and pH effects on biodegradation of hexachlorocyclohexane isomers in water and soil slurry. *Journal of Agricultural and Food Chemistry*, **50**, 5070–5076, https://doi.org/10.1021/jf0204304

Singer A. C. (2006). The chemical ecology of pollutant biodegradation: bioremediation and phytoremediation from mechanistic and ecological perspectives. In: Phytoremediation Rhizoremediation, M. Mackova, D. Dowling, T. Macek (eds.), Springer, Dordrecht, pp. 5–21.

Singh B. K., Millard P., Whiteley A. S. and Murrell J. C. (2004). Unravelling rhizosphere microbial interactions: opportunities and limitations. *Trends in Microbiol*, **12**(8), 386–393, https://doi.org/10.1016/j.tim.2004.06.008

Singh B. K., Walker A. and Wright D. J. (2006). Bioremedial potential of fenamiphos and chlorpyrifos degrading isolates: influence of different environmental conditions. *Soil Biology and Biochemistry*, **38**, 2682–2693, https://doi.org/10.1016/j.soilbio.2006.04.019

Syed J. H. and Malik R. N. (2011). Occurrence and source identification of organochlorine pesticides in the surrounding surface soils of the Ittehad Chemical Industries Kalashah Kaku, Pakistan. *Environmental Earth Sciences*, **62**(6), 1311–1321, https://doi.org/10.1007/s12665-010-0618-z

Syed J. H., Malik R. N., Liu D., Xu Y., Wang Y., Li J., Zhang G. and Jones K. C. (2013). Organochlorine pesticides in air and soil and estimated air and soil exchange in Punjab, Pakistan. *The Science of the Total Environment*, **444**, 491–497, https://doi.org/10.1016/j.scitotenv.2012.12.018

Syed J. H., Malik R. N., Li J., Chaemfa C., Zhang G. and Jones K. C. (2014). Status, distribution and ecological risk of organochlorines (OCs) in the surface sediments from the Ravi River, Pakistan. *The Science of the Total Environment*, **472**, 204–211, https://doi.org/10.1016/j.scitotenv.2013.10.109

Tariq M. I., Afzal S., Hussain I. and Sultana N. (2007). Pesticides exposure in Pakistan: a review. *Environment International*, **33**(8), 1107–1122, https://doi.org/10.1016/j.envint.2007.07.012

Terzaghi E., Zanardini E., Morosini C., Raspa G., Borin S., Mapelli F., Vergani L. and Di Guardo A. (2018). Rhizoremediation half-lives of PCBs: role of congener composition, organic carbon forms, bioavailability, microbial activity, plant species and soil conditions, on the prediction of fate and persistence in soil. *The Science of the Total Environment*, **612**, 544–560, https://doi.org/10.1016/j.scitotenv.2017.08.189

Thijs S., Sillen W., Rineau F., Weyens N. and Vangronsveld J. (2016). Towards an enhanced understanding of plant microbiome interactions to improve phytoremediation: engineering the metaorganism. *Frontiers in Microbiology*, **7**, 341, https://doi.org/10.3389/fmicb.2016.00341

Van Hees P. A., Jones D. L., Finlay R., Godbold D. L. and Lundström U. S. (2005). The carbon we do not seed the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. *Soil Biology and Biochemistry*, **37**(1), 1–13, https://doi.org/10.1016/j.soilbio.2004.06.010

Vancura V. and Hovadik A. (1965). Root exudates of plants: II. Composition of root exudates of some vegetables. *Plant and Soil*, **22**, 21–32, https://doi.org/10.1007/BF01377686

Vergani L., Mapelli F., Zanardini E., Terzaghi E., Di Guardo A., Morosini C., Raspa G. and Borin S. (2017). Phytorhizoremediation of polychlorinated biphenyl contaminated soils: an outlook on plant-microbe beneficial interactions. *The Science of the Total Environment*, **575**, 1395–1406, https://doi.org/10.1016/j.scitotenv.2016.09.218

Vos J. G., Dybing E., Greim H. A., Ladefoged O., Lambré C., Tarazona J. V., Brandt I. and Vethaak A. D. (2000). Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Critical Reviews in Toxicology*, **30**(1), 71–133, https://doi.org/10.1080/10408440091159176

Walker T. S., Bais H. P., Grotewold E. and Vivanco J. M. 2003a. Root exudation and rhizosphere biology. *Plant Physiology*, **132**(1), 44–51, https://doi.org/10.1104/pp.102.019661

Walker T. S., Bais H. P., Halligan K. M., Stermitz F. R. and Vivanco J. M. 2003b. Metabolic profiling of root exudates of *Arabidopsis thaliana*. *Journal of Agricultural and Food Chemistry*, **51**, 2548–2554, https://doi.org/10.1021/jf021166h

Walton B. T. and Anderson T. A. (1990). Microbial degradation of trichloroethylene in the rhizosphere: potential application to biological remediation of waste sites. *Applied and Environmental Microbiology*, **56**(4), 1012–1016, https://doi.org/10.1128/aem.56.4.1012-1016.1990

Yadav D. V., Mittal P. K., Agarwal H. C. and Pillai M. K. (1981). Organochlorine insecticide residues in soil and earthworms in the Delhi area, India, AugusteOctober, 1974. *Pesticides Monitoring Journal*, **15**(2), 80–85.

Yee D. C., Maynard J. A. and Wood T. K. (1998). Rhizoremediation of trichloroethylene by a recombinant, root-colonizing *Pseudomonas fluorescens* strain expressing toluene ortho-monooxygenase constitutively. *Applied and Environmental Microbiology*, **64**(1), 112–118, https://doi.org/10.1128/AEM.64.1.112-118.1998

Yoshitomi K. J. and Shann J. R. (2001). Corn (*Zea mays* L.) root exudates and their impact on 14C-pyrene mineralization. *Soil Biology and Biochemistry*, **33**, 1769–1776, https://doi.org/10.1016/S0038-0717(01)00102-X

Yuste L., Canosa I. and Rojo F. (1998). Carbon-source-dependent expression of the PalkB promoter from the *Pseudomonas oleovorans* alkane degradation pathway. *Journal of Bacteriology*, **180**(19), 5218–5226, https://doi.org/10.1128/JB.180.19.5218-5226.1998

Zablotowicz R. M., Hoagland R. E. and Locke M. A. (1994). Glutathione S-transferase activity in rhizosphere bacteria and the potential for herbicide detoxification. In: Bioremediation Through Rhizosphere Technology, T. A. Anderson and J. R. Coats (eds.), American Chemical Society, Washington, DC, pp. 184–198.

Zhang G., Chakraborty P., Li J., Sampathkumar P., Balasubramanian T., Kathiresan K., Takahashi S., Subramanian A., Tanabe S. and Jones K. C. (2008). Passive atmospheric sampling of organochlorine pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers in urban, rural, and wetland sites along the coastal length of India. *Environmental Science and Technology*, **42**(22), 8218–8223, https://doi.org/10.1021/es8016667

Zhang Y. and Miller R. M. (1994). Effect of a *Pseudomonas* rhamnolipid biosurfactant on cell hydrophobicity and biodegradation of octadecane. *Applied and Environmental Microbiology*, **60**(6), 2101–2106, https://doi.org/10.1128/aem.60.6.2101-2106.1994

Zhang H. B., Luo Y. M., Wong M. H., Zhao Q. G. and Zhang G. L. (2007). Concentrations and possible sources of polychlorinated biphenyls in the soils of Hong Kong. *Geoderma*, **138**(3–4), 244–251, https://doi.org/10.1016/j.geoderma.2006.11.016

doi: 10.2166/9781789063783_0231

Chapter 12

Role of microorganisms in reclaiming 1,4-dioxane-contaminated sites: perspective analysis

Anil Kumar Singh[1] *and Ria Rautela Rana*[2]

[1]*Department of Agriculture, Sant Baba Bhag Singh University, Khiala, Punjab 144030, India*
[2]*Gujarat Ecological Education and Research (GEER) Foundation, Gandhinagar, Gujarat 382007, India*
**Corresponding author: anilimtech@gmail.com*

ABSTRACT

1,4-Dioxane is an emerging organic pollutant with suspected carcinogenic effect. It is produced as a by-product in several petrochemical-based industrial processes. It has also been utilized as a stabilizer in several chlorinated solvents. Physical and chemical properties of 1,4-dioxane has facilitated widespread contamination of land, surface, and groundwater resources. The extensive occurrence of 1,4-dioxane has highlighted the need for developing treatment methods that remediate contaminated water sources and prevent human exposure to 1,4-dioxane through drinking water. Removing 1,4-dioxane from contaminated sites and refurbishing it to uncontaminated condition is a challenging task. Remediation of 1,4-dioxane-contaminated sites can be achieved by physical, chemical, and biological processes. However, large-scale *in situ* remediation is not feasible by physical and chemical processes. Exploiting metabolic capabilities of 1,4-dioxane utilizing microorganisms for restoration and reclamation of contaminated sites is often seen as a most realistic option. Microorganisms can metabolize or co-metabolize 1,4-dioxane and bring about bioremediation of contaminated sites. The present chapter provides an overview and future perspective of microorganism-assisted reclamation of 1,4-dioxane-polluted sites. This chapter also discusses chemistry of dioxane, toxicity, and fate of dioxane in environment, reclamation strategies, different factors influencing reclamation, dioxane-degrading monooxygenase enzymes, and microbial degradation pathways.

Keywords: carcinogenic, biostimulation, bioattenuation, bioaugmentation, monooxygenases.

12.1 INTRODUCTION

Environment pollution is a serious problem faced by mankind. In simple words, environment pollution can be explained as the introduction of any contaminant that has the ability to adversely affect the biological, physical, and chemical properties of natural surroundings. Broadly speaking, pollution can have natural or anthropogenic origin. Natural pollution is caused by natural sources such as volcanic eruption, release of methane from paddy fields, and cattle. Generally, pollution caused by natural sources does not execute a severe hazardous effect on environment. In comparison to natural pollutants, man-made pollutants cause more serious problems as they have persistent nature and ability to get transported long distances. Owing to their persistent nature, man-made pollutants stay longer in the natural environment and enter the food chain and thus, affect the health of animals and humans. Man-made pollutants can also alter microbe-assisted natural processes by adversely affecting microbial population and diversity.

Man-made pollution can be inorganic or organic in nature. Inorganic pollutants cannot be degraded but transformed from toxic to non-toxic forms. Contrarily, organic pollutants can be degraded to simpler and non-toxic forms by natural processes. In spite of degradability, pollution due to man-made organic pollutants has become a common feature of present day human society. Unplanned and excessive expansion of industries has added an implausible amount of organic pollutants to air, soil, and water. Modern agriculture practice uses several agrochemicals that can adversely alter environmental condition by adding organic pollutants to the surroundings. In developing countries, aggressive agriculture practices, overpopulation, facile rule enforcement, and unplanned urbanization have augmented the problem of environment pollution.

Generally under natural conditions, organic pollutants are mitigated from environment by physical, chemical, and biological degradation processes. Degradation of organic pollutants under natural conditions may take a few hours to several years. Pollutants with short half-life do not cause any serious problem to environment. Some of the pollutants with short half-life may produce more toxic and persistent degradation intermediates resulting in a serious secondary pollution (Gheorghe & Ion, 2011). Contrarily, organic pollutants with longer half-life get accumulated in environment causing serious problems to all living organisms. Persistent man-made pollutants are long lasting, penetrable, and unbreakable making them more vulnerable to humans as well as all other life forms on earth. Their ability to get transported longer distances has made it a global problem. Moreover, their presence in the food chain has brought a lot of worries to mankind. Organic pollutants have infiltrated through the lowest member of the food chain making their way to the highest member. Gradual accumulation of organic pollutants in environment alters population dynamics and disturbs interaction of organisms at various tropic levels. This further aggravates to disturb the natural community structure of the polluted ecosystem. Man-made organic pollutants may have immunotoxicity, neurotoxicity, carcinogenicity, mutagenicity, and endocrine disruption potentials toward animals (Ossai *et al*., 2020). Plants are also adversely affected by man-made organic pollutants. In recent past, organic pollutants have drawn scientific and political apprehension due to their wide distribution, bioaccumulation behavior, and potential harmful effects.

Conventionally, remediation of organic pollutant-contaminated sites can be achieved by physical, chemical, and biological processes (Ossai *et al*., 2020). Very often single process fails to achieve a desirable level of toxic neutralization due to recalcitrant nature of pollutant and generation of toxic degradation intermediates. In such scenario, combination of different processes helps in achieving a desirable level of toxic neutralization.

Use of biological systems such as microorganisms, plants, and their products for reclamation is a sustainable and cost-effective method (Ossai *et al*., 2020). Reclamation of organic pollutant-contaminated sites by biodegradation and bioremediation techniques uses metabolic capabilities of living organisms for rendering hazardous pollutants to non-hazardous forms (Ossai *et al*., 2020). Despite decades of research, successful reclamation of organic pollutant-contaminated sites by biological means remains a challenging task. 1,4-Dioxane is one such organic pollutant that has contaminated soil and water resources at several places throughout the world. It is often suspected as a carcinogenic pollutant. The Agency for Toxic Substances and Disease Registry (ATSDR) included 1,4-dioxane as one of the priority substances for removal from drinking water (ATSDR, 2017).

This chapter focuses on microorganism-assisted reclamation of 1,4-dioxane-polluted sites. It also discusses the chemistry of 1,4-dioxane, distribution in environment, toxicity, reclamation strategies, microbial degradation pathways, 1,4-dioxane-degrading monooxygenase, and factors influencing reclamation.

12.2 CHEMICAL NATURE OF DIOXANE

1,4-Dioxane (CAS no. 123-91-1) is a synthetic heterocyclic organic compound with faint sweet odor. It has a molar mass of 88.106 g/mol and its chemical formula is $C_4H_8O_2$. 1,4-Dioxacyclohexane is a systematic International Union of Pure and Applied Chemistry (IUPAC) name of 1,4-dioxane. In 1,4-dioxane, oxygen atom is directly positioned opposite to each other and this results in the formation of stable symmetric ether linkage (Surprenant, 2000). It is simply known as dioxane because the other dioxane isomers (1,2- and 1,3-) are rarely encountered. 1,4-Dioxane structure is very stable to reaction with acids, oxides, and oxidizing agents (Surprenant, 2000). Unlike other organic pollutants, 1,4-dioxane is highly miscible in water.

Historically, 1,4-dioxane was used as a stabilizer in chlorinated industrial solvents. It is extensively used as a solvent during the production of a variety of organic chemicals present in deodorants, dyes, paints, antifreeze, shampoos, and cosmetics. 1,4-Dioxane is also part of pesticide formulations, food additives, and food-packaging adhesives (Surprenant, 2000).

12.3 DISTRIBUTION OF 1,4-DIOXANE IN THE ENVIRONMENT

Extensive use and unregulated disposal has made 1,4-dioxane contamination a global problem. 1,4-Dioxane can contaminate air, water, and soil (Godri Pollitt *et al.*, 2019). It is non-volatile, hence chances of air pollution by 1,4-dioxane is very rare. Moreover, in the gaseous phase, 1,4-dioxane has half-life of 35 h due to photo-oxidation. It has high water solubility of nearly 100 mg/mL, hence chances of water getting polluted is very high. Federal agencies in the United States have recognized 1,4-dioxane as emerging drinking water contaminant at several sites (Godri Pollitt *et al.*, 2019). Anderson *et al.* (2012) reported that 1,4-dioxane is present in 17.4% of the groundwater samples examined by their group. 1,4-Dioxane has been reported as a second most prevalent prior contaminant in public water supplies of United the States (Godri Pollitt *et al.*, 2019). The presence of 1,4-dioxane in the natural water system has also been reported from several other countries such as Japan, Korea, Canada, United Kingdom, and Germany. The occurrence of 1,4-dioxane in the groundwater has been often associated with other chlorinated solvents such as 1,1,1-trichloroethane (1,1,1-TCA), 1,1-dichloroethane (1,1-DCA), and trichloroethylene (Adamson *et al.*, 2015).

Consumer products can also become a source of 1,4-dioxane exposure and pollution. Personal care products containing ethoxylated surfactants may have 1,4-dioxane as contaminate. Black *et al.* (2001) reported the presence of 1,4-dioxane in various cosmetic products (279 mg/kg) and children's shampoo (85 mg/kg) used in the United States. It is estimated that women have greater chances of 1,4-dioxane exposure than men or children (Godri Pollitt *et al.*, 2019). The high exposure is mainly attributed to inhalation of the volatilized 1,4-dioxane from personal care products. Rarely, 1,4-dioxane get absorbed in the body during topical application of 1,4-dioxane-contaminated personal care product.

1,4-Dioxane adsorb weakly into mineral phases and organic matter (Jackson & Dwarakanath, 1999). This causes 1,4-dioxane to easily leach into water systems and subsurface environments. 1,4-Dioxane has half-life of 2–5 years and 56 days in groundwater and surface water, respectively (Adamson *et al.*, 2015). Shorter half-life of 1,4-dioxane in surface water as compared to subsurface water may be linked to microbial degradation in the former environment (Godri Pollitt *et al.*, 2019). Microbial population and diversity is relatively more in surface water as compared to subsurface water. Thus, there are more chances of microbial degradation of 1,4-dioxane in surface water bodies.

12.4 TOXIC EFFECT OF DIOXANE

1,4-Dioxane is documented hazardous substance for humans and animals (Göen *et al.*, 2016). The United States Environment Protection Agency has classified dioxane as a probable human carcinogen. International Agency for Research on Cancer has classified dioxane as a group 2B agent. This means it is possibly carcinogenic to humans due to the inadequate evidence in humans but sufficient research evidence in experimental animals for carcinogenicity. The average half-life of 1,4-dioxane in human body is approximately 1 h (Göen *et al.*, 2016). An 8 h of inhalation can result in the detection of 1,4-dioxane and 2-hydroxyethoxyacetic acid (HEAA) within first few hours of exposure. Dioxane and HEAA can also be detected in urine after 4 and 24 h after exposure, respectively (Göen *et al.*, 2016).

Studies have shown that dioxane primarily affect liver and kidney (Godri Pollitt *et al.*, 2019). Oral administration of dioxane-contaminated water to rats and mice induced dose-dependent liver toxicity, degeneration of hepatic cells, and development of pre-neoplastic lesions (Kano *et al.*, 2009; Stott *et al.*, 1981). Chronic exposure of dioxane-contaminated drinking water has also been reported to adversely affect kidney (EPA 2013; Woo *et al.*, 1977). As evident from Figure 12.1, 1,4-dioxane metabolism results in the formation of HEAA and diglycolic acids. HEAA formation takes place through multiple pathways occurring in liver, kidney, and nasal mucosa (Woo *et al.*, 1977). Cytochrome P450 plays a key role in converting 1,4-dioxane to HEAA and diglycolic acids (Nannelli *et al.*, 2005). Another view is that initially dioxane is metabolized to diethylene glycol and then converted to 2-hydroxyethoxyacetaldehyde which is rapidly metabolized to HEAA (CDCP, 2017; Schier *et al.*, 2013).

Toxicological studies in rats and mice demonstrated that 1,4-dioxane increased the occurrence of tumors in these animals (Kano *et al.*, 2009). Chronic exposure through inhalation or ingestion induces tumor formation in liver and nasal cavity (Dourson *et al.*, 2017; Kano *et al.*, 2009). However, *in vitro* studies did not exhibit any genotoxic effects even after prolong exposure to dioxane (EPA, 2013). Undoubtedly, dioxane can induce cancer but the mechanisms responsible for carcinogenic effect are not very clear (EPA, 2013). HEAA is a primary metabolite detected in urine after exposure to dioxane. HEAA is not known to have high toxic or carcinogenic effect on humans. Other metabolites such as diethylene glycol and diglycolic acid are known to have toxic effect on liver and kidney (Landry *et al.*, 2011, 2015; Schier *et al.*, 2013).

Oral administration of dioxane has also been reported to result in the formation of cytochrome P450 isoforms. Cytochrome P450 isoforms along with toxic metabolites such as diethylene glycol and diglycolic acid can cause hepatic tumor formation (Nannelli *et al.*, 2005). Co-contaminates of dioxane such as 1,1,1-TCA and 1,1-DCA are known carcinogens in animals. Metabolism of co-contaminates

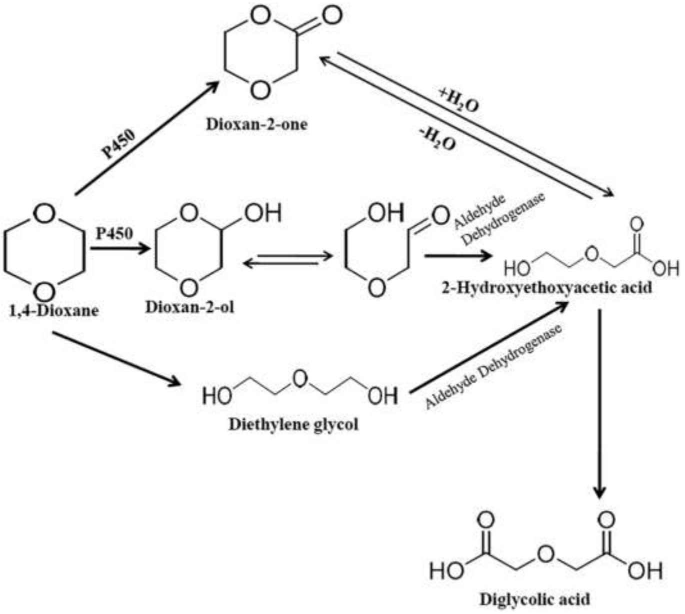


Figure 12.1 Proposed metabolic degradation pathways of 1,4-dioxane in humans (Woo *et al.*, 1977).

produces metabolites that can induce cancer in liver and kidney. Some researchers are of the opinion that dioxane acts as a tumor promoter rather than tumor inducer (Inoue *et al.*, 1989).

12.5 STRATEGIES FOR RECLAIMING DIOXANE-CONTAMINATED SITES

The distinctive physical and chemical characteristics of 1,4-dioxane confer challenges in the removal and reclamation of contaminated sites (Stepien *et al.*, 2014). Effective remediation of contaminated sites must convert toxic pollutants to non-toxic or less toxic form, approximately to pre-polluted conditions (Kumar *et al.*, 2015). The methods selected for remediation play a crucial role in complete removal, cleaning, reclamation, and restoration of 1,4-dioxane-contaminated sites. Unlike most of the organic pollutants, dioxane exhibits good water solubility as a result it favors partitioning into the aqueous phase making common physical separation methods such as air stripping or adsorption inefficient (Godri Pollitt *et al.*, 2019). Dioxane removal by membrane filtration is also not very effective. Linares *et al.* (2011) reported that reverse osmosis is not efficient in removing dioxane from contaminated water. Oxidation is considered as a most effective method for removing dioxane from contaminated sites (Godri Pollitt *et al.*, 2019). Dioxane oxidation can be achieved by both abiotic and biotic processes.

Conventional oxidation methods are not effective due to the formation of more toxic degradation intermediates. An advanced oxidation process that uses reactive radicals such as hydroxyl ($^{\cdot}OH$) and sulfate radicals ($SO_4^{\cdot -}$) has been demonstrated to mineralize 1,4-dioxane (Lee *et al.*, 2015). As evident from Figure 12.2, degradation of dioxane by advanced oxidation processes results in the formation of low molecular weight fatty acids such as glycolic, formic acid, glyoxal, acetic, and oxalic acids

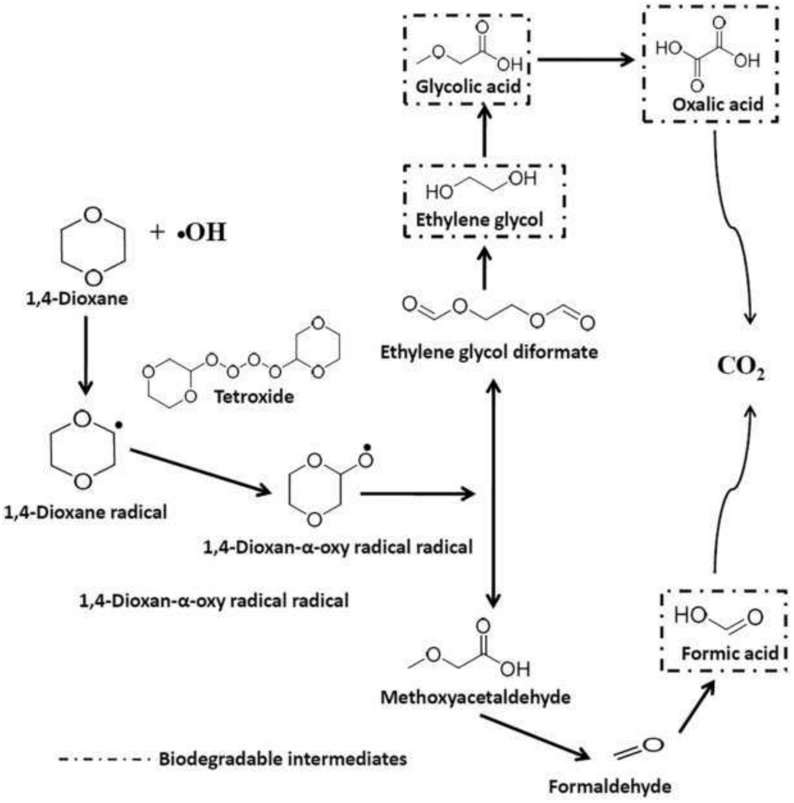


Figure 12.2 1,4-Dioxane degradation by advanced oxidation processes. Molecules shown in the dotted box can be assimilated by microorganisms under available natural conditions.

(Merayo *et al.*, 2014). Low molecular weight fatty acids can be removed by conventional biological treatments (Merayo *et al.*, 2014). However, feasibility of applying advanced oxidation processes at the contaminated sites is a challenging task (Lee *et al.*, 2015). Oxidation of dioxane can also be carried out in electrochemical cells. However, electrochemical oxidation is expensive and lacks selectivity. Boron-doped diamond electrodes used in electrochemical cells are expensive (Jasmann *et al.*, 2016). Photocatalytic advanced oxidation processes coupled with membrane filtration has also been demonstrated to efficiently remove dioxane from contaminated water. Extensive application of the abovementioned method is restricted due to high-energy requirement for generating ultraviolet and pressure (Hwangbo *et al.*, 2019).

Oxidation by biological means is a most suitable method for *in situ* removal of dioxane from contaminated sites due to environment friendly and inexpensive nature. Biological oxidation utilizes metabolic capabilities of microorganisms to transform toxic contaminates to benign form. Biodegradation, bioattenuation, biostimulation, and bioaugmentation have been demonstrated as effective strategies for *in situ* removal of 1,4- dioxane from contaminated sites (Godri Pollitt *et al.*, 2019). Unlike aforementioned abiotic strategies, biological methods of 1,4-dioxane removal do not require high-energy input or require expensive set ups. Moreover, biological degradation methods do not generate any toxic degradation intermediates (Godri Pollitt *et al.*, 2019).

12.6 DIOXANE-DEGRADING MICROORGANISMS AND DEGRADATION PATHWAYS

Dioxane is a man-made compound, hence microorganisms dwelling in soil and water have not encounter it in their pristine natural surroundings. Thus, chances of finding 1,4-dioxane degrader in natural environment are grim. Contrarily, in the past few decades, several researchers have reported isolation of 1,4-dioxane-degrading microorganisms from environmental samples (Kim *et al.*, 2009; Parales *et al.*, 1994). Probably, microorganisms have developed 1,4-dioxane utilization ability due to exposure to compounds with similar structure. Dioxane-degrading bacteria have been reported to degrade other cyclic ethers such as tetrahydrofuran (THF) and some naturally occurring ether compounds (Godri Pollitt *et al.*, 2019). For instance, cyclic ethers containing 5- and 6-membered rings are extensively present in natural environment. These cyclic ethers are utilized by microorganisms for biosynthesizing certain antibiotics (Martın *et al.*, 2014). Microorganisms having capacity to degrade these antibiotics have survival advantage over other non-degrading microbes (Topp *et al.*, 2013). Several dioxane- and THF-degrading microorganisms produce lactones as degradation intermediates (Sales *et al.*, 2013). Under natural conditions microorganisms encounter lactones as chemical signal molecules for accomplishing quorum sensing (Rehman & Leiknes, 2018). Many bacteria produce lactones degrading enzymes to disrupt signals and subsequently interfere with quorum sensing. Disrupting quorum sensing enables microorganisms to compete against the bacteria that produce them (Rehman & Leiknes, 2018). Aforementioned finding suggests that biodegradation of simple cyclic ethers such as THF and dioxane by microorganisms may be a survival outcome of backward evolution of catabolic pathways. Lactones assimilating microorganisms growing under depleted lactone conditions are likely to experience selective pressure for transforming other compounds such as dioxane into lactones as their survival strategy (He *et al.*, 2017).

Microorganisms can degrade 1,4-dioxane by metabolizing it as a source of carbon and energy (Godri Pollitt *et al.*, 2019). There are also reports that some microorganisms co-metabolize 1,4-dioxane while growing on other carbon sources such as propane, THF, toluene, and methane (He *et al.*, 2017). 1,4-Dioxane is assimilated as a primary substrate and co-substrate in metabolizing and co-metabolizing microorganisms, respectively (Figure 12.3). As 1,4-dioxane co-metabolizing microorganisms do not use co-substrates as major carbon or energy sources, they can often degrade co-substrates to concentrations well below those that can be achieved by organisms that metabolize and grow on these co-substrates.

Pseudonocardia dioxanivorans CB1190 is well-studied 1,4-dioxane-degrading bacteria. Initially, it was isolated as *Actinomycete* CB1190 from industrial-activated sludge (Parales *et al.*, 1994). Strain

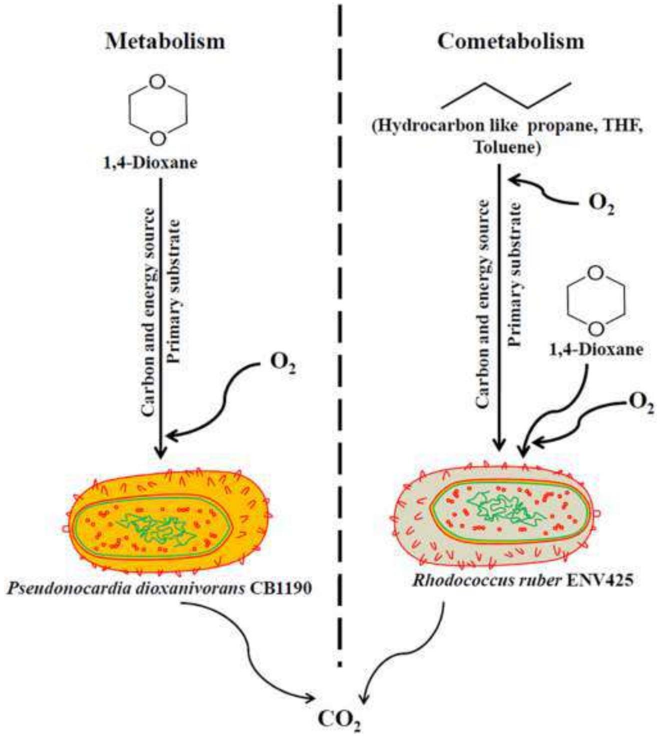


Figure 12.3 Simple illustration of microbial metabolism and co-metabolism of 1,4-dioxane.

CB1190 can use 1,4-dioxane as a sole carbon and energy source. Strain CB1190 has capability to metabolize 1,4-dioxane at the rate of 0.33 mg dioxane/h/mg protein (Parales *et al.*, 1994).

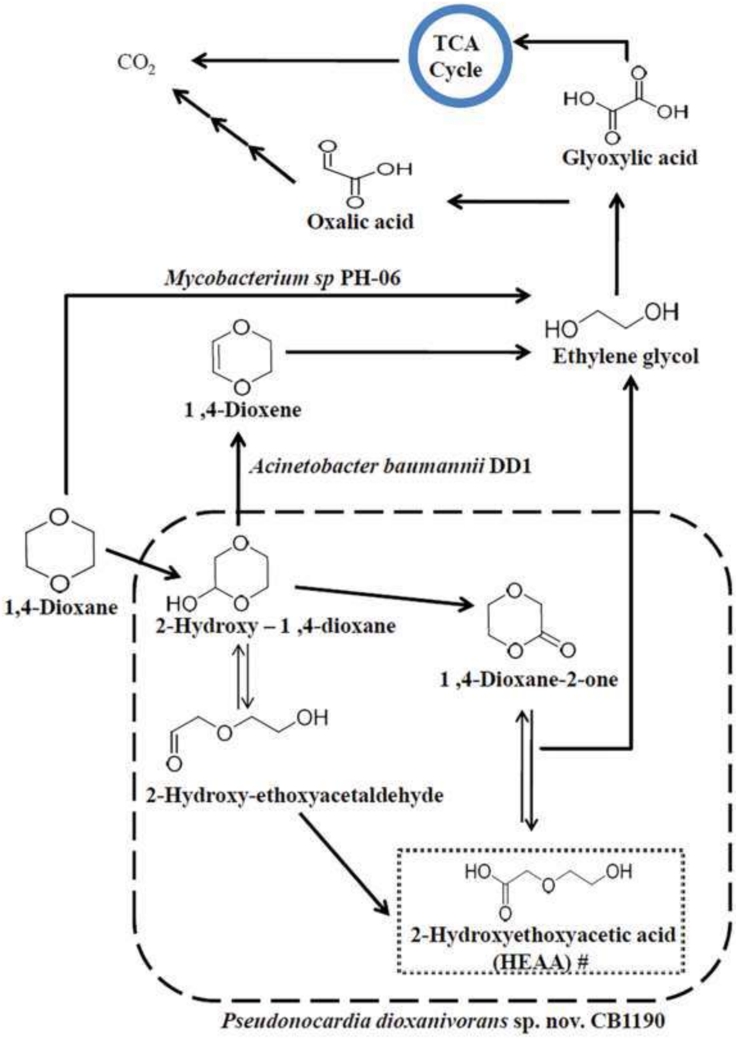
1,4-Dioxane metabolizing *Mycobacterium* sp. PH-06 was isolated from contaminated river sediments (Kim *et al.*, 2009). Strain PH-06 has ability to metabolize 1,4-dioxane by the formation of 1,4-dioxane-2-ol and ethylene glycol as major metabolites. Sei *et al.* (2013) reported isolation of 1,4-dioxane metabolizing *Afipia* sp. D1 from drainage of a chemical factory. Strain D1 was able to tolerate co-contaminant ethylene glycol when grown on 1,4-dioxane (Sei *et al.*, 2013). Vainberg *et al.* (2006) reported isolation of *Pseudonocardia* sp. strain ENV478 by enrichment culturing of liquor collected from a membrane bioreactor system that was treating wastes of fine chemical manufacturer. Strain ENV478 degraded 1,4-dioxane to HEAA at a rate of 21 mg/h/g total suspended solids. 1,4-Dioxane degradation was initiated after growth on THF, sucrose, lactate, yeast extract, 2-propanol, and propane (Vainberg *et al.*, 2006).

Aoyagi *et al.* (2018) used stable isotope probing (SIP) of rRNA for identifying active and diverse 1,4-dioxane-degrading microorganisms in activated sludge. There group reported a total of 9 OTUs that increased the expression of 16S rRNAs after SIP incubation, thus indicating their involvement in ^{13}C-labeled 1,4-dioxane degradation. During the study, Aoyagi *et al.* (2018) observed *P. dioxanivorans*, an ammonia-oxidizing bacterium and phylogenetically novel bacteria as dioxane degraders.

Generally, contaminated environment is believed to be a good source for isolating pollutant-degrading microorganisms. Contrarily, He *et al.* (2017) reported isolation of two consortia from a pristine environment. Consortia were capable of removing dioxane even at a low concentration of 300 μg/L within 3 days. Consortium A was primarily constituted by *Aminobacter* sp., *Ralstonia* spp, *Chitinophaga* spp, *Cupriavidus* spp, *Afipia* spp, *Ensifer* spp, and *Sediminibacterium* spp. Consortium B was mainly composed of *Chitinophaga* spp, *Aminobacter* spp, *Terrimonas* spp, *Acidovorax* spp, *Variovorax* spp, *Brevundimonas* spp, and *Sediminibacterium* spp (He *et al.*, 2017).

Most of the 1,4-dioxane degradation studies have been reported under aerobic conditions. There are very few studies showing anaerobic 1,4-dioxane degradation (Godri Pollitt *et al.*, 2019). Shen *et al.* (2008) reported anaerobic growth of iron-reducing bacterium in 1,4-dioxane-containing medium. In one of the recent microcosm studies, Ramalingam and Cupples (2020) reported anaerobic biodegradation of 1,4-dioxane in sediments collected from contaminated sites. Research was also carried out in uncontaminated agricultural soils and river sediments. Most efficient 1,4-dioxane biodegradation was reported in the nitrate-amended treatments. The soil microcosms were primarily dominated by *Rhodanobacter* with less abundance of *Pseudomonas*, *Mycobacterium*, and *Acinetobacter* (Ramalingam & Cupples, 2020). Study by Ramalingam and Cupples (2020) also highlighted the fact that natural attenuation is suitable for *in situ* cleanup technology when time is not constrained.

Literature suggests that bacteria are more dominant 1,4-dioxane degrader compared to fungi (Godri Pollitt *et al.*, 2019). Nakamiya *et al.* (2005) reported isolation of 1,4-dioxane-metabolizing



#*Pseudomonas mendocina* KR1 and *Pseudocardia sp* strain ENV478 produce HEAA as degradation end product while cometabolizing 1,4-dioxane.

Figure 12.4 Simple illustration of biological degradation pathways of 1,4-dioxane. The degradation intermediates shown in the pathways are key recognized products observed during microbial degradation of 1,4-dioxane.

fungus *Cordyceps sinensis* strain A from garden soil of the National Institute for Environmental Studies. Strain A exhibited optimum growth on 0.034 M 1,4-dioxane. Fungal metabolism of 1,4-dioxane resulted in the formation of ethylene glycol, glycolic acid, and oxalic acid as degradation intermediates. Strain A was also able to utilize several other cyclic ethers such as THF, tetrahydropyran, 1,3-dioxane, and *s*-trioxane (Nakamiya *et al.*, 2005). Fungi have also been known to exhibit co-metabolic degradation of 1,4-dioxane (Skinner *et al.*, 2009). Growth of *Aureobasidium pullulans* and *Graphium* sp. on THF has been reported to induce 1,4-dioxane degradation ability in both fungi (Patt & Abebe, 1995; Skinner *et al.*, 2009). Skinner *et al.* (2009) reported that propane and THF induced monooxygenase activity in fungus *Graphium* sp. and degraded 1,4-dioxane. Propane-grown mycelia of fungus *Graphium* sp. could consume 50% of 1 mM 1,4-dioxane within 12 h of exposure (Skinner *et al.*, 2009).

Microbial metabolism as well as co-metabolism of 1,4-dioxane result in the formation of degradation intermediates such as 2-HEAA, 1,4-dioxane-2-ol, ethylene glycol, glycolic acid, glyoxylic acid, and oxalic acid (Figure 12.4). Glyoxylic acid or oxalic acid is further consumed in the tricarboxylic acid cycle to complete biodegradation of 1,4-dioxane (Nakamiya *et al.*, 2005). Overall, the 1,4-dioxane biodegradation pathway suggests that microbial metabolites generated during degradation are unlikely to have persistent or toxic nature (McElroy *et al.*, 2019).

12.7 RECLAMATION OF CONTAMINATED SITES BY *IN SITU* BIOREMEDIATION

Exploiting *in situ* bioremediation for reclaiming 1,4-dioxane-contaminated sites is simplest, cost-efficient, environmentally safe, and sustainable method. Godri Pollitt *et al.* (2019) highlighted *in situ* biodegradation of 1,4-dioxane as the best method of removing contamination from large area and diluted plume. *Ex situ* technologies are expensive and inefficient under environmental conditions. However, successful field-scale accomplishment using bioremediation to remove 1,4-dioxane are still scarce. 1,4-Dioxane metabolizing and 1,4-dioxane co-metabolizing microorganisms can be exploited for reclaiming contaminated sites (Godri Pollitt *et al.*, 2019).

Microorganisms metabolizing 1,4-dioxane do not require additional nutrient amendments, oxygen requirement is also low, and chances of aquifer getting clogged due to excessive growth is very less. However, plumes with low 1,4-dioxane concentration may not support growth of microorganisms, and thus restricting the effectiveness of bioremediation with the aid of 1,4-dioxane-metabolizing microbes. Contrarily, 1,4-dioxane co-metabolizing microorganisms can carry out bioremediation even at low concentrations of 1,4-dioxane. Co-metabolizing microorganisms degrade 1,4-dioxane as a co-substrate by utilizing primary substrates for growth and reproduction (Godri Pollitt *et al.*, 2019). 1,4-Dioxane co-metabolizing microorganisms can be employed in biostimulation and bioaugmentation for removing 1,4-dioxane from contaminated sites (Zenker *et al.*, 2004). There are several microorganisms that can co-metabolize and degrade dioxane (Godri Pollitt *et al.*, 2019; Vainberg *et al.*, 2006). Mahendra and Alvarez-Cohen (2006) reported that *Burkholderia cepacia* G4 degraded 1,4-dioxane at a rate of 0.1 ± 0.006 mg/h/mg protein by toluene-induced monooxygenase. *Pseudonocardia* sp. strain ENV478 degraded 1,4-dioxane by utilizing THF as the primary growth substrate (Vainberg *et al.*, 2006).

According to most of the guidelines safe groundwater must have dioxane concentrations below 1 g/L. However, achieving such low concentrations by exploiting microbial metabolic capabilities is a challenging task. Microbial growth rate at environmentally relevant dioxane concentrations is very slow. Dioxane co-metabolizing microorganisms help in overcoming this hurdle as they utilize primary substrates for growth and reproduction. Rolston *et al.* (2019) reported that *Rhodococcus rhodochrous* strain ATCC 21198 could transform low and environmentally relevant concentrations of dioxane while growing on isobutene. Microcosms constructed with aquifer solids from Fort Carson, Colorado were biostimulated with isobutane and inorganic nutrients. Biostimulation of native microorganisms help in transforming dioxane to acceptable concentration. Bioaugmentation with strain 21198 also helped to achieve acceptable 1,4-dioxane concentration in the microcosms (Rolston *et al.*, 2019).

Biostimulation enhances microbial degradation activity by addition of microbial growth supporting material and optimizing of various parameters that limits remediation at contaminated sites (Wu *et al.*, 2016). Li *et al.* (2010) reported 1,4-dioxane (50 mg/L) degradation in microcosm consisting of contaminated groundwater and soil from Alaska. Abiotic processes such as evaporation, adsorption, and diffusion into fine grain caused 15.0 ± 8.7% removal of 1,4-dioxane over a period of 6 months. Biosimulation of indigenous bacteria by adding 1-butanol and inorganic nutrients significantly enhanced the removal of 1,4-dioxane. This observation by Li *et al.* (2010) suggests that Arctic groundwater samples contain 1,4-dioxane degraders.

Bioattenuation exploits a variety of chemical, physical, and biological processes to remove, neutralize, transform, and reduce toxicity or concentration of dioxane at contaminated sites (Ossai *et al.*, 2020). Indigenous microbial population degrades or transforms dioxane to less toxic form depending upon their metabolic capabilities. Bioattenuation is useful at contaminated sites having low concentration of dioxane, where adopting other remediation methods is not feasible and time is not a limiting factor (Vásquez-Murrieta *et al.*, 2016). Li *et al.* (2015) demonstrated natural attenuation of dioxane in groundwater microcosms. A site impacted by 1,1,1-TCA and 1,1-dichloroethene (1,1-DCE) exhibited nearly 100% removal of dioxane in 20 weeks (Li *et al.*, 2015). Natural attenuation has been demonstrated to play a key role in cleaning dioxane-contaminated sites located in California (Adamson *et al.*, 2015).

Bioaugmentation involves the addition of exogenous microbial cultures, microbial consortia, or genetically engineered microorganisms for removing dioxane from contaminated sites. Microorganisms selected for bioaugmentation must be adapted to the environmental conditions prevalent at contaminated sites and have proven record to degrade dioxane. Kelley *et al.* (2001) reported that poplar root extract (40 mg/L) stimulated dioxane degradation in soil bioaugmented with *Amycolata* sp. CB1190. Within 45 days of incubation nearly 100 mg/L dioxane was removed from contaminated soil. Bioaugmentation of microcosms with *P. dioxanivorans* CB1190 exhibited rapid removal of dioxane at a rate of 0.16 ± 0.04 mg dioxane/day/mg protein (Li *et al.*, 2010). In another experiment, Li *et al.* (2014) carried out a bench-scale microcosm study in 1,4-dioxane-contaminated groundwater samples collected from California. The highest rate of 1,4-dioxane removal in the microcosm study was reported to be 3449 ± 459 μg/L/week (Li *et al.*, 2014).

In one of the microcosm studies, Lippincott *et al.* (2015) applied propane biosparging and *Rhodococcus ruber* ENV425 bioaugmentation to enhance *in situ* biodegradation of dioxane at Vandenberg Air Force Base site. Biosparging and bioaugmentation demonstrated dioxane decay rate in the range of 0.021–0.036/day. Dioxane concentration in wells reduced from 1090 to <2 μg/L after the reclamation process (Lippincott *et al.*, 2015).

Microbial consortia have also been reported to remove 1,4 dioxane from contaminated samples (Saul, 2012; Xiong *et al.*, 2020). Microbial consortia-based bioremediation of 1,4-dioxane is often considered better than pure culture-based remediation. Biological reactors used for water treatments comprise microbial consortia as maintaining pure culture free from contamination is very difficult. Also some bacteria are unable to completely mineralize 1,4-dioxane and produce degradation intermediates. These intermediates can be further mineralized by microbial members of consortia (Xiong *et al.*, 2020). Saul (2012) patented a method for removing 1,4-dioxane from contaminated groundwater with the help of microbial consortia. Bacterial consortium consisting of *Pseudomonas putida* strain B, *P. putida* stain E, and *Pseudomonas fluorescens* strain G was able to co-metabolize 1,4-dioxane and chlorinated solvents (Saul, 2012).

12.8 FACTORS INFLUENCING RECLAMATION OF PETROLEUM HYDROCARBON-CONTAMINATED SITES

Biological cleaning of 1,4-dioxane-contaminated sites is influenced by several biotic and abiotic factors. Microorganisms employed for cleaning of contaminated sites is the most important biotic factor influencing reclamation processes. In natural ecosystems, microbial community grows together in a synergistic relationship and produce bioactive metabolites (including oxidative and hydrolytic enzymes) to implicate the mineralization of dioxane (Adamson *et al.*, 2015; Li *et al.*, 2014). Selection of microorganisms for *in situ* remediation is decided keeping in view the environmental conditions and locally available resources. Several microcosm studies have been carried out to understand the influence of environmental conditions on reclamation processes (Li *et al.*, 2010; Lippincott *et al.*, 2015). Apart from nutrients, pH, temperature, oxygen, heavy metals, and co-contaminates are important abiotic factors influencing reclamation processes.

Oxygen is prerequisite for aerobic biodegradation of 1,4-dioxane. There are very few reports suggesting anaerobic biodegradation of 1,4-dioxane (Ramalingam & Cupples, 2020; Shen *et al.*, 2008). Generally, a low concentration of dissolved oxygen particularly in groundwater (below 3 mg/L) significantly reduces the efficiency of bioremediation (Lee *et al.*, 2014). Da Silva *et al.* (2020) observed that bioaugmentation efficiency increased by aeration of flow-through aquifer columns. To overcome problem arising due to anoxia, oxygen-releasing compounds (such as calcium dioxide, magnesium peroxide) are added to enhance *in situ* biodegradation (Kao *et al.*, 2003). However, such additions are likely to alter pH and adversely affect degradation processes. Lee *et al.* (2014) designed oxygen-releasing alginate beads (ORABs) to overcome oxygen deficiency during 1,4-dioxane degradation. In the presence of ORABs, 99.0% and 68.1% degradation of 1,4-dioxane was achieved under batch and column conditions, respectively. Batch and column without ORABs showed 34.3% and 18% degradation of 1,4-dioxane, respectively (Lee *et al.*, 2014).

The pH and temperature of growth medium or surrounding medium affect 1,4-dioxane-cleaning processes by influencing microbial growth, oxygen availability, and enzyme activity (Lee *et al.*, 2014). He *et al.* (2017) reported maximum 1,4-dioxane degradation by microbial consortium at pH 7 and 30°C. Generally, temperature supporting optimum microbial growth is conducive for pollutant degradation. Most of the studies have reported that 30°C as suitable temperature for 1,4-dioxane degradation (He *et al.*, 2017; Inoue *et al.*, 2018). Dioxane degradation at low temperatures (14 and 4°C) has been reported by Li *et al.* (2010). At low temperature (4°C), microcosms demonstrated a lag period of 3 months before a notable degradation of 1,4-dioxane (Li *et al.*, 2010). Inoue *et al.* (2018) reported that *Rhodococcus aetherivorans* JCM 14343 can degrade 1,4-dioxane at a wide range of temperatures (5–40°C) and pH (4–9). Strain JCM 14343 exhibited maximum degradation rate at pH 7 and 28°C (Inoue *et al.*, 2018). *Pseudonocardia* sp. N23 exhibited 1,4-dioxane degradation under a wide pH (3.8–8.2) and temperature (20–35°C) range. Maximum 1,4-dioxane degradation was observed at pH 7 and 30°C (Inoue *et al.*, 2018).

Microbial encounter with metals cannot be ruled out in natural environment. Usually, metal toxicity hinders microbial growth and reduces or even prevents biodegradation of pollutants (Inoue *et al.*, 2020). High metal concentrations in growth medium execute cellular toxicity and suppress enzymatic activity. Transition metals namely Co(II), Cu(II), Fe(II), Mo(VI), Ni(II), and Zn(II) reduce rate of dioxane degradation (Inoue *et al.*, 2020). Johnson *et al.* (2020) reported that *Mycobacterium austroafricanum* JOB5 can metabolize dioxane in planktonic pure cultures and biofilms even in the presence of 10 mg/L of Cr(VI).

Metal ions may not always have inhibitory effect on dioxane degradation. Inoue *et al.* (2020) reported that Fe(III) enhanced 1,4-dioxane degradation by *Mycobacterium* sp. D6 and *R. aetherivorans* JCM 14343. The presence of Mn(II) in growth medium enhanced 1,4-dioxane degradation by Pseudonocardia sp. D17 and Pseudonocardia sp. N23. Most of the 1,4-dioxane degraders have soluble di-iron monooxygenases (SDIMOs) to catalyze initial steps of biodegradation. Transition metals such as Mn and Fe act as cofactors in the catalytic site of SDIMOs (Inoue *et al.*, 2020).

1,4-Dioxane degradation is significantly affected by co-contaminates such as 1,1-DCE, *cis*-1,2-DCE, trichloroethene, and Cu(II) ions (Adamson *et al.*, 2015; Li *et al.*, 2015; Zhao *et al.*, 2018). The co-occurring compounds can influence reclamation processes by altering the activity of 1,4-dioxane-degrading enzymes. Zhao *et al.* (2018) observed that co-contaminants, 1,1-DCE and Cu(II) ions inhibited 1,4-dioxane degradation in soil columns. Cyclic ethers analogues of 1,4-dioxane are known to induce competitive inhibition in degradative enzymes (Mahendra *et al.*, 2013). Co-contaminates can inhibit dioxane biodegradation via complex mechanisms. It is believed that competition, damage of dioxane-degrading enzymes (i.e., SDIMOs), suppression of dioxane-degrading gene expression, and toxic effects on dioxane-degrading bacteria can inhibit 1,4-dioxane degradation (Mahendra *et al.*, 2013).

Some compounds (such as butanol and propane) can induce dioxane degradation (Hand *et al.*, 2015). Hand *et al.* (2015) reported 1-butanol as a better inducer compared to propane for *in situ* bioaugmentation of 1,4-dioxane-contaminated groundwater. *Mycobacterium vaccae* JOB5 and *Rhodococcus jostii* RHA1 were grown in Reasoner's 2A broth medium until an optical density of 0.8–1.0 was achieved. Bacterial cells collected from RHA1 medium were induced for expressing either butane- or propane-monooxygenases by exposing them to 1-butanol or propane, respectively. Bacterial cells induced by butane performed better than propane-induced cells (Hand *et al.*, 2015).

12.9 SOLUBLE DI-IRON MONOOXYGENASES

The microbial degradation pathways indicate that the rate-limiting step in 1,4-dioxane biodegradation is the initial oxidation of the carbon atom adjacent to the oxygen atom. Oxidation of carbon results in the cleavage of high-energy C–O bond of cyclic ether. Initial oxidation of 1,4-dioxane is catalyzed by monooxygenase enzymes known as SDIMOs. Monooxygenases are defined as a group of enzymes that catalyze the incorporation of one atom of molecular oxygen into a substrate, with the other being reduced to water. Dioxane biodegradation in metabolizing and co-metabolizing microorganisms is initiated by SDIMOs. 1,4-Dioxane-oxidizing SDIMOs are divided into six groups based on the arrangement of components, sequence similarity, and substrate specificity. However, monooxygenase enzymes responsible for metabolizing 1,4-dioxane are limited to group-5 (dioxane/THF monooxygenase) and group-6 SDIMOs (propane monooxygenase) (He *et al.*, 2017; Ramos-Garcia *et al.*, 2018). Mahendra and Alvarez-Cohen (2006) reported the presence of other two types of SDIMOs namely soluble methane monooxygenases and toluene monooxygenases in some dioxane-co-metabolizing microbial strains. SDIMOs are family of multi-component enzymes consisting of three or four components namely an oxygenase, a reductase, a coupling protein (in most cases), and additional ferredoxin (in several cases). An active site of SDIMOs contains a di-iron center (Li *et al.*, 2013).

In renowned dioxane degrader *P. dioxanivorans* CB1190, monooxygenase enzyme (THF monooxygenase or dioxane monooxygenase) expressing gene cluster *thmADBC* is located on plasmid pPSED02 (Sales *et al.*, 2013). Mahendra and Alvarez-Cohen (2006) reported *Escherichia coli* TG1/pBS(Kan) containing recombinant plasmids derived from toluene monooxygenases of *B. cepacia* G4, *Pseudomonas mendocina* KR1, and *Ralstonia pickettii* PKO1 was able to co-metabolize dioxane by utilizing toluene as a primary substrate. The recombinant strains transformed 0.17–0.60 mg of 1,4-dioxane/h/mg protein (Mahendra & Alvarez-Cohen, 2006).

Genes encoding monooxygenase enzymes have been used as biomarkers for detecting 1,4-dioxane degradation (Li *et al.*, 2013). This approach is effective for monitoring the known microorganisms employing monooxygenase-based 1,4-dioxane degradation. However, this approach cannot report new and unknown 1,4-dioxane degraders that lack monooxygenase-encoding genes or possess some novel degradation pathway involving new types of the genes (Aoyagi *et al.*, 2018). He *et al.* (2018) did not observe THF or dioxane monooxygenase gene in bacterial consortia that has ability to utilize dioxane as a carbon and energy source.

12.10 CONCLUSIONS

1,4-Dioxane has not only polluted land but also groundwater and surface water at several places throughout the world. Effective measures must be taken to manage and reclaim dioxane-contaminated sites. Among limited treatment options available, use of biological processes for remediating dioxane-contaminated sites is the most effective and environment-friendly option. Most of the biodegradation studies reported to date have focused on laboratory-cultured microorganisms. Dioxane-contaminated environment may contain many unknown and unidentified microorganisms. Theses dioxane degraders can play a critical role in the remediation of 1,4-dioxane and associated co-contaminants. Phylogenetic diversity of 1,4-dioxane degraders makes it difficult to identify functional microorganisms with a single biomarker. Use of modern approaches such as metagenomics and high-throughput sequencing can aid in discovery of novel degrading microorganisms, enzymes, and biotransformation pathways.

REFERENCES

Adamson D. T., Anderson R. H., Mahendra S. and Newell C. J. (2015). Evidence of 1,4-dioxane attenuation at groundwater sites contaminated with chlorinated solvents and 1,4-dioxane. *Environmental Science & Technology*, **49**, 6510–6518, https://doi.org/10.1021/acs.est.5b00964

Anderson R. H., Anderson J. K. and Bower P. A. (2012). Co-occurrence of 1,4-dioxane with trichloroethylene in chlorinated solvent groundwater plumes at US Air Force installations: fact or fiction. *Integrated Environmental Assessment and Management*, **8**, 731–737, https://doi.org/10.1002/ieam.1306

Aoyagi T., Morishita F., Sugiyama Y., Ichikawa D., Mayumi D., Kikuchi Y., Ogata A., Muraoka K., Habe H. and Hori T. (2018). Identification of active and taxonomically diverse 1,4-dioxane degraders in a full-scale activated sludge system by high-sensitivity stable isotope probing. *The ISME Journal*, **12**, 2376–2388, https://doi.org/10.1038/s41396-018-0201-2

ATSDR (2017). Support document to the 2017 substance priority list (candidates for toxicological profiles). Division of Toxicology and Human Health Sciences, Atlanta, GA.

Black R. E., Hurley F. J. and Havery D. C. (2001). Occurrence of 1,4-dioxane in cosmetic raw materials and finished cosmetic products. *Journal of AOAC International*, **84**, 660–670, https://doi.org/10.1093/jaoac/84.3.666

Centers for Disease Control and Prevention (2017). National health and nutrition examination survey: volatile organic compounds (VOCs) and trihalomethanes/MTBE-blood-special sample (VOCWBSH). 2013–2014 Data Documentation, Codebook, and Frequencies.

Da Silva M. L. B., He Y., Mathieu J. and Alvarez P. J. J. (2020). Enhanced long-term attenuation of 1,4-dioxane in bioaugmented flow-through aquifer columns. *Biodegradation*, **31**, 201–211, https://doi.org/10.1007/s10532-020-09903-0

Dourson M. L., Higginbotham J., Crum J., Burleigh-Flayer H., Nance P., Forsberg N. D., Lafranconi M. and Reichard J. (2017). Update: mode of action (MOA) for liver tumors induced by oral exposure to 1,4-dioxane. *Regulatory Toxicology and Pharmacology*, **88**, 45–55, https://doi.org/10.1016/j.yrtph.2017.02.025

EPA US (2013). Toxicological Review of 1,4-Dioxane (with Inhalation Update). United States Environmental Protection Agency, Washington, DC.

Gheorghe I. F. and Ion B. (2011). The effects of air pollutants on vegetation and the role of vegetation in reducing atmospheric pollution. In: The Impact of Air Pollution on Health, Economy, Environment and Agricultural Sources, M. K. Khallaf (ed.), Intech Croatia, 241–280.

Godri Pollitt K. J., Kim J. H., Peccia J., Elimelech M., Zhang Y., Charkoftaki G., Hodges B., Zucker I., Huang H., Deziel N. C., Murphy. K., Ishii M., Johnson C. H., Boissevain A., O'Keefe E., Anastas P. T., Orlicky D., Thompson D. C. and Vasiliou V. (2019). 1,4-Dioxane as an emerging water contaminant: state of the science and evaluation of research needs. *Science of the Total Environment*, **690**, 853–866, https://doi.org/10.1016/j.scitotenv.2019.06.443

Göen T., von Helden F., Eckert E., Knecht U., Drexler H. and Walter D. (2016). Metabolism and toxicokinetics of 1,4-dioxane in humans after inhalational exposure at rest and under physical stress. *Archives of Toxicology*, **90**, 1315–1324, https://doi.org/10.1007/s00204-015-1567-9

Hand S., Wang B. and Chu K.-H. (2015). Biodegradation of 1,4-dioxane: effects of enzyme inducers and trichloroethylene. *Science of the Total Environment*, **520**, 154–159, https://doi.org/10.1016/j.scitotenv.2015.03.031

He Y., Mathieu J., da Silva M. L. B., Li M. and Alvarez P. J. J. (2018). 1,4-Dioxane-degrading consortia can be enriched from uncontaminated soils: prevalence of *Mycobacterium* and soluble di-iron monooxygenase genes. *Microbial Biotechnology*, **11**(1), 189–198, https://doi.org/10.1111/1751-7915.12850

Hwangbo M., Claycomb E. C., Liu Y., Alivio T. E. G., Banerjee S. and Chu K. H. (2019). Effectiveness of zinc oxide-assisted photocatalysis for concerned constituents in reclaimed wastewater: 1,4-dioxane, trihalomethanes, antibiotics, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs). *Science of the Total Environment*, **649**, 1189–1197, https://doi.org/10.1016/j.scitotenv.2018.08.360

Inoue O., Seiji K., Kawai T., Jin C., Liu Y. T., Chen Z., Cai S. X., Yin S. N., Li G. L. and Nakatsuka H. (1989). Relationship between vapor exposure and urinary metabolite excretion among workers exposed to trichloroethylene. *American Journal of Industrial Medicine*, **15**, 103–110, https://doi.org/10.1002/ajim.4700150111

Inoue D., Tsunoda T., Yamamoto N., Ike M. and Sei K. (2018). 1,4-Dioxane degradation characteristics of *Rhodococcus aetherivorans* JCM 14343. *Biodegradation*, **29**(3), 301–310, https://doi.org/10.1007/s10532-018-9832-2

Inoue D., Tsunoda T., Sawada K., Yamamoto N., Sei K. and Ike M. (2020). Stimulatory and inhibitory effects of metals on 1,4-dioxane degradation by four different 1,4-dioxane-degrading bacteria. *Chemosphere*, **238**, 124606, https://doi.org/10.1016/j.chemosphere.2019.124606

Jackson R. E. and Dwarakanath V. (1999). Chlorinated decreasing solvents: physical-chemical properties affecting aquifer contamination and remediation. *Groundwater Monitoring & Remediation*, **19**, 102–110, https://doi.org/10.1111/j.1745-6592.1999.tb00246.x

Jasmann J. R., Borch T., Sale T. C. and Blotevogel J. (2016). Advanced electrochemical oxidation of 1,4-dioxane via dark catalysis by novel titanium dioxide (TiO_2) pellets. *Environmental Science & Technology*, **50**, 8817–8826, https://doi.org/10.1021/acs.est.6b02183

Johnson N. W., Gedalanga P. B., Zhao L., Gu B. and Mahendra S. (2020). Cometabolic biotransformation of 1,4-dioxane in mixtures with hexavalent chromium using attached and planktonic bacteria. *Science of the Total Environment*, **706**, 135734, https://doi.org/10.1016/j.scitotenv.2019.135734

Kano H., Umeda Y., Kasai T., Sasaki T., Matsumoto M., Yamazaki K., Nagano K., Arito H. and Fukushima S. (2009). Carcinogenicity studies of 1,4-dioxane administered in drinking-water to rats and mice for 2 years. *Food and Chemical Toxicology*, **47**, 2776–2784, https://doi.org/10.1016/j.fct.2009.08.012

Kao C. M., Chen S. C., Wang J. Y., Chen Y. L. and Lee S. Z. (2003). Remediation of 20 PCE-contaminated aquifer by an in situ two-layer biobarrier: laboratory batch 21 and column studies. *Water Research*, **37**, 27–38, https://doi.org/10.1016/S0043-1354(02)00254-3

Kelley S. L., Aitchison E. W., Deshpande M., Schnoor J. L. and Alvarez P. J. J. (2001). Biodegradation of 1,4-dioxane in planted and unplanted soil: effect of bioaugmentation with *Amycolata* sp CB1190. *Water Research*, **35**, 3791–3800, https://doi.org/10.1016/S0043-1354(01)00129-4

Kim Y. M., Jeon J. R., Murugesan K., Kim E. J. and Chang Y. S. (2009). Biodegradation of 1,4-dioxane and transformation of related cyclic compounds by a newly isolated *Mycobacterium* sp. PH-06. *Biodegradation*, **20**, 511–519, https://doi.org/10.1007/s10532-008-9240-0

Kumar R., Das A. J. and Juwarkar A. A. (2015). Reclamation of petrol oil contaminated soil by rhamnolipids producing PGPR strains for growing *Withania somnifera* a medicinal shrub. *World Journal of Microbiology and Biotechnology*, **31**, 307–313, https://doi.org/10.1007/s11274-014-1782-1

Landry G. M., Martin S. and McMartin K. E. (2011). Diglycolic acid is the nephrotoxic metabolite in diethylene glycol poisoning inducing necrosis in human proximal tubule cells in vitro. *Toxicological Sciences*, **124**, 35–44, https://doi.org/10.1093/toxsci/kfr204

Landry G. M., Dunning C. L., Abreo F., Latimer B., Orchard E. and McMartin K. E. (2015). Diethylene glycol-induced toxicities show marked threshold dose response in rats. *Toxicology and Applied Pharmacology*, **282**, 244–251, https://doi.org/10.1016/j.taap.2014.12.010

Lee C. S., Le Thanh T., Kim E. J., Gong J., Chang Y. Y. and Chang Y. S. (2014). Fabrication of novel oxygen-releasing alginate beads as an efficient oxygen carrier for the enhancement of aerobic bioremediation of 1,4-dioxane contaminated groundwater. *Bioresource Technology*, **171**, 59–65, https://doi.org/10.1016/j.biortech.2014.08.039

Lee K. C., Beak H. J. and Choo K. H. (2015). Membrane photoreactor treatment of 1,4-dioxane containing textile wastewater effluent: performance, modeling, and fouling control. *Water Research*, **86**, 58–65, https://doi.org/10.1016/j.watres.2015.05.017

Li M. Y., Fiorenza S., Chatham J. R., Mahendra S. and Alvarez P. J. J. (2010). 1,4-Dioxane biodegradation at low temperatures in Arctic groundwater samples. *Water Research*, **44**, 2894–2900, https://doi.org/10.1016/j.watres.2010.02.007

Li M., Mathieu J., Yang Y., Fiorenza S., Deng Y., He Z., Zhou J. and Alvarez P. J. (2013). Widespread distribution of soluble di-iron monooxygenase (SDIMO) genes in Arctic groundwater impacted by 1,4-dioxane. *Environmental Science & Technology*, **47**, 9950–9958, https://doi:10.1021/es402228x

Li M., Van Orden E. T., DeVries D. J., Xiong Z., Hinchee R. and Alvarez P. J. (2015). Bench-scale biodegradation tests to assess natural attenuation potential of 1,4-dioxane at three sites in California. *Biodegradation*, **26**, 39–50, https://doi.org/10.1007/s10532-014-9714-1

Linares R. V., Yangali-Quintanilla V., Li Z. and Amy G. (2011). Rejection of micropollutants by clean and fouled forward osmosis membrane. *Water Research*, **45**, 6737–6744, https://doi.org/10.1016/j.watres.2011.10.037

Lippincott D., Streger S. H., Schaefer C. E., Hinkle J., Stormo J. and Steffan R. J. (2015). Bioaugmentation and propane biosparging for in situ biodegradation of 1,4-dioxane. *Groundwater Monitoring & Remediation*, **35**, 81–92, https://doi.org/10.1111/gwmr.12093

Mahendra S. and Alvarez-Cohen L. (2006). Kinetics of 1,4-dioxane biodegradation by monooxygenase-expressing bacteria. *Environment Sciences Technology*, **40**, 5435–5442, https://doi:10.1021/es060714v

Mahendra S., Grostern A. and Alvarez-Cohen L. (2013). The impact of chlorinated solvent co-contaminants on the biodegradation kinetics of 1,4-dioxane. *Chemosphere*, **91**, 88–92, https://doi.org/10.1016/j.chemosphere.2012.10.104

Martın T., Padron J. I. and Martın V. S. (2014). Strategies for the synthesis of cyclic ethers of marine natural products. *Synlett*, **25**, 12–32.

McElroy A. C., Hyman M. R. and Knappe D. R. U. (2019). 1,4-Dioxane in drinking water: emerging for 40 years and still unregulated. *Current Opinion in Environmental Science & Health*, **7**, 117–125, https://doi.org/10.1016/j.coesh.2019.01.003

Merayo N., Hermosilla D., Cortijo L. and Blanco A. (2014). Optimization of the Fenton treatment of 1,4-dioxane and on-line FTIR monitoring of the reaction. *Journal of Hazardous Materials*, **268**, 102–109, https://doi.org/10.1016/j.jhazmat.2014.01.008

Nakamiya K., Hashimoto S., Ito H., Edmonds J. S. and Morita M. (2005). Degradation of degradation of 1,4-dioxane and cyclic ethers by an isolated fungus. *Applied and Environmental Microbiology*, **71**, 1254–1258, https://doi.org/10.1128/AEM.71.3.1254-1258.2005

Nannelli A., De Rubertis A., Longo V. and Gervasi P. G. (2005). Effects of dioxane on cytochrome P450 enzymes in liver, kidney, lung and nasal mucosa of rat. *Archives of Toxicology*, **79**, 74–82, https://doi.org/10.1007/s00204-004-0590-z

Ossai I. C., Ahmed A., Hassan A. and Hamid F. S. (2020). Remediation of soil and water contaminated with petroleum hydrocarbon: a review. *Environmental Technology & Innovation*, **17**, 100526, https://doi.org/10.1016/j.eti.2019.100526

Parales R. E., Adamus J. E., White N. and May H. D. (1994). Degradation of 1,4-dioxane by an *Actinomycete* in pure culture. *Applied and Environmental Microbiology*, **60**, 4527–4530, https://doi.org/10.1128/aem.60.12.4527-4530.1994

Patt T. E. and Abebe H. M. (1995). Microbial degradation of chemical pollutants; Patent Number: 5399495.

Ramalingam V. and Cupples A. M. (2020). Anaerobic 1,4-dioxane biodegradation and microbial community analysis in microcosms inoculated with soils or sediments and different electron acceptors. *Applied Microbiology and Biotechnology*, **104**, 4155–4170, https://doi.org/10.1007/s00253-020-10512-3

Ramos-Garcia A. A., Shankar V., Saski C. A., Hsiang T. and Freedman D. L. (2018). Draft genome sequence of the 1,4-dioxane-degrading bacterium *Pseudonocardia dioxanivorans* BERK-1. *Genome Announcements*, **6**, e00207–18, https://doi.org/10.1128/genomeA.00207-18

Rehman Z. U. and Leiknes T. (2018). Quorum-quenching bacteria isolated from Red Sea sediments reduce biofilm formation by *Pseudomonas aeruginosa*. *Frontiers in Microbiology*, **9**, 1354, https://doi.org/10.3389/fmicb.2018.01354

Rolston H. M., Hyman M. R. and Semprini L. (2019). Aerobic cometabolism of 1,4-dioxane by isobutane-utilizing microorganisms including *Rhodococcus rhodochrous* strain 21198 in aquifer microcosms: experimental and modeling study. *Science of the Total Environment*, **694**, 133688, https://doi.org/10.1016/j.scitotenv.2019.133688

Sales C. M., Grostern A., Parales J. V., Parales R. E. and Alvarez-Cohen L. (2013). Oxidation of the cyclic ethers 1,4-dioxane and tetrahydrofuran by a monooxygenase in two *Pseudonocardia* species. *Applied and Environmental Microbiology*, **79**, 7702–7708, https://doi.org/10.1128/AEM.02418-13

Saul M. T. (2012). Bioaugmentation to remediate dioxane in groundwater. US20100140168 A1.

Schier J. G., Hunt D. R., Perala A., McMartin K. E., Bartels M. J., Lewis L. S., McGeehin M. A. and Flanders W. D. (2013). Characterizing concentrations of diethylene glycol and suspected metabolites in human serum, urine, and cerebrospinal fluid samples from the Panama DEG mass poisoning. *Clinical Toxicology*, **51**, 923–929, https://doi.org/10.3109/15563650.2013.850504

Sei K., Miyagaki K., Kakinoki T., Fukugasako K., Inoue D. and Ike M. (2013). Isolation and 816 characterization of bacterial strains that have high ability to degrade 1,4-dioxane as a sole carbon and energy source. *Biodegradation*, **24**, 665–674, https://doi.org/10.1007/s10532-012-9614-1

Shen W., Chen H. and Pan S. (2008). Anaerobic biodegradation of 1,4-dioxane by sludge enriched with iron-reducing microorganisms. *Bioresource Technology*, **99**, 2483–2487, https://doi.org/10.1016/j.biortech.2007.04.054

Skinner K., Cuiffetti L. and Hyman M. (2009). Metabolism and cometabolism of cyclic ethers by a filamentous fungus, a *Graphium* sp. *Applied and Environmental Microbiology*, **75**, 5514–5522, https://doi.org/10.1128/AEM.00078-09

Stepien D. K., Diehl P., Helm J., Thoms A. and Puttmann W. (2014). Fate of 1,4-dioxane in the aquatic environment: from sewage to drinking water. *Water Research*, **48**, 406–419, https://doi.org/10.1016/j.watres.2013.09.057

Stott W. T., Quast J. F. and Watanabe P. G. (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. *Toxicology and Applied Pharmacology*, **60**, 287–300, https://doi.org/10.1016/0041-008X(91)90232-4

Surprenant K. S. (2000). Dioxane. In Ullmann's encyclopedia of industrial chemistry (ed.).

Topp E., Chapman R., Devers-Lamrani M., Hartmann A., Marti R., Martin-Laurent F., Sabourin L., Scott A. and Sumarah M. (2013). Accelerated biodegradation of veterinary antibiotics in agricultural soil following long-term exposure, and isolation of a sulfamethazine-degrading sp. *Journal of Environmental Quality*, **42**, 173–178, https://doi.org/10.2134/jeq2012.0162

Vainberg S., McClay K., Masuda H., Root D., Condee C., Zylstra G. J. and Steffan R. J. (2006). Biodegradation of ether pollutants by *Pseudonocardia* sp. strain ENV478. *Applied and Environmental Microbiology*, **72**, 5218–5224, https://doi.org/10.1128/AEM.00160-06

Vásquez-Murrieta M. S., Hernández-Hernández O. J., Cruz-Maya J. A., Cancino-Díaz J. C. and Jan-Roblero J. (2016). Approaches for removal of PAHs in soils: bioaugmentation, biostimulation and bioattenuation. In: Environmental Sciences. Soil Contamination – Current Consequences and Further Solutions, M. L. Larramendy and S. Soloneski (eds.), InTech, Croatia, 329–340.

Woo Y., Arcos J. C., Argus M. F., Griffin G. W. and Nishiyama K. (1977). Metabolism in vivo of dioxane: identification of *p*-dioxane-2-one as the major urinary metabolite. *Biochemical Pharmacology*, **26**, 1535–1538, https://doi.org/10.1016/0006-2952(77)90430-0

Wu M., Dick W. A., Li W., Wang X., Yang Q., Wang T., Xu L., Zhang M. and Chen L. (2016). Bioaugmentation and biostimulation of hydrocarbon degradation and the microbial community in a petroleum contaminated soil. *International Biodeterioration & Biodegradation*, **107**, 158–164, https://doi.org/10.1016/j.ibiod.2015.11.019

Xiong Y., Mason O. U., Lowe A., Zhang Z., Zhou C., Chen G., Villalonga M. J. and Tang Y. (2020). Investigating promising substrates for promoting 1,4-dioxane biodegradation: effects of ethane and tetrahydrofuran on microbial consortia. *Biodegradation*, **31**, 171–182, https://doi.org/10.1007/s10532-020-09901-2

Zenker M. J., Borden R. C. and Barlaz M. A. (2004). Biodegradation of 1,4-dioxane using trickling filter. *Journal of Environmental Engineering*, **130**, 926–931, https://doi.org/10.1061/(ASCE)0733-9372(2004)130:9(926)

Zhao L., Lu X., Polasko A., Johnson N. W., Miao Y., Yang Z., Mahendra S. and Gu B. (2018). Co-contaminant effects on 1,4-dioxane biodegradation in packed soil column flow-through systems. *Environmental Pollution*, **243**(Pt A), 573–581, https://doi.org/10.1016/j.envpol.2018.09.018

doi: 10.2166/9781789063783_0247

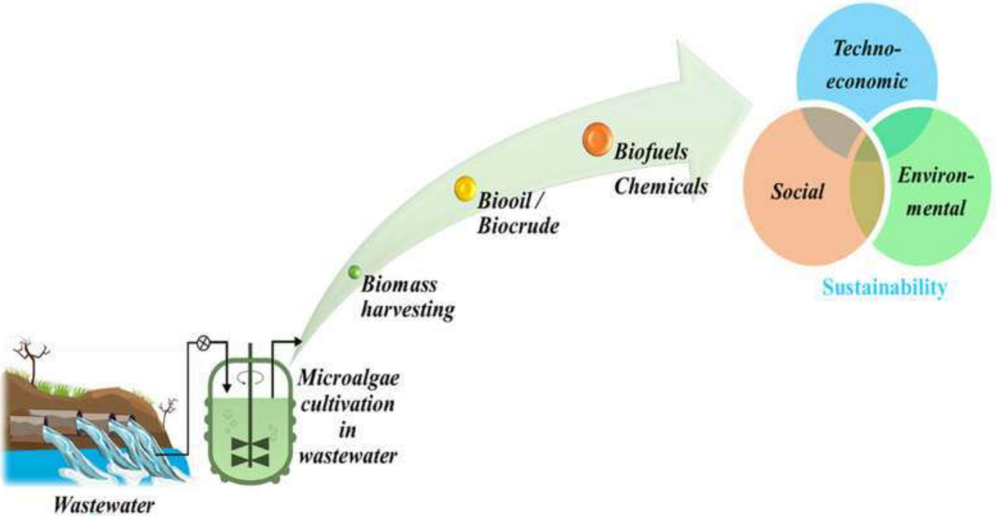
Chapter 13

Role of biomass-based biorefinery in mitigating environmental pollution

Pankaj Garkoti[1], Harshit Tiwari[1], Priyanka Sarkar[2] and Biswanath Bhunia[3]*

[1]Department of Hydro and Renewable Energy, Indian Institute of Technology, Roorkee 247667, India
[2]Department of Biotechnology, Techno India University, Salt Lake, Sector 5, Kolkata 700091, West Bengal, India
[3]Department of Bio Engineering, National Institute of Technology, Agartala 799046, India
**Corresponding author: Priyanka Sarkar. Email address: Priyanka.g@technoindiaeducation.com; priyankasarkar91@gmail.com*

ABSTRACT



About 400 trillion liters of wastewater is generated yearly due to widespread and inefficient water use. The wastewater from different sources contains many nutrients, including nitrogen, phosphorous, and carbon. Therefore, these nutrients must be recovered using appropriate sustainable processes. The selection of the proper process depends upon the type of waste and its generation. Hence, there is a need to implement a biorefinery approach to utilize organic and biological pollutants to recover energy and bioproducts. For sustainable and inexpensive wastewater treatment, microalgae-based processes have garnered considerable interest. The biomass produced by microalgae can be used in bioenergy production and other value-added products. Thus, this chapter provides state-of-the art information regarding the potential of biomass-based integrated biorefineries for effluent treatment.

Keywords: microalgae, biorefinery, wastewater treatment, bioenergy, biomass, resource recovery

The chapter is from the book *Clean Technologies Towards a Sustainable Future: Physicochemical, Biochemical and Biotechnological Approaches*, Pradeep Verma, Maulin P. Shah (Editors).

13.1 INTRODUCTION

Water is one of the essential resources on earth which plays a vital role in the survival and well-being of all living organisms (Wollmann *et al.*, 2019). In general, 97% of fresh water is found in the oceans, whereas 3% occurs in polar ice caps, glaciers, soil, and the atmosphere. However, 0.4–0.5% of freshwater is accessible for human consumption (Bureau of Reclamation, 2020). Population growth and industrialization consume vast amounts of freshwater, generating enormous volumes of effluent. Further, according to Corcoran (2010), rising population, urban expansion, and increasing agricultural and economic activities degrade natural water resources, especially in developing and developed nations. High-income nations treat an average of 70% of their produced wastewater, whereas middle and low-income countries treat 38% and 28%, respectively (United *et al.*, 2016). In addition, Qadir *et al.* (2020) reported that around 400 trillion liters of wastewater are produced annually across the world.

Wherein, Asia is the primary producer of wastewater (~160 trillion liters/year), followed by European and North American nations (~68 and 67 trillion liters/year, respectively). Moreover, Asia is a prominent producer of domestic effluent, which generates ~42% of the total effluent (Qadir *et al.*, 2020). This proportion is further projected to expand to 43.9% by the end of 2030. According to the Central Pollution Control Board, New Delhi, India, about 40 billion liters per day of wastewater was generated in 2011 (Singh *et al.*, 2016). Likewise, Bhuvaneshwari and Devika (2005) stated that the amount of effluent generation in India's metropolitan cities (~120 L/day) was twice as high as in other small cities (~60 L/day).

Wastewater can be classified according to its source, such as municipal wastewater, industrial wastewater, and agricultural runoff. However, their nutrient load varies with varying wastewater. In general, the BOD, COD, total nitrogen, and total phosphate are in the range of 27–96,000 mg/ L, 80–134,400 mg/L, 0.6–4200 mg/L, and 100–3030 mg/L, respectively. Moreover, wastewater contains various types of heavy metals such as zinc, copper, mercury, selenium, arsenic, and chromium, and so on) (Pancha *et al.*, 2019). The direct discharge of effluent into the environment endangered aquatic and terrestrial ecosystems (oxygen depletion in the aquatic environment). The presence of excessive levels of BOD, COD, total nitrogen, and total phosphate in water bodies leads to eutrophication (Ibrahim & Heikal, 2019). According to Qu and Fan (2010), about 80% of rivers in China are contaminated due to the presence of high concentrations of nitrate, phosphate, organic matter, heavy metals, and micronutrients. Eutrophication results in the proliferation of toxic microbes and damaging effects on aquatic creatures. Besides, industrial effluents such as polyaromatic hydrocarbons, petroleum chemicals, phenolic compounds, pesticides, polychlorinated biphenyls, and antibiotics, threaten terrestrial and aquatic ecosystems. According to the studies by the United Nations (WWAP, 2017), about 80% of effluent is directly discharged into open water bodies without prior treatment. Sato *et al.* (2013) reported that developed or highly developed nations treat more than 70% of industrial, and municipal wastewater, whereas developing and less-developed countries treat only 38% and 8%, respectively, due to technological availability and economic feasibility. Further, in 2012, ~13 million individuals died because of various forms of pollution, including water, air, soil, and chemical exposure (Neira & Prüss-Ustün, 2016). Therefore, each form of pollution must be treated before being released into the environment. Several previous treatments such as physical, chemical, and biological processes have been developed for the removal of toxic substances from wastewater; however, they have several disadvantages (Akubude *et al.*, 2019; Udaiyappan *et al.*, 2017). Moreover, conventional treatment methods are not equitable due to the following reasons (Abdelgadir *et al.*, 2014; Ezugbe & Rathilal, 2020; Gurung *et al.*, 2018; Li *et al.*, 2019; Pancha *et al.*, 2019; Qadir *et al.*, 2020; Sato *et al.*, 2013):

(i) It requires a high energy input (4000–3,91,000 Wh)
(ii) Time-consuming (120–1800 h)
(iii) Less efficient in nutrient removal (removal efficiency ~10–90%)
(iv) Produce an excessive amount of by-product 'sludge' that requires further treatment.

According to a report published by SAMCO (2016), the cost of wastewater treatment facilities varies from $200,000 to $700,000 at peak flow rates of 21 and 114 m^3/min, respectively. In Europe, wastewater treatment facilities consume roughly 1% of the overall electrical usage. The power used to remove 1 kg of COD ranged between 850 and 970 W-h/kg, with an average electricity cost rate of 140 $/W-h (Longo *et al.*, 2016; Panepinto *et al.*, 2016). However, advanced treatment methods require less energy (200 W-h/t) compared with conventional technologies (Li *et al.*, 2017). The demand for the development of effective and cost-effective technologies is critical in the middle- and low-income countries because of insufficient access to the above elements. Overall, conventional methods are neither environmentally viable nor capable of resolving the issue of water scarcity. Furthermore, recent research has progressively emphasized the recovery of several valuable resources from wastewater for bioenergy generation. Microalgae-mediated wastewater treatments and bioenergy generation are highly advantageous owing to its significant nutrient bioaccumulation efficiency, high biomass productivity, and biomolecule content (especially lipid and protein) that can be utilized for bioenergy production (Rawat *et al.*, 2013). Microalgae are aquatic microorganisms that utilize nutrients from multiple wastes to generate new biomass that can be used as feedstock for the bioenergy production and value-added bioproducts (Yang *et al.*, 2015). This is one of the viable techniques for sustainable socio-economic development based on the concept of circular bioeconomy.

13.2 BIOREFINERY

The increasing demand for energy and chemicals has led the researcher's attention toward renewable energy. Renewable resource utilization is the key to moving toward a sustainable economy from today's fossil fuel-based economy. It is strongly advised that biofuels (ethanol, biodiesel, butanol, Fischer–Tropsch diesel) be utilized specifically in heavy-duty vehicles and aviation. However, biofuel production is not profitable compared to current crude oil prices. The promising approach for reducing the biofuel production cost is constructing and implementing a biorefinery model. The co-production of value-added products (chemicals, materials) can reduce the production cost of biofuels by 30% using biorefinery models. International Energy Agency defines Biorefining as 'the sustainable processing of biomass into bio-based products and bioenergy.' The biorefinery model includes different steps, such as the collection of biomass, its pretreatment, hydrolysis, transformation, and product recovery. The major goal of the biorefinery concept is to replace fossil fuels and reduce negative environmental emissions produced through the disposal of organic matter. The biorefinery concept can complete the constant demand for energy, building block chemicals, and employment locally. The critical factors of a successful biorefinery are the integration of the biorefinery model with existing industrial infrastructure. The types of biorefinery are classified based on the biomass type and its origin. The wastewater biorefinery relies on the wastewater or sludge produced by households or different industries. Similarly, the agricultural, industrial, and municipal biorefinery depends on agriculture, industrial waste, municipal waste, and algae biomass, respectively. Different types of biorefineries are as follows:

(i) Agriculture-based biorefinery
(ii) Forestry-based biorefinery
(iii) Industrial waste biorefinery
(iv) Food waste biorefinery
(v) Animal waste biorefinery
(vi) Wastewater biorefinery
(vii) Plastic waste biorefinery
(viii) Algae-based biorefinery

13.3 WASTEWATER BIOREFINERY

The wastewater contains different biological, chemical, and physical pollutants; removing these pollutants is crucial to minimize water pollution and environmental security. The wastewater biorefinery aims to close the material and energy flow to improve economic and environmental sustainability. The most traditional way to produce energy from wastewater is to utilize co-produced sludge. The sludge, after thermal hydrolysis, can be fed to the anaerobic digester to produce biogas. The methane-rich biogas may be used directly in combined heat and power (CHP) units or purified to further use for transportation. The anaerobic treatment processes are the core of the wastewater biorefinery concept. The nutrients from the wastewater can be used as biofertilizers in agriculture. The nitrogen and phosphorous recovery from the wastewater are essential as it promotes eutrophication in the aquatic environment. Other than these nutrients, there is cellulosic sludge separated by upstream dynamic sieving, and polyhydroxyalkanoate (PHA) can be recovered from primary or secondary sludge. The cellulosic sludge is a feed for an anaerobic digester, and PHA can be used for the synthesis of various chemicals. Table 13.1 presents the different anaerobic bioreactor processes for treating wastewater and recovering energy as biogas. It can be noticed from the table that the anaerobic bioreactor used to treat wastewater are upflow anaerobic sludge blanket reactor (UASB), fixed-bed reactor (FBR), anaerobic baffled reactor (ABR), anaerobic moving biofilm reactor (AMBR), and continuous stirred tank reactor (CSTR) which have COD removal efficiency within range of 80%–95%. The biogas production from the UASB is observed to be more than other types of reactors using municipal or domestic wastewater (Akyol *et al.*, 2020; Hu *et al.*, 2018).

The other way to generate energy from wastewater is by producing biohydrogen using a dark fermentation process (Arooj *et al.*, 2007; Kim *et al.*, 2008; Mu *et al.*, 2006). Different studies have been performed to utilize wastewater for biohydrogen production. The high organic-rich cassava wastewater for biohydrogen production study shows that the increase in organic load reduces biohydrogen production. The highest hydrogen production achieved was 2.41 mol H_2/mol glucose (Mari *et al.*, 2020). A similar study for dairy wastewater showed that rate of the substrate to biohydrogen production becomes slow when protein degradation occurs. The study observed that dairy industry wastewater has low potential to generate biohydrogen (Xing *et al.*, 2010). However, adding nanoparticles (hematite or nickel oxide) to the dairy wastewater showed an improvement in biohydrogen yield by 43%. It is concluded from these studies that biohydrogen production from industrial wastewater is good from an economic point of view.

The wastewater biorefinery utilizes biological agents to remove these pollutants and utilize them to produce value-added products (ethanol, biodiesel, polylactic acid). The cultivation of microorganisms or algae in wastewater allows the removal of pollutants (a rich source of nitrogen, phosphorous, and ammonium) cost-effectively and biomass as a co-product which can be utilized for the production of bioproducts (carbohydrates, lipids, and proteins). The cultivation of *Saccharomyces cerevisiae* for olive mill wastewater treatment showed decolorization and phenol removal efficiency of about 63% and 34%, respectively. The microbial culture after treatment showed the potential for lipid and bioethanol production. The co-culturing of microalgae and bacteria for wastewater treatment has been studied in a biorefinery approach. The author concluded that the COD removal efficiency was about 82.7% and the biomass productivity was 343.3 mg/L/d.

The available methods for resource recovery from wastewater require chemicals and catalysts. Therefore, more attention is now given to the biological approach to recover resources to make the process more sustainable. The biological approach includes the utilization of different microorganisms such as bacteria, fungi, algae, and plants. Among all these, microalgae-based wastewater treatment seems to be more promising due to algae biosorption/bioaccumulation efficiency, high biomass productivity, and nutrients (nitrogen and phosphorous) utilization. The algae-based wastewater treatment has been studied by various researchers and is discussed in the next section.

Table 13.1 Anaerobic bioreactor for wastewater treatment and biogas production.

Country	Wastewater	Reactor Type	Temperature (°C)	HRT (h)	COD Removal Efficiency (%)	Methane (m^3 CH_4/kg COD Removed)	References
Spain	Municipal wastewater	UASB	18 ± 2	7	80	0.24	Gouveia *et al.* (2015)
Australia	Synthetic domestic wastewater	UAGB	20	/	91	0.16	Chen *et al.* (2017)
China	Domestic wastewater	UASB	22–25	8	92	0.45	Hu *et al.* (2018)
Poland	Synthetic dairy wastewater	AMBR	20	132	86.6	0.002	Zielińska *et al.* (2018)
Germany	Raw dairy effluent	CSTR, ABR	38	7.6	91	0.264	Jürgensen *et al.* (2018)
Spain	Real municipal wastewater	–	10–25	10	94	0.14	Peña *et al.* (2019)
United States	Municipal wastewater	–	12.7–31.5	6–20	88	0.12	Lim *et al.* (2019)
United States	Domestic wastewater	FBR	13–32	3.9 ± 1	90	0.17	Evans *et al.* (2019)
Taiwan	Domestic wastewater	FBR	20–35	12	95	0.37	Chen *et al.* (2019)
Singapore	Domestic wastewater	CSTR	30 ± 3	17	91	0.07	Xu *et al.* (2020)
Italy	Municipal wastewater	UASB	30	5–6	83	0.56	Akyol *et al.* (2020)

13.4 ALGAE-BASED WASTEWATER BIOREFINERY

The algae growth requires nutrients and water, which can be completed with wastewater. The microalgae accumulate phosphorous and nitrogen from the wastewater and utilize CO_2 for their growth using a process known as bio-fixation (Nizami *et al.*, 2017). A pilot project on palm oil mill effluent using a microalgae system with biogas production in Indonesia is designed to generate 1 MW of electricity (Ichsan *et al.*, 2014). Biohydrogen using dark fermentation is another way of generating bioenergy. Literature suggests that the wastewater treatment with microalgae reduced the COD by 70–90%. The energy consumption analysis concluded that the microalgae-based wastewater treatment consumes 10 times ($0.2\,kW/h/m^3$) less energy compared to conventional wastewater treatment ($2\,kW/h/m^3$) (Sivaramakrishnan *et al.*, 2022). The microalgae can grow in both open ponds and in the photobioreactor. The photobioreactor has a low contamination risk, easy to operate, and has high biomass productivity. Microalgae cultivation in the open pond is preferable from an economic point of view (Wijffels *et al.*, 2010). Table 13.2 presents the economic feasibility of microalgae-based wastewater treatment. The comparative economic study of microalgae cultivation in an open pond and photobioreactor shows that algae oil production in the open pond is 2.12 times cheaper than in a photobioreactor (Davis *et al.*, 2011). A biorefinery approach for producing glycerol, biogas, and biodiesel from microalgae showed that the system's payback period is around 3.3 years (Wijffels *et al.*, 2010). The production of biofuels and other value-added from microalgae depends on the composition of the strain. Therefore, selecting microalgal strains and coupling different bioprocesses to produce different bio-products becomes much more critical for the profitability of microalgal biorefinery. Freshwater-based microalgae cultivation is expensive and threatens water security. The wastewater for microalgae cultivation not only provides nutrients but also reduces the demand of freshwater, making the process cost-effective. Microalgae cultivation in wastewater and flue gas increases biomass productivity and bioenergy and bioactive compounds. It is reported that a microalgae–bacteria consortium is a novel approach to treating wastewater. The result showed that the consortia helped in reducing COD and NO_3 with greater efficiency than the monoculture. The immobilization of microalgae is another approach to treating wastewater efficiently (Goswami *et al.*, 2021). The immobilization of microalgae on sodium alginate showed 23% higher COD reduction efficiency than non-immobilized algal cells (Chu *et al.*, 2020). Table 13.2 presents different case studies and their economic feasibility. The sustainability of microalgae biorefinery can be increased if it produces multiple products such as fertilizer, fuels, food, pharmaceuticals, and cosmetics. It can be seen from the table that there are different routes to utilize the produced biomass, such as liquification, transesterification, anaerobic digestion, and pyrolysis. The utilization of biomass will produce bioproducts such as bio-oil, biochar, syngas, and biogas (Figure 13.1).

13.5 POTENTIAL PRODUCTS FROM WASTEWATER BIOREFINERY

Sewage sludge management is a cost-intensive part of the wastewater treatment process. In commercial-scale wastewater plants, the sewage sludge is usually utilized in the anaerobic digestion plants to produce biogas and utilized to produce heat and electricity, which completes the industrial plant's heat and electricity demand. However, the recovery of nutrients from the sludge in the biorefinery approach can help increase the wastewater plants' profitability.

13.5.1 Volatile fatty acid

The acidogenic fermentation of the biomass yields volatile fatty acids. The sludge from the wastewater is gaining attention of researchers as a potential feedstock for VFA production (Peces *et al.*, 2016). The VFA is a precursor for different bioproducts such as PHA, esters, ketones, alkanes, and biofuels (Lin *et al.*, 2018). Thus, makes it a high economic value-added product. The important properties of VFA are its high flexibility, low stiffness, and high tensile strength. The utilization of carbon from sludge to VFA is an energy and cost-effective strategy. However, VFA production from the sludge could reduce the energy recovery from the sludge in the form of biogas (Peces *et al.*, 2016). The optimization of both

Table 13.2 Economic feasibility of microalgae-based biorefineries.

Strain	Feedstock	Cultivation / Biomass Utilized	Value-added Products	Capital Cost (million $)	Operating Cost (million $)	NPV (million $)	Reference
Dunaliella salina	FW, CO_2, salts	OP	Fertilizer, carotene	11.87	14.20	9.22	Thomassen *et al.* (2016)
Chlorella vulgaris	Municipal WW, CO_2	PBR/PL	Bio-oil, syngas, biochar	5.35	1.24	–	Xin *et al.* (2016)
-	FW, CO_2, salts	OP/TE	Diesel	381.2	292.4	6.27/gallon	Hoffman *et al.* (2017)
Chlorella vulgaris	WW, CO_2	PBR/PL	Bio-oil Syngas Biochar	47.07	8.94	$1.85/gallon bio-oil	Xin *et al.* (2018)
Arthrospira	FW, CO_2, salts	–	Phycocyanin Polysaccharide	1273	–	Negative	Chaiklahan *et al.* (2018)
Chlorella vulgaris	WW, CO_2	OP/ LF	Bio-oil	1.56	4.19	173.82	Musa *et al.* (2019)
Synechocystis GMO	FW, CO_2, salts	PBR	Bioethanol Fertilizer Biogas	38.37	–	Negative	Lopes *et al.* (2019)
Chlorella vulgaris	WW, CO_2	OP/LF	Bio-oil	1.34	22.52	74.82	Musa *et al.* (2019)
Chlorella vulgaris	Paper WW, flue gas	OP/AD	Lipids Biogas	22.47	4.40	16.29	Silva *et al.* (2021)

FW = freshwater; WW = wastewater; OP = open pond; PL = pyrolysis; PBR = photobioreactor; TE = transesterification; LF = liquefaction; AD = anaerobic digestion.

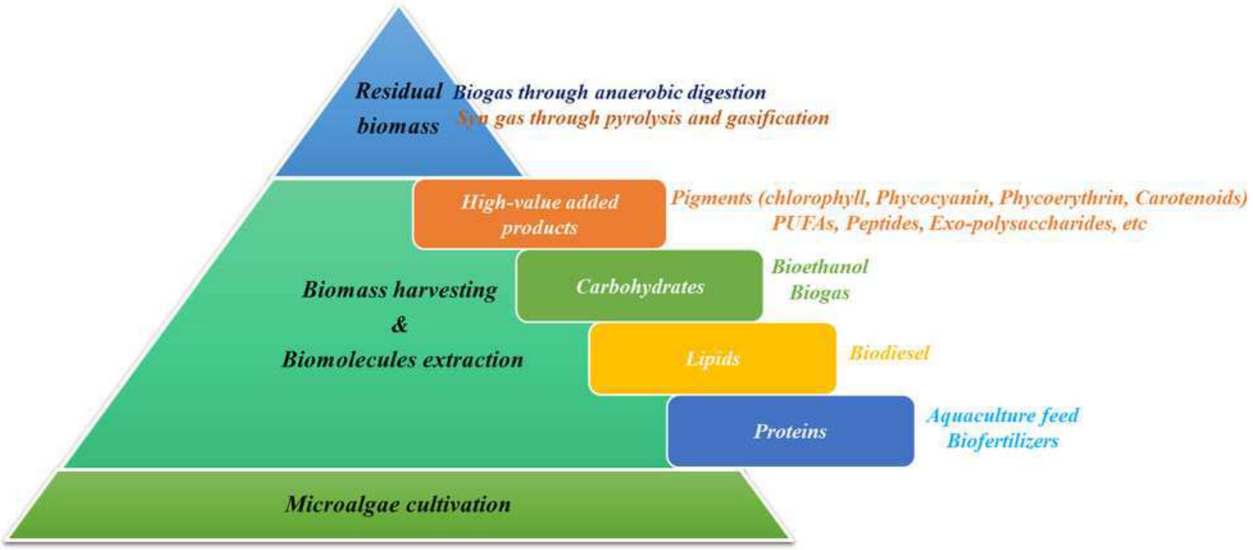


Figure 13.1 Microalgae and their by-products.

processes has been studied, and it concluded that the optimization should focus on the cost of VFA production and the impact on methane generation (Akyol *et al.*, 2020).

13.5.2 Nutrients

The energy-efficient nutrient recovery by using the biorefinery concept in wastewater can decrease the cost and increase the sustainability of wastewater treatment plants. Nitrogen and phosphorous as nutrients, PHA as a biopolymer, and cellulose are currently verified biorefinery products from wastewater treatment plants (Raheem *et al.*, 2018). The process of crystallization of phosphorous and magnesium salt usually accomplishes phosphorus recovery. The process is also known as struvite recovery. The phosphorus recovery reduces environmental disposal issues and increases the sustainability of the plant as its market value is 0.5 times of imported phosphorous (Ghosh *et al.*, 2019). The struvite as fertilizer is preferred in agricultural applications as it releases Mg, N, and P simultaneously and with slow rate than other fertilizers (Puchongkawarin *et al.*, 2015). The struvite recovery from the sludge also decreases the volume of sludge produced, which saves the sludge disposal cost of the plant. The phosphorous recovery from wastewater has been carried out commercially by various technologies such as CalPrex^{TM} reactor, AirPrex^{TM}, WASSTRIP^{TM}, and Ostara Pearl^{TM} (Akyol *et al.*, 2020). Other than phosphorous, nitrogen recovery is necessary as it also causes eutrophication. The conventional method to remove nitrogen from the wastewater is by nitrification, denitrification, and anammox process. However, the traditional process mostly focuses on the removal of nutrients rather than their recovery (Rodríguez Arredondo *et al.*, 2019). The recovered nitrogen in the form of ammonia has fertilizer application. Therefore, its recovery makes the wastewater plant a sustainable process. Various studies have used microbial fuel cells (MFC) for nitrogen recovery (Yadav *et al.*, 2022). The nitrogen recovery from the anode and cathode is achieved 47% and 83% when an output voltage of 600–700 mV and external load of $500\,\Omega$ is supplied in three-chamber MFC (Xiao *et al.*, 2016). In another study, the single-chamber MFC recovered 85 mg N/m^2 (Littfinski *et al.*, 2022). The data show that only 20% of total nitrogen is recovered through recovery (Yadav *et al.*, 2022). Various other processes are in the development phase, such as ion exchange, ammonia stripping, and nanofiltration. Microalgal-based wastewater treatment with nitrogen recovery has been studied, and algal biomass is used as animal feed (Sharma *et al.*, 2022). The recovery of these nutrients using microalgae is reported to be cost-effective. However, energy and sustainability analysis is required before implementation on a commercial scale (Khan *et al.*, 2022).

13.5.3 Polyhydroxyalkanoate

Polyhydroxyalkaonate (PHA) is produced from a renewable source and has properties similar to petroleum-based plastics (Raheem *et al.*, 2018). The advantage of PHA is its biodegradable nature and non-toxicity (Mannina *et al.*, 2020). It has been reported that PHA production cost using pure cultures is more than mixed cultures (Puyol *et al.*, 2017). The microbial cultures that produce these polymers are well-grown in activated sludge and used as a carbon source. The PHA production in full-scale activated sludge at the pilot scale reported a production efficiency of about 0.47 kg PHA/kg VSS (Bengtsson *et al.*, 2017). Various studies have reported that PHA can be produced from waste in sufficient quantities. The economic and environmental aspects of the production of PHA should be evaluated to implement the technology at a commercial scale (Fernández-Dacosta *et al.*, 2015).

13.5.4 Cellulose

Domestic or municipal wastewater contains about 30–50% cellulose fiber (Behera *et al.*, 2018). Cellulose biodegradation is difficult, but if recovered, it has a great potential to increase the economy of the plant (Crutchik *et al.*, 2018). The application of recovered fiber is in the paper industry as a building material, biofuel, and bioplastics (Van Der Hoek *et al.*, 2016). The cellulose enters the wastewater majorly in the form of toilet paper. The cellulose fiber can be recovered from the wastewater by sieving. The recovered fiber is dewatered, which minimizes chemical consumption, reduces electricity consumption, and reduces the phosphate release and volume of the sludge. The technical feasibility and energy cost to recover cellulose should be evaluated before implementation as the recovery process might affect the efficiency of the wastewater plant (Lin *et al.*, 2015).

13.6 POTENTIAL PRODUCTS FROM ALGAL BIOMASS

The wastewater treatment through microalgae will also have algae biomass other than sludge. Microalgae biomass may be used to make a variety of bio-products such as biogas, bioethanol, bio-oil, charcoal, and biodiesel. The bioproducts made from microalgae have been discussed below.

13.6.1 Biogas

Biogas production from algal biomass is more technically feasible than other bioenergy products. However, for its commercial use, the microalgae feedstock price should be decreased by 75% of its current market value (Goswami *et al.*, 2021). The produced biomass can be utilized in the anaerobic digester for biogas production. Biogas is a mixture of methane, carbon dioxide, and trace amounts of hydrogen sulfide and other gases (Singh *et al.*, 2022). The biogas production is reported to be more for the algal biomass having lipid content of 40% lower. The biogas generated may be utilized in CHP plants to generate heat and power in wastewater treatment plants. Preheating the algae biomass has been reported to increase the biomethane content in the biogas. The preheating of microalgae biomass at 70°C–90°C enhanced the biomethane production by 40% (Wang *et al.*, 2016; Wollmann *et al.*, 2019).

13.6.2 Biodiesel

The lipid is converted into biodiesel by a process known as transesterification. The process of transesterification involves the transformation of lipids into low-molecular-weight fatty acid alkyl esters (Trivedi *et al.*, 2015). The catalyst used for the reaction could be an alkaline catalyst, enzyme catalyst, heterogeneous inorganic catalyst, or acid catalyst (Goswami *et al.*, 2021). The lipid concentration in microalgal cells varies from 10% to 50% and, therefore, can be used for biodiesel production. The lipid content in the microalgal cell varies among the different species. The *N. oculate* species reported to have 16% lipid (Converti *et al.*, 2009), *C. protothecoides* have 55% lipid (Xu *et al.*, 2006), *Picochlorum* sp. contains 25% lipid (Pereira *et al.*, 2013), and *Chlorella* sp. BTA contains about 25% lipid (Mondal *et al.*, 2016). The environmental stress can increase the lipid concentration in the microalgal biomass by 38–40%. During biodiesel production, glycerol is a by-product that can be further utilized by yeast or fungi to convert it into organic acids, mannitol, or single-cell oil.

13.6.3 Bioethanol

The microalgae cells are rich in carbohydrate molecules, and fermentation results in bioethanol. The carbohydrate concentration varies from 15% to 50% in microalgae, depending on the species (Markou & Nerantzis, 2013). Carbohydrates may be found in many forms, including cellulose, mannitol, and agar, and can be extracted via physical, chemical, or biological processes. The major species used for bioethanol production are *Cholorococcum*, *Chlorella,* and *Chlamydomonas* (Trivedi *et al.*, 2015). Several studies have reported that brown algae might be used for making bioethanol due to their high carbohydrate content. Despite these benefits, bioethanol production from microalgae is not yet commercialized due to the high downstream process cost (Goswami *et al.*, 2021).

13.6.4 Syngas

The syngas can be produced through the thermochemical combustion of algae biomass. Different thermochemical processes are gasification, pyrolysis, and direct combustion (Milano *et al.*, 2016; Razzak *et al.*, 2013). Syngas is a mixture of methane, carbon dioxide, hydrogen, and carbon monoxide gas. The species that are reported to have the highest syngas production rate (50–90%) are *Chlorella* sp., and *Scenedesmus* sp. (Raheem *et al.*, 2018). The syngas can be directly burnt or used to produce electricity. The experimental study on the gasification of microalgal biomass in the presence of CaO and Fe_2O_3 (oxygen carrier) resulted in a higher production rate of syngas (Liu *et al.*, 2018).

13.6.5 Other applications

Various microalgal species have been reported to have pharmaceutical, nutraceutical, antimicrobial, anti-inflammatory, and antiproliferative properties (Goswami *et al.*, 2021). However, few of these species can grow on wastewater as well as produce high-quality biomass. The microalgae *Haematococcus pluvialis* cultivation on piggery wastewater showed an astaxanthin concentration in the cell of about 83.9 ppm. The author reported that biomass could not be further used for producing biomolecules or biomass due to the presence of toxic compounds (Kang *et al.*, 2006). The removal of these toxic compounds requires a high purification level, therefore, high operating cost. On the contrary, several studies reported that microalgae cultivated in wastewater could be used as a biofertilizer. The study on the cultivation of *C. minutissima* in brewery wastewater reported that the algal biomass could be used as a biofertilizer to promote organic farming (Amenorfenyo *et al.*, 2019).

13.7 CHALLENGES AND FUTURE PERSPECTIVE

Conventional wastewater treatment requires much energy and cost to remove contaminants and their treatment. Microalgae-based biorefinery is cost-effective as it utilizes atmospheric CO_2 and solar energy for its growth. Microalgae are reported to be 10–15 times faster than plants to fix carbon dioxide (Onyeaka *et al.*, 2021). The biorefinery presently focuses on producing a single type of value-added product, such as biofuel. The merits of the single-step biorefinery are high-quality products, easy recovery, and well-established (Gifuni *et al.*, 2019). However, high cost is the major challenge for its commercialization. The feasibility analysis of microalgae cultivation for biofuel production reported that the biodiesel cost for an open pond system was $9.84/gallon and for a photobioreactor was $20.53/gallon (Davis *et al.*, 2011). The production cost was reported to be much higher than the present fossil fuel prices. The reason for the high price (20–30% of the capital cost) was accounted by the cost of nutrients and freshwater for algae cultivation and the high downstream processing cost (50–60% of capital cost (Javed *et al.*, 2019; Lam *et al.*, 2018). Other than the economic aspects, the presence of bacteria, fungi, and protozoa also affects microalgae growth. The contamination can be removed with the pretreatment step but is cost and energy intensive. Different wastewater contains different types of contaminants. Therefore, there is a need for the selection of microalgal species based on the type of wastewater. The species should be selected based on nutrient removal capacity, high lipid accumulation, and biomass productivity. The low C:N ratio of wastewater is preferred for microalgae biomass productivity. However, a high C:N ratio is preferred for lipid accumulation. Another challenge for wastewater treatment using microalgae is internal shading. The light could not penetrate inside the wastewater due to the presence of suspended solids. It reduces the growth of microalgae inside the water. Therefore, highly efficient reactors are required to be designed. Process parameter such as temperature also affects microalgal growth. Hence, for cold regions, the heating requirement is one of the major challenges. A heating source like solar energy can be utilized to overcome the heating issue, but it requires a large amount of capital cost. The high hydraulic retention time (HRT) of microalgae is another major challenge. The major commercial technologies for wastewater treatment have an HRT of less than 1 day (Acién Fernández *et al*., 2018; Matamoros *et al*., 2015). The extraction of the biomolecule is still complex and expensive. The challenges are required to be overcome for the commercialization of microalgal-based biorefinery.

REFERENCES

Abdelgadir A., Chen X., Liu J., Xie X., Zhang J., Zhang K., Wang H. and Liu N. (2014). Characteristics, process parameters, and inner components of anaerobic bioreactors. *BioMed Research International*, **2014**, 1–10, https://doi.org/10.1155/2014/841573

Acién Fernández F. G., Gómez-Serrano C. and Fernández-Sevilla J. M. (2018). Recovery of nutrients from wastewaters using microalgae. *Frontiers in Sustainable Food Systems*, **2**(September), 1–13, https://doi.org/10.3389/fsufs.2018.00059

Akubude V. C., Nwaigwe K. N. and Dintwa E. (2019). Production of biodiesel from microalgae via nanocatalyzed transesterification process: a review. *Materials Science for Energy Technologies*, **2**(2), 216–225, https://doi.org/10.1016/j.mset.2018.12.006

Akyol Ç, Foglia A., Ozbayram E. G., Frison N., Katsou E., Eusebi A. L. and Fatone F. (2020). Validated innovative approaches for energy-efficient resource recovery and re-use from municipal wastewater: from anaerobic treatment systems to a biorefinery concept. *Critical Reviews in Environmental Science and Technology*, **50**(9), 869–902, https://doi.org/10.1080/10643389.2019.1634456

Amenorfenyo D. K., Huang X., Zhang Y., Zeng Q., Zhang N., Ren J. and Huang Q. (2019). Microalgae brewery wastewater treatment: potentials, benefits and the challenges. *International Journal of Environmental Research and Public Health*, **16**(11), 1910, https://doi.org/10.3390/ijerph16111910

Arooj M. F., Han S. K., Kim S. H., Kim D. H. and Shin H. S. (2007). Sludge characteristics in anaerobic SBR system producing hydrogen gas. *Water Research*, **41**(6), 1177–1184, https://doi.org/10.1016/j.watres.2006.11.052

Behera C. R., Santoro D., Gernaey K. V. and Sin G. (2018). Organic carbon recovery modeling for a rotating belt filter and its impact assessment on a plant-wide scale. *Chemical Engineering Journal*, **334**(November 2017), 1965–1976, https://doi.org/10.1016/j.cej.2017.11.091

Bengtsson S., Werker A., Visser C. and Korving L. (2017). PHARIO: Stepping Stone to a Sustainable Value Chain for PHA Bioplastic Using Municipal Activated Sludge. Stichting Toegepast Onderzoek Waterbeheer Amersfoort, The Netherlands.

Bhuvaneshwari B. and Devika R. (2005). Studies on the physico-chemical and biological characteristics of Coovum river. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, **7**(3), 449–451.

Bureau of Reclamation (2020). Water Facts – Worldwide Water Supply. https://www.usbr.gov/mp/arwec/water-facts-ww-water-sup.html

Chaiklahan R., Chirasuwan N., Loha V., Tia S. and Bunnag B. (2018). Stepwise extraction of high-value chemicals from *Arthrospira* (*Spirulina*) and an economic feasibility study. *Biotechnology Reports*, **20**, e00280, https://doi.org/10.1016/j.btre.2018.e00280

Chen, Guo W., Ngo H. H., Chang S. W., Duc Nguyen D., Dan Nguyen P., Bui X. T. and Wu Y. (2017). Impact of reactor configurations on the performance of a granular anaerobic membrane bioreactor for municipal wastewater treatment. *International Biodeterioration and Biodegradation*, **121**, 131–138, https://doi.org/10.1016/j.ibiod.2017.03.021

Chen W.-H., Tsai C.-Y., Chen S.-Y., Sung S. and Lin J.-G. (2019). Treatment of campus domestic wastewater using ambient-temperature anaerobic fluidized membrane bioreactors with zeolites as carriers. *International Biodeterioration & Biodegradation*, **136**(October 2018), 49–54, https://doi.org/10.1016/j.ibiod.2018.10.010

Chu F.-J., Wan T.-J., Chen H., Wu C.-H. and Kao P.-M. (2020). Magnetophoretic harvesting of *Nannochloropsis oculata* using iron oxide immobilized beads. *Water*, **12**(1), 236, https://doi.org/10.3390/w12010236

Converti A., Casazza A. A., Ortiz E. Y., Perego P. and Del Borghi M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing: Process Intensification*, **48**(6), 1146–1151, https://doi.org/10.1016/j.cep.2009.03.006

Corcoran E., Nellemann C., Bos R., Osborn D. and Savelli H. (2010). Sick water? The central role of wastewater management in sustainable development: a rapid response assessment. In UNEP, 23–73, www.grida.no.

Crutchik D., Frison N., Eusebi A. L. and Fatone F. (2018). Biorefinery of cellulosic primary sludge towards targeted short chain fatty acids, phosphorus and methane recovery. *Water Research*, **136**, 112–119, https://doi.org/10.1016/j.watres.2018.02.047

Davis R., Aden A. and Pienkos P. T. (2011). Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy*, **88**(10), 3524–3531, https://doi.org/10.1016/j.apenergy.2011.04.018

Evans P. J., Parameswaran P., Lim K., Bae J., Shin C., Ho J. and McCarty P. L. (2019). A comparative pilot-scale evaluation of gas-sparged and granular activated carbon-fluidized anaerobic membrane bioreactors for domestic wastewater treatment. *Bioresource Technology*, **288**(November 2018), 120949, https://doi.org/10.1016/j.biortech.2019.01.072

Ezugbe E. O. and Rathilal S. (2020). Membrane technologies in wastewater treatment: a review. *Membranes*, **10**(5), 89, https://doi.org/10.3390/membranes10050089

Fernández-Dacosta C., Posada J. A., Kleerebezem R., Cuellar M. C. and Ramirez A. (2015). Microbial community-based polyhydroxyalkanoates (PHAs) production from wastewater: techno-economic analysis and ex-ante environmental assessment. *Bioresource Technology*, **185**, 368–377, https://doi.org/10.1016/j.biortech.2015.03.025

Ghosh S., Lobanov S. and Lo V. K. (2019). An overview of technologies to recover phosphorus as struvite from wastewater: advantages and shortcomings. *Environmental Science and Pollution Research*, **26**(19), 19063–19077, https://doi.org/10.1007/s11356-019-05378-6

Gifuni I., Pollio A., Safi C., Marzocchella A. and Olivieri G. (2019). Current bottlenecks and challenges of the microalgal biorefinery. *Trends in Biotechnology*, **37**(3), 242–252, https://doi.org/10.1016/j.tibtech.2018.09.006

Goswami R. K., Mehariya S., Verma P., Lavecchia R. and Zuorro A. (2021). Microalgae-based biorefineries for sustainable resource recovery from wastewater. *Journal of Water Process Engineering*, **40**(July 2020), 101747, https://doi.org/10.1016/j.jwpe.2020.101747

Gouveia J., Plaza F., Garralon G., Fdz-Polanco F. and Peña M. (2015). Long-term operation of a pilot scale anaerobic membrane bioreactor (AnMBR) for the treatment of municipal wastewater under psychrophilic conditions. *Bioresource Technology*, **185**, 225–233, https://doi.org/10.1016/j.biortech.2015.03.002

Gurung K., Tang W. Z. and Sillanpää M. (2018). Correction to: unit energy consumption as benchmark to select energy positive retrofitting strategies for Finnish wastewater treatment plants (WWTPs): a case study of Mikkeli WWTP. *Environmental Processes*, **5**(3), 667–681. In Environmental Processes. https://doi.org/10.1007/s40710-018-0340-5, https://doi.org/10.1007/s40710-018-0310-y

Hoffman J., Pate R. C., Drennen T. and Quinn J. C. (2017). Techno-economic assessment of open microalgae production systems. *Algal Research*, **23**, 51–57, https://doi.org/10.1016/j.algal.2017.01.005

Hu Y., Yang Y., Yu S., Wang X. C. and Tang J. (2018). Psychrophilic anaerobic dynamic membrane bioreactor for domestic wastewater treatment: effects of organic loading and sludge recycling. *Bioresource Technology*, **270**(June), 62–69, https://doi.org/10.1016/j.biortech.2018.08.128

Ibrahim and Heikal S. M. (2019). Bioremediation of wastewater from an agricultural drain by the use of microalgae. *SVU–International Journal of Basic Sciences*, **1**(1), 6–15.

Ichsan, Hadiyanto H. and Hendroko R. (2014). Integrated biogas-microalgae from waste waters as the potential biorefinery sources in Indonesia. *Energy Procedia*, **47**, 143–148, https://doi.org/10.1016/j.egypro.2014.01.207

Javed F., Aslam M., Rashid N., Shamair Z., Khan A. L., Yasin M., Fazal T., Hafeez A., Rehman F., Rehman M. S. U., Khan Z., Iqbal J. and Bazmi A. A. (2019). Microalgae-based biofuels, resource recovery and wastewater treatment: a pathway towards sustainable biorefinery. *Fuel*, **255**(February), 115826, https://doi.org/10.1016/j.fuel.2019.115826

Jürgensen L., Ehimen E. A., Born J. and Holm-Nielsen J. B. (2018). A combination anaerobic digestion scheme for biogas production from dairy effluent – CSTR and ABR, and biogas upgrading. *Biomass and Bioenergy*, **111**, 241–247, https://doi.org/10.1016/j.biombioe.2017.04.007

Kang C. D., An J. Y., Park T. H. and Sim S. J. (2006). Astaxanthin biosynthesis from simultaneous N and P uptake by the green alga *Haematococcus pluvialis* in primary-treated wastewater. *Biochemical Engineering Journal*, **31**(3), 234–238, https://doi.org/10.1016/j.bej.2006.08.002

Khan M. J., Das S., Vinayak V., Pant D. and Ghangrekar M. M. (2022). Live diatoms as potential biocatalyst in a microbial fuel cell for harvesting continuous diafuel, carotenoids and bioelectricity. *Chemosphere*, **291**(P1), 132841, https://doi.org/10.1016/j.chemosphere.2021.132841

Kim D. H., Kim S. H., Ko I. B., Lee C. Y. and Shin H. S. (2008). Start-up strategy for continuous fermentative hydrogen production: early switchover from batch to continuous operation. *International Journal of Hydrogen Energy*, **33**(5), 1532–1541, https://doi.org/10.1016/j.ijhydene.2008.01.012

Lam G. P., Vermuë M. H., Eppink M. H. M., Wijffels R. H. and van den Berg C. (2018). Multi-product microalgae biorefineries: from concept towards reality. *Trends in Biotechnology*, **36**(2), 216–227, https://doi.org/10.1016/j.tibtech.2017.10.011

Li W., Li L. and Qiu G. (2017). Energy consumption and economic cost of typical wastewater treatment systems in Shenzhen, China. *Journal of Cleaner Production*, **163**, S374–S378, https://doi.org/10.1016/j.jclepro.2015.12.109

Li, Liu Q., Fang F., Luo R., Lu Q., Zhou W., Huo S., Cheng P., Liu J., Addy M., Chen P., Chen D. and Ruan R. (2019). Microalgae-based wastewater treatment for nutrients recovery: a review. *Bioresource Technology*, **291**, 121934, https://doi.org/10.1016/j.biortech.2019.121934

Lim K., Evans P. J. and Parameswaran P. (2019). Long-term performance of a pilot-scale gas-sparged anaerobic membrane bioreactor under ambient temperatures for holistic wastewater treatment. *Environmental Science and Technology*, **53**(13), 7347–7354, https://doi.org/10.1021/acs.est.8b06198

Lin, Nierop K. G. J., Girbal-Neuhauser E., Adriaanse M. and van Loosdrecht M. C. M. (2015). Sustainable polysaccharide-based biomaterial recovered from waste aerobic granular sludge as a surface coating material. *Sustainable Materials and Technologies*, **4**, 24–29, https://doi.org/10.1016/j.susmat.2015.06.002

Lin L., Li R. H. and Li X. Y. (2018). Recovery of organic resources from sewage sludge of Al-enhanced primary sedimentation by alkali pretreatment and acidogenic fermentation. *Journal of Cleaner Production*, **172**, 3334–3341, https://doi.org/10.1016/j.jclepro.2017.11.199

Littfinski T., Beckmann J., Gehring T., Stricker M., Nettmann E., Krimmler S., Murnleitner E., Lübken M., Pant D. and Wichern M. (2022). Model-based identification of biological and pH gradient driven removal pathways of total ammonia nitrogen in single-chamber microbial fuel cells. *Chemical Engineering Journal*, **431**(P1), 133987, https://doi.org/10.1016/j.cej.2021.133987

Liu G., Liao Y., Wu Y. and Ma X. (2018). Synthesis gas production from microalgae gasification in the presence of Fe_2O_3 oxygen carrier and CaO additive. *Applied Energy*, **212**(January), 955–965, https://doi.org/10.1016/j.apenergy.2017.12.110

Longo S., d'Antoni B. M., Bongards M., Chaparro A., Cronrath A., Fatone F., Lema J. M., Mauricio-Iglesias M., Soares A. and Hospido A. (2016). Monitoring and diagnosis of energy consumption in wastewater treatment plants. A state of the art and proposals for improvement. *Applied Energy*, **179**, 1251–1268, https://doi.org/10.1016/j.apenergy.2016.07.043

Lopes T. F., Cabanas C., Silva A., Fonseca D., Santos E., Guerra L. T., Sheahan C., Reis A. and Gírio F. (2019). Process simulation and techno-economic assessment for direct production of advanced bioethanol using a genetically modified *Synechocystis* sp. *Bioresource Technology Reports*, **6**(February), 113–122, https://doi.org/10.1016/j.biteb.2019.02.010

Mannina G., Presti D., Montiel-Jarillo G., Carrera J. and Suárez-Ojeda M. E. (2020). Recovery of polyhydroxyalkanoates (PHAs) from wastewater: a review. *Bioresource Technology*, **297**(November 2019), 122478, https://doi.org/10.1016/j.biortech.2019.122478

Mari A. G., Andreani C. L., Tonello T. U., Leite L. C. C., Fernandes J. R., Lopes D. D., Rodrigues J. A. D. and Gomes S. D. (2020). Biohydrogen and biomethane production from cassava wastewater in a two-stage anaerobic sequencing batch biofilm reactor. *International Journal of Hydrogen Energy*, **45**(8), 5165–5174, https://doi.org/10.1016/j.ijhydene.2019.07.054

Markou G. and Nerantzis E. (2013). Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. *Biotechnology Advances*, **31**(8), 1532–1542, https://doi.org/10.1016/j.biotechadv.2013.07.011

Matamoros V., Gutiérrez R., Ferrer I., García J. and Bayona J. M. (2015). Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study. *Journal of Hazardous Materials*, **288**, 34–42, https://doi.org/10.1016/j.jhazmat.2015.02.002

Milano J., Ong H. C., Masjuki H. H., Chong W. T., Lam M. K., Loh P. K. and Vellayan V. (2016). Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews*, **58**, 180–197, https://doi.org/10.1016/j.rser.2015.12.150

Mondal M., Ghosh A., Sharma A. S., Tiwari O. N., Gayen K., Mandal M. K. and Halder G. N. (2016). Mixotrophic cultivation of *Chlorella* sp. BTA 9031 and *Chlamydomonas* sp. BTA 9032 isolated from coal field using various carbon sources for biodiesel production. *Energy Conversion and Management*, **124**, 297–304, https://doi.org/10.1016/j.enconman.2016.07.033

Mu Y., Yu H. Q. and Wang Y. (2006). The role of pH in the fermentative H_2 production from an acidogenic granule-based reactor. *Chemosphere*, **64**(3), 350–358, https://doi.org/10.1016/j.chemosphere.2005.12.048

Musa M., Doshi A., Brown R. and Rainey T. J. (2019). Microalgae dewatering for biofuels: a comparative techno-economic assessment using single and two-stage technologies. *Journal of Cleaner Production*, **229**, 325–336, https://doi.org/10.1016/j.jclepro.2019.05.039

Neira M. and Prüss-Ustün A. (2016). Preventing disease through healthy environments: a global assessment of the environmental burden of disease. *Toxicology Letters*, **259**, S1–S3, https://doi.org/10.1016/j.toxlet.2016.07.028

Nizami A. S., Rehan M., Waqas M., Naqvi M., Ouda O. K., Shahzad K., Miandad R., Khan M. Z., Syamsiro M., Ismail I. M. I. and Pant D. (2017). Waste biorefineries: enabling circular economies in developing countries. *Bioresource Technology*, **241**, 1101–1117, https://doi.org/10.1016/j.biortech.2017.05.097

Onyeaka H., Miri T., Obileke K., Hart A., Anumudu C. and Al-Sharify Z. T. (2021). Minimizing carbon footprint via microalgae as a biological capture. *Carbon Capture Science & Technology*, **1**(October), 100007, https://doi.org/10.1016/j.ccst.2021.100007

Pancha I., Chokshi K. and Mishra S. (2019). Industrial wastewater-based microalgal biorefinery: a dual strategy to remediate waste and produce microalgal bioproducts. *In Application of Microalgae in Wastewater Treatment*, **2**, 173–193, https://doi.org/10.1007/978-3-030-13909-4_8

Panepinto D., Fiore S., Zappone M., Genon G. and Meucci L. (2016). Evaluation of the energy efficiency of a large wastewater treatment plant in Italy. *Applied Energy*, **161**, 404–411, https://doi.org/10.1016/j.apenergy.2015.10.027

Peces M., Astals S., Clarke W. P. and Jensen P. D. (2016). Semi-aerobic fermentation as a novel pre-treatment to obtain VFA and increase methane yield from primary sludge. *Bioresource Technology*, **200**, 631–638, https://doi.org/10.1016/j.biortech.2015.10.085

Peña M., do Nascimento T., Gouveia J., Escudero J., Gómez A., Letona A., Arrieta J. and Fdz-Polanco F. (2019). Anaerobic submerged membrane bioreactor (AnSMBR) treating municipal wastewater at ambient temperature: operation and potential use for agricultural irrigation. *Bioresource Technology*, **282**(March), 285–293, https://doi.org/10.1016/j.biortech.2019.03.019

Pereira H., Barreira L., Custódio L., Alrokayan S., Mouffouk F., Varela J., Abu-Salah K. M. and Ben-Hamadou R. (2013). Isolation and fatty acid profile of selected microalgae strains from the red sea for biofuel production. *Energies*, **6**(6), 2773–2783, https://doi.org/10.3390/en6062773

Puchongkawarin C., Gomez-Mont C., Stuckey D. C. and Chachuat B. (2015). Optimization-based methodology for the development of wastewater facilities for energy and nutrient recovery. *Chemosphere*, **140**, 150–158, https://doi.org/10.1016/j.chemosphere.2014.08.061

Puyol D., Batstone D. J., Hülsen T., Astals S., Peces M. and Krömer J. O. (2017). Resource recovery from wastewater by biological technologies: opportunities, challenges, and prospects. *Frontiers in Microbiology*, **7**(JAN), 1–23, https://doi.org/10.3389/fmicb.2016.02106

Qadir M., Drechsel P., Jiménez Cisneros B., Kim Y., Pramanik A., Mehta P. and Olaniyan O. (2020). Global and regional potential of wastewater as a water, nutrient and energy source. *Natural Resources Forum*, **44**(1), 40–51, https://doi.org/10.1111/1477-8947.12187

Qu J. and Fan M. (2010). The current state of water quality and technology development for water pollution control in China. *Critical Reviews in Environmental Science and Technology*, **40**(6), 519–560, https://doi.org/10.1080/10643380802451953

Raheem A., Sikarwar V. S., He J., Dastyar W., Dionysiou D. D., Wang W. and Zhao M. (2018). Opportunities and challenges in sustainable treatment and resource reuse of sewage sludge: a review. *Chemical Engineering Journal*, **337**(October 2017), 616–641, https://doi.org/10.1016/j.cej.2017.12.149

Rawat I., Ranjith Kumar R., Mutanda T. and Bux F. (2013). Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. *Applied Energy*, **103**, 444–467, https://doi.org/10.1016/j.apenergy.2012.10.004

Razzak S. A., Hossain M. M., Lucky R. A., Bassi A. S. and De Lasa H. (2013). Integrated CO_2 capture, wastewater treatment and biofuel production by microalgae culturing – a review. *Renewable and Sustainable Energy Reviews*, **27**, 622–653, https://doi.org/10.1016/j.rser.2013.05.063

Rodríguez Arredondo M., Kuntke P., ter Heijne A. and Buisman C. J. N. (2019). The concept of load ratio applied to bioelectrochemical systems for ammonia recovery. *Journal of Chemical Technology and Biotechnology*, **94**(6), 2055–2061, https://doi.org/10.1002/jctb.5992

SAMCO (2016). How Much Does a Wastewater Treatment System Cost? (Pricing, Factors, Etc.). https://samcotech.com/cost-wastewater-treatment-system/

Sato T., Qadir M., Yamamoto S., Endo T. and Zahoor A. (2013). Global, regional, and country level need for data on wastewater generation, treatment, and use. *In Agricultural Water Management*, **130**(1), 1–13, https://doi.org/10.1016/j.agwat.2013.08.007

Sharma R., Mishra A., Pant D. and Malaviya P. (2022). Recent advances in microalgae-based remediation of industrial and non-industrial wastewaters with simultaneous recovery of value-added products. *Bioresource Technology*, **344**(PB), 126129, https://doi.org/10.1016/j.biortech.2021.126129

Silva M. I., Gonçalves A. L., Vilar V. J. P. and Pires J. C. M. (2021). Experimental and techno-economic study on the use of microalgae for paper industry effluents remediation. *Sustainability*, **13**(3), 1314, https://doi.org/10.3390/su13031314

Singh Tiwari A. and Das M. (2016). Phyco-remediation of industrial waste-water and flue gases with algal-diesel engenderment from micro-algae: a review. *In Fuel*, **173**, 90–97, https://doi.org/10.1016/j.fuel.2016.01.031

Singh R., Paritosh K., Pareek N. and Vivekanand V. (2022). Integrated system of anaerobic digestion and pyrolysis for valorization of agricultural and food waste towards circular bioeconomy: review. *Bioresource Technology*, **360**(July), 127596, https://doi.org/10.1016/j.biortech.2022.127596

Sivaramakrishnan R., Suresh S., Kanwal S., Ramadoss G., Ramprakash B. and Incharoensakdi A. (2022). Microalgal biorefinery concepts' developments for biofuel and bioproducts: current perspective and bottlenecks. *International Journal of Molecular Sciences*, **23**(5), 2623, https://doi.org/10.3390/ijms23052623

Thomassen G., Egiguren Vila U., Van Dael M., Lemmens B. and Van Passel S. (2016). A techno-economic assessment of an algal-based biorefinery. *Clean Technologies and Environmental Policy*, **18**(6), 1849–1862, https://doi.org/10.1007/s10098-016-1159-2

Trivedi J., Aila M., Bangwal D. P., Kaul S. and Garg M. O. (2015). Algae based biorefinery – how to make sense? *Renewable and Sustainable Energy Reviews*, **47**, 295–307, https://doi.org/10.1016/j.rser.2015.03.052

Udaiyappan A. F., Abu Hasan H., Takriff M. S. and Sheikh Abdullah S. R. (2017). A review of the potentials, challenges and current status of microalgae biomass applications in industrial wastewater treatment. *Journal of Water Process Engineering*, **20**, 8–21, https://doi.org/10.1016/j.jwpe.2017.09.006

United T., World N., Development W., Tran M., Koncagul E. and Connor R. (2016). The United Nations World Water Development Report 2016: WATER AND JOBS Facts and Figures. Report: UN WWDR4-2016.

Van Der Hoek J. P., De Fooij H. and Struker A. (2016). Wastewater as a resource: strategies to recover resources from Amsterdam's wastewater. *Resources, Conservation and Recycling*, **113**, 53–64, https://doi.org/10.1016/j.resconrec.2016.05.012

Wang Y., Ho S. H., Cheng C. L., Guo W. Q., Nagarajan D., Ren N. Q., Lee D. J. and Chang J. S. (2016). Perspectives on the feasibility of using microalgae for industrial wastewater treatment. *Bioresource Technology*, **222**, 485–497, https://doi.org/10.1016/j.biortech.2016.09.106

Wijffels R. H., Barbosa M. J. and Eppink M. H. M. (2010). Microalgae for the production of bulk chemicals and biofuels. *Biofuels, Bioproducts and Biorefining*, **4**(3), 287–295, https://doi.org/10.1002/bbb.215

Wollmann F., Dietze S., Ackermann J. U., Bley T., Walther T., Steingroewer J. and Krujatz F. (2019). Microalgae wastewater treatment: biological and technological approaches. *Engineering in Life Sciences*, **19**(12), 860–871, https://doi.org/10.1002/elsc.201900071

WWAP (2017). Wastewater: The Untapped Resource. In The United Nations World Water Development Report. https://wedocs.unep.org/20.500.11822/20448

Xiao Y., Zheng Y., Wu S., Yang Z. H. and Zhao F. (2016). Nitrogen recovery from wastewater using microbial fuel cells. *Frontiers of Environmental Science and Engineering*, **10**(1), 185–191, https://doi.org/10.1007/s11783-014-0730-5

Xin C., Addy M. M., Zhao J., Cheng Y., Cheng S., Mu D., Liu Y., Ding R., Chen P. and Ruan R. (2016). Comprehensive techno-economic analysis of wastewater-based algal biofuel production: a case study. *Bioresource Technology*, **211**, 584–593, https://doi.org/10.1016/j.biortech.2016.03.102

Xin C., Addy M. M., Zhao J., Cheng Y., Ma Y., Liu S., Mu D., Liu Y., Chen P. and Ruan R. (2018). Waste-to-biofuel integrated system and its comprehensive techno-economic assessment in wastewater treatment plants. *Bioresource Technology*, **250**(August 2017), 523–531, https://doi.org/10.1016/j.biortech.2017.11.040

Xing Y., Li Z., Fan Y. and Hou H. (2010). Biohydrogen production from dairy manures with acidification pretreatment by anaerobic fermentation. *Environmental Science and Pollution Research*, **17**(2), 392–399, https://doi.org/10.1007/s11356-009-0187-4

Xu H., Miao X. and Wu Q. (2006). High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *Journal of Biotechnology*, **126**(4), 499–507, https://doi.org/10.1016/j.jbiotec.2006.05.002

Xu Ng T. C. A., Huang S. and Ng H. Y. (2020). Effect of quorum quenching on EPS and size-fractioned particles and organics in anaerobic membrane bioreactor for domestic wastewater treatment. *Water Research*, **179**, 115850, https://doi.org/10.1016/j.watres.2020.115850

Yadav A., Rene E. R., Sharma M., Jatain I., Mandal M. K. and Dubey K. K. (2022). Valorization of wastewater to recover value-added products: a comprehensive insight and perspective on different technologies. *Environmental Research*, **214**(P2), 113957, https://doi.org/10.1016/j.envres.2022.113957

Yang J. S., Cao J., Xing G. L. and Yuan H. L. (2015). Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae *Chlorella minutissima* UTEX2341. *Bioresource Technology*, **175**, 537–544, https://doi.org/10.1016/j.biortech.2014.10.124

Zielińska M., Zieliński M. and Dębowski M. (2018). Organic compounds and phosphorus removal from dairy wastewater by biofilm on iron-containing supports. *Journal of Environmental Engineering*, **144**(1), 1, https://doi.org/10.1061/(ASCE)EE.1943-7870.0001309

doi: 10.2166/9781789063783_0263

Chapter 14

Role of nanotechnology in environmental cleaning

Jayato Nayak[1]* *and Sankha Chakrabortty*[2]

[1]*Center for Life Sciences, Mahindra University, Hyderabad, Telangana 500043, India*
[2]*Department of Chemical Technology, KIIT University, Bhubaneswar, Odisha 751024, India*
**Corresponding author: nayak.jayato@gmail.com*

ABSTRACT

Air, water, and soil pollution pose serious threats to ecosystem health and should be prioritized as a global issue of paramount importance. Emerging technologies such as 'nanoremediation,' which embraces the concept of nanotechnology through the development of nanoparticles (NPs), join established procedures such as thermal treatment, pump-and-treat, and chemical oxidation in the arsenal of remediation approaches. Nanoremediation is the process of using designed NPs to clean up polluted medium; it is more efficient and cost-effective than conventional approaches. Nanomaterials' unique properties such as their nanostructure and cost-effectiveness are the main factors of their growing popularity for use in environmental clean-up. NPs have excellent electrical characteristics, catalytic behavior, and sensitivity because of their large surface area to mass ratio. Remediation by NPs can primarily occur through catalysis and chemical reduction. Because of their high surface area, random distribution of active sites, and versatility in coating changes, NPs have also been used in adsorption-based removal processes. The ability of NPs to spread in tight places also increases their usefulness for cleaning up polluted water and soil. Water nanofiltration utilizes nanomaterial-based membranes because the membrane pores can trap substantial components in water waste streams. In addition, the membrane contact selectively separates the less abundant molecules. Metal oxides, carbon nanotubes, quantum dots, and biopolymers are just a few of the nanomaterials used in water, soil, and air purification. Environmentally, NPs are useful for cleaning soil and water. Organic solvents, pesticides, metals, and fertilizers such as nitrates can all be dechlorinated, detoxified, and transformed by injecting it into the affected area. Besides carbon nanotubes and bimetallic NPs, there are other NPs that can be used to address these problems. Food processing, disinfectants, water purification, and even common household appliances all make use of NPs. The use of nanotechnology in pollution prevention, monitoring, and remediation has been gaining popularity.

Keywords: nanotechnology, wastewater treatment, adsorption, catalysis, filtration, nanoremediation

14.1 INTRODUCTION

Currently, nanomaterial-based emerging technologies have turned quintessential to guarantee no waste generation and environmental friendliness in the face of the rising tide of anthropogenic discarded volumes of wastewater from industrial houses that link to various health issues of living beings (Lin *et al.*, 2020). A number of infectious disorders, including parasitic attacks, diarrhea, and dermatitis, are the typical issues rising from such water contaminations (Adegoke *et al.*, 2018). There are four main categories of treatment: physical, chemical, biological, and sludge. Adsorption, ion exchange, and reverse osmosis are just few of the conventional technologies that aren't very efficient or reusable. But, in-fact, separation of exhausted adsorbents is reckoned as a crucial problem till date. Emerging technologies include fuel cell development using microbes, treatments using algae and membrane separations, and so on. But, the slowness of the processes which include living species turns out to be the major drawback where membrane separation gets affected due to fouling issues. Under such circumstances, nanotechnology in wastewater decontamination offers a promising alternative solution that can be used to address the problems now facing society.

Nanotechnology's versatility, in terms of both surface area and reactivity, makes it the ideal treatment approach (Crane & Scott, 2012). Nanoparticles are highly efficient in removing pollutants such heavy metals, dyes, and organic and inorganic chemicals (Kumar *et al.*, 2014). A variety of nanostructures, including graphene, silver nanoparticles, nanomagnets, and nanotubes, have shown promise as wastewater treatment options. Nanoadsorbents such as silicas and chitosan-based particles (Vunain *et al.*, 2016), were employed in water treatment for the heavy metal removal. Titanium dioxide (TiO_2) photodegradation is garnering a lot of attention from scientists in recent years as a potential method for reducing the impact of these hazardous chemicals (Al-Mamun *et al.*, 2019). Under solar or ultraviolet irradiation, these nanocatalytic semiconductor materials show remarkable aptitude for photocatalytic remediation (Feizpoor & Habibi-Yangjeh, 2018). TiO_2 is the first transition metal oxide to undergo oxidative deterioration when exposed to sunlight because of its low-energy band gap and sufficiently positive valence band. The other such widely used nanocatalytic particles utilized in wastewater treatment are zinc oxide (ZnO) and magnesium oxide. One of the promising methods for the synthesis of nanomaterials is the use of ultrasound methodology (Low *et al.*, 2021a, 2021b, 2022). Heavy metals in wastewater are detected using nanomaterial-based biosensors (Hara & Singh, 2021). One of the capable materials for adsorptive decontamination of wastewater is biomass-derived renewable biochar, by the virtue of its microporous volume, active functional groups, and appreciable specific surface area which could be tailor-made using temperature and choice of feed stocks (Ahmad *et al.*, 2014; Mohanty *et al.*, 2013; Tan *et al.*, 2021). Biochar made through pyrolysis is useful for removing non-polar organic contaminants because of its high surface area (Mohanty *et al.*, 2013). In this chapter, a special focus on nanotechnology-based waste remediation therapies has been described.

14.2 BIOMASS-DERIVED ADSORPTIVE PROCESS FOR WASTE TREATMENT

Thermochemical degradation of diverse biomass feedstocks yields biochar, a porous carbonaceous substance. The processes of pyrolysis, gasification, and hydrothermal carbonization are the means by which biochar is manufactured (Park *et al.*, 2016). Adsorption onto biochar is an affordable and sustainable process (Zazycki *et al.*, 2018). Biochar is an appealing substrate for wastewater treatment because of its high surface area and abundance of surface functional groups. Feedstock choice, pretreatment method, and thermochemical decomposition method all play significant roles in determining biochar's adsorption capacity (Yu *et al.*, 2019). Biochar's porous characteristics and adsorptivity can be improved after being functionalized with acid or base. Impregnating certain materials with biochar and then allowing them to cure produces biochar composites with improved surface properties. Biochar's surface can also be altered with the use of steam or gases. Recently, surface modification techniques have included the use of ultrasound, plasma, and microwave energy (Karim *et al.*, 2017). Thus, biochar functions as an effective adsorbent, demonstrating a level of adsorption ability that is on par with, and in some cases even exceeds, that of commercial activated carbon. Bagasse biochar efficiently removes lead from wastewater created during battery production (Bharti & Kumar, 2018). In heavy metal removal, mixed biochar–chitosan substrates excel. Biochar made from digested sludge has been shown to be effective in the treatment of municipal wastewater for the removal of ammonia (Tang *et al.*, 2019). Biochars made from municipal solid wastes have been employed extensively as adsorbents to eliminate chromium, ammonia nitrogen (NH_4^+-N), phosphate (PO_4^{3-}-P), F^-, acetaminophen, and methylene blue from wastewater (Zhou *et al.*, 2019). Persulfate activation using biochar produced from municipal solid waste has been used to detoxify water of organic contaminants such as azo dyes and antibiotics (Meng *et al.*, 2020). The elimination of sulfamethazine by steam-activated biochar was reported by Rajapaksha *et al.* (2015). Agricultural wastewater has had Cu^{2+}, As^{5+}, Cd^{2+}, and Pb^{2+} successfully removed by biochar adsorption technology (Higashikawa *et al.*, 2016). The effectiveness of rice-husk biochar against malachite green was 99.98%. Biochar-based filters have become increasingly common for use in wastewater treatment systems in recent years (Kaetzl *et al.*, 2019; Perez-Mercado *et al.*, 2019). Biochar from cotton gin waste has been shown to remove halogens, dyes, and chlorinated hydrocarbons from aqueous solutions (Haque *et al.*, 2020). The physicochemical properties of biochar derived from cotton gin waste and walnut shells possess the desired characteristics needed to remove pharmaceuticals from aqueous solution (Nodun *et al.*, 2020). Biochar is a carbon sink, soil fertility booster, and a catalyst that has been used in wastewater treatment. Regeneration has a significant impact on the cost-effectiveness of biochar adsorption. The effectiveness of regeneration is affected by three factors: the adsorbent employed, the regeneration process, and the regeneration dose (Ambaye *et al.*, 2021). pH and temperature are adjusted, and chemicals such as aniline and dyes are added, all in the name of regeneration (Fagbohungbe *et al.*, 2017). Biochar adsorption is mostly determined by pH (Shang *et al.*, 2017). Degradation efficiency for organic pollutants is improved by biochar-supported photocatalysts such as Au–TiO_2 composite (Ambaye *et al.*, 2021). TiO_2-impregnated biochar with nanoparticles of softwood, bamboo, reed straw, or corn cob improved its sorption capacity by boosting its physicochemical qualities and active site.

14.3 SEPARATION OF CONTAMINANTS BY NANOFILTRATION

In recent years, thin film composite (TFC) polyamide (PA) membranes have been the standard commercial nanofiltration (NF) membrane. These membranes are created by interfacial polymerization (IP) of two monomers (such as piperazine and tricarbonyl chloride) over a porous substrate (Li *et al.*, 2021). As a consequence of the development of nanotechnology, the incorporation of functional nanoparticles into PA layers has become a common practice for tuning the performance of TFC membranes (Liao *et al.*, 2021). Adding nanomaterials to an aqueous or organic phase and subsequently performing IP are two methods that have been demonstrated to significantly improve the hydrophilicity, fouling resistance, and separation properties of TFC membranes (Zhao *et al.*, 2021). As per the reported research (Jeong *et al.*, 2007), the membrane's water permeability can be improved by incorporating nanoscale particles of the mineral clay zeolite into the PA matrix. The size of the nanoparticle is a significant component in influencing the quality of the thin film nanofilter membranes (Lind *et al.*, 2009). Linde Type A (LTA) zeolites and a PA matrix are utilized to generate nanofilters by utilizing IP. The manufacturing of rougher membranes was the consequence of utilizing larger nanoparticles, whilst the formation of thin film membranes with high permeability was the result of using smaller nanoparticles. It is feasible to manufacture a nanoparticle from a nanomaterial that has improved performance by making slight adjustments to the production technique. During the process of producing a modified membrane filter, an IP technique was utilized to produce a PA material, and polyether sulfone was used as a support for nanoparticles (Dong *et al.*, 2016). This was accomplished by fabricating the PA material from polyether sulfone. They discovered that by making this modification to the production process, it is possible to produce a nanocomposite that has nanoparticles more evenly distributed throughout it and that has more constant NF performance. As a result of their capacity to eradicate bacteria, silver nanoparticles have established themselves as a lucrative business. The membrane that is utilized in commercial nanofilters has been modified in order to accommodate the presence of silver nanoparticles. The *in situ* synthesis of silver nanoparticles on the surface of the membrane made this outcome attainable. Following the application of polyvinyl alcohol as a coating, silver salts were next added to the membrane. It was discovered that silver nanoparticles ranged in size from 10 to 20 nanometers. Utilizing this refined method for the fabrication of membranes, we were able to produce a membrane with anti-biofouling qualities and minimize the pace at which silver nanoparticles leached out. Adding graphene oxide (GO) particles to the membrane matrix improved the physicochemical properties of the polysulfone (PSF) substrate and the PA selective layer, capitalizing on the strong hydrophilicity and electronegativity of GO to boost the membrane's water flux and salt rejection performance (Lai *et al.*, 2016). However, the high price of GO makes industrial scale-up by heavy doping with the filler prohibitive. Emerging nanomaterial O-MoS2 has a structure comparable to GO but at a far more reasonable cost. In our prior work, we suggested using high-energy ultrasonication in conjunction with the Hummers' approach to fabricate O-MoS2. It has high electronegativity and is highly hydrophilic. It also has good non-swelling qualities and a stable chemical composition. Improved water flux and salt rejection were achieved in a TFC NF membrane produced by Yang *et al.* by adding hydrophilic O-MoS2 into the PA layer (Yang *et al.*, 2021). Hollow fiber membranes made from the more mechanically and chemically stable O-MoS2 have been shown to have improved performance and resistance to fouling (Jiang *et al.*, 2019). Embedding O-MoS2 into the substrate layer in order to make use of O-MoS2's characteristics holds great promise for enhancing the separation performance and stability of the TFC NF membrane. Development of such an NF membrane is shown in Figure 14.1.

In addition to nanoparticles, carbon nanotubes (CNTs) have been evaluated as potential components of a nanofilter composite. Poly (dopamine)-modified multi-walled CNTs are incorporated into a polyethylenimine/trimethyl carbonate-based polymeric system developed by Zhao *et al.* (2017). This increased water flux was evidence of the high-water permeability of the NF membrane. In order to increase the efficiency of nanofilter membranes, a lot of time and energy is spent researching material chemistry and creating new membrane synthesis procedures that can accept nanoparticles.

14.4 DECONTAMINATION USING CNTS AND CARBON NANOPARTICLES (CNPS)

Many studies are being conducted, each one concentrating on one of the various nanoadsorbents currently in use. Pores in CNTs serve as binding sites, making them more approachable than activated carbon for big compounds like tetracycline (Zhang *et al.*, 2019). CNTs can be used as adsorbents for both persistent pollutants and detecting poisons, in addition to their pre-concentration role. Ionic interaction and complex formation are the mechanisms by which CNTs adsorb metal ions. In addition, CNTs' antibacterial activities manifest themselves via producing oxidative stress in pathogens and causing cellular membrane breakdown. In contrast to conventional cleaning methods such as chlorination and ozonation, no toxic metabolic end-products would be created if chemical reactions took place (Liu *et al.*,

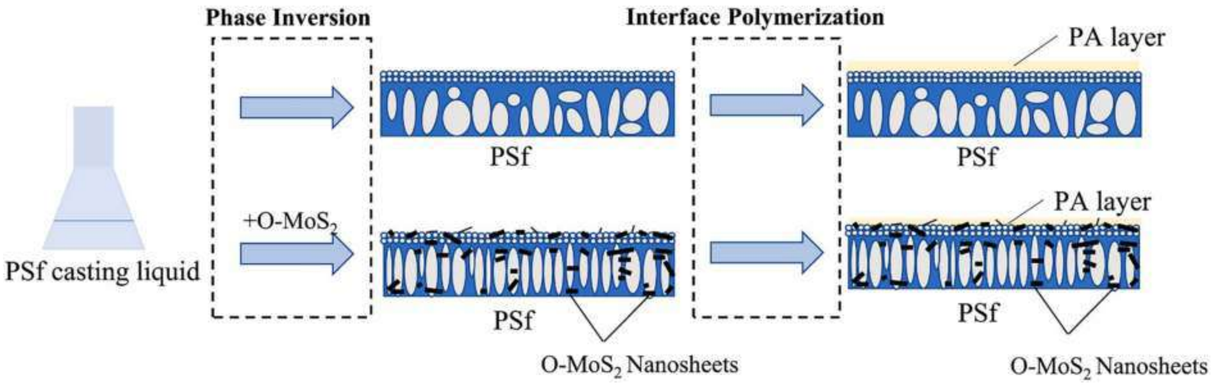


Figure 14.1 Development scheme of a PSF/O–MoS2 nanocomposite substrate-supported TFC NF membrane. Reproduced with copyright from Jiang *et al.* (2023).

2013). To improve their effectiveness, CNT standards are constantly upgraded with newer technologies. Examples of such advancements include the design of new types of multi-wall carbon nanotubes (MWCNTs) and the incorporation of magnetic characteristics, such as polyacrylamide-functionalized MWCNTs that selectively dissolved humic acid and lead (II) (Kausar, 2016). Functionalization of CNTs employing oxygen-containing agents (nitric acid, sulfuric acid, potassium permanganate) imposes surface charge negativity that is responsible in improvization of performance through electrostatic contact (Jeon *et al.*, 2011). Surface-modified CNT exhibited the following heavy metal ion removal pattern as: Pb2+ > Ni2+ > Zn2+ > Cu2+ > Cd2+ (Rao *et al.*, 2007). It has also been discovered that CNTs can be utilized to get rid of organic compounds and colors. The removal of the reactive red M-2BE textile dye from water using MWCNT was optimized. Rather than commercially available powdered activated carbon, the developed MWCNT showed better color removal (about 99%) (Machado *et al.*, 2011). Kuo *et al.* (2008) found that adsorption efficiency is proportional to target molecule concentration when studying the removal of C.I. Direct Yellow 86 (DY86) and C.I. Direct Red (DR224). They found that increasing concentrations of CNTs and ionic strengths resulted in greater dye removals. Adsorption, substance degradation, photodegradation, and compound sanitation are some of the methods used to treat or remediate water. Photocatalytic degradation mechanism using CNT has been represented in Figure 14.2. Nanomaterials made of metal and non-metal oxides are commonly used to remove harmful toxins from wastewater. Ferric oxide nanomolecules are useful for a variety of applications due to the abundance of iron and the simple amalgamation process required to create them (Joshi *et al.*, 2019). Innovations in nanotechnology point to fresh approaches to addressing these issues, such as increasing energy efficiency and lowering water desalination costs. Adsorption-based methods are crucial and simple to apply for water-filtering devices, but they have limited capacity to evacuate salts compared to conventional desalination techniques, which are energy-intensive and time-consuming. When comparing plasma-modified ultra-long CNTs to conventional carbon-based water treatment systems, the former's substantially greater explicit adsorption limit concerning salt (over 4-fold by weight) stands out.

Heavy metals, hazardous chemicals, and dyes all find their way into the wastewater produced by sectors as diverse as mining, chemistry, and textiles. One reliable technique to purify the contaminated discharge and turning it benign prior to its return to the natural water cycle is through adsorptive capturing of these pollutants utilizing CNP. Physisorption is the term used to describe physical interactions through weak molecular forces or electrostatic forces. Chemisorption is the process of binding to an adsorbent through chemical means. Both chemisorption and physisorption are temperature-dependent, with the former increasing at higher temperatures and the latter being

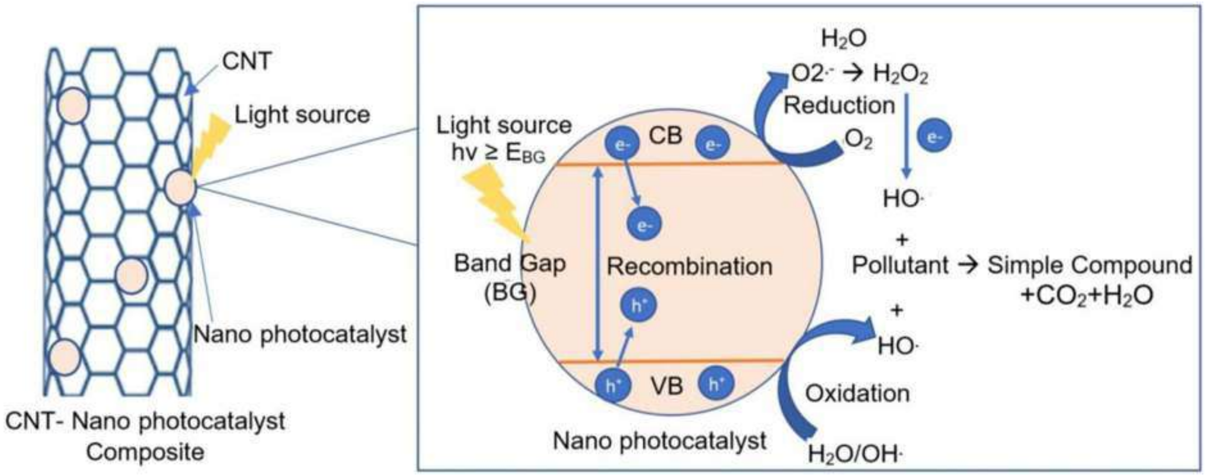


Figure 14.2 Photocatalytic degradation using CNT. Reproduced with copyright from Mishra and Sundaram (2023).

affected by them. In order to use adsorption in wastewater treatments, one must first determine the optimal parameters for doing so, such as the dosage ratio of pollutant to CNPs, the treatment period, the pH, and the temperature (Krishnamurthy & Agarwal, 2013). CNPs have a large surface area, making them ideal for removing pollutants during treatment. Also, in physisorption, the electrostatic connection between the nanoparticle and the pollutant is considerably greater than the weaker van der Waals forces because functionalization could be performed on the nanoparticle to match the molecule's charge, whether it be positive or negative. Researchers looked into the efficacy of CNPs with added functions (Mubaraka *et al.*, 2012). According to the results of their research, CNTs functionalized with sulfuric acid and nitric acid employed for the treatment of wastewater for 101 min at 90°C and at a pH of 5 were more effective in removing heavy metals, particularly copper. Utilization of CNPs as photocatalysts has been shown in Table 14.1. Exploitation of CNTs as an adsorbent has been exhibited in Table 14.2 and that for membrane filtration is displayed in Table 14.3.

14.5 REMEDIATION USING GRAPHENE AND NANOPOROUS-ACTIVATED CARBON

Graphene's enhanced mechanical and electrical stability, as well as their ability to absorb pollutants, can be attributed to their two-dimensional structure. Deng *et al.* (2010) produced functional graphene nanosheets in potassium hexafluorophosphate electrolyte solution and utilized them to remove heavy metals such as lead and cadmium from wastewater. Lead (406 mg/g) and cadmium (73.42 mg/g) were both removed using the GNSPF6 graphene nanosheet that was produced. Treatment of graphene sheets with heat improved their ability to adsorb heavy metals, as reported by Huang *et al.* (2011). Several studies have shown that graphene may be used to effectively remove colors from wastewater. Dye removal capacity between graphite and graphene oxide (GO) was evaluated by Bradder *et al.* (2011), and GO was found to have a 140% better efficiency than graphite. Due to the rising cost of carbon nanomaterials, GO nanocomposites are being used as a replacement for metallic and organic contaminants. Graphene-based materials are efficient adsorbers of a variety of anions, including fluoride and perchlorates. When it comes to eliminating cationic dyes, GO and its composites perform exceptionally well, while graphene and its composites perform best when dealing with anionic dyes. Metal-embedded nanomaterials (Me/MeO ENMs) are one kind of nanomaterials, while nanomaterials composed of two or more metals and oxides form a second class. There are several types of Me/MeO ENMs, but nanosized zerovalent iron, TiO_2 (Ren *et al.*, 2013), and ZnO are currently the most widely employed for treating water/natural remediation. By adding oxygen or nitrogen functionalizing groups to the activated carbon with acid or alkali, the carbon can be transformed into nanoporous-activated carbon. Such activated carbon will have pores measuring around 1–2 nm in size. Nanoporous-activated carbon has a stellar reputation for its ability to purge wastewaters of unsightly impurities. Textiles benefit greatly from the lightfastness of azo dyes. The dye's azo bonds are responsible for its stability and resistance to degradation under extreme conditions. Reactive dyes can be eliminated with the use of nanoporous-activated carbon in such situations. When trying to get rid of reactive black dye, Ahmad and Hameed (2010) produced granules of activated charcoal made from bamboo waste. An 80 mM tall adsorption column filled with granules of activated charcoal derived from bamboo biomass was created where the effective ratio of dye to activated carbon was found to be 39.9 mg/g. Adsorption of basic dyes onto granular-activated carbon and neutral zeolite was investigated in an experiment by Meshko *et al.* (2001). The adsorbent effectiveness of neutral zeolite and granular-activated carbon was compared in a batch study. The adsorptive performance of granular-activated charcoal was 77 mg/g of dye-activated carbon, demonstrating its superior efficiency.

14.6 RISKS IN NANOREMEDIATION

Tailor-made nanomaterials can be used in a variety of cutting-edge applications, but their use in treatment of water and industrial discharges can have unintended consequences for both the environment and human health (Yao *et al.*, 2013). According to Westerhoff *et al.* (2018), when nanotechnology is applied

Table 14.1 Effect of CNT as a photocatalyst in wastewater treatment.

Photocatalyst	Synthesis Method	Light Source	Target Pollutant	Operating Parameters	Removal Efficiency	Reference
MWCNT + TiO_2	Sol–gel	Visible	Methylene blue (MB)	MWCNT: TiO_2 molar ratio (by weight) = 0.05:1 Light intensity = 15 W Calcination temperature = 400°C Time = 180 min Pollutant concentration = 1×10^{-5} M	With TiO_2 only = 22% MWCNT + TiO_2 = 70%	Wongaree *et al.* (2015)
MWCNT + C_3N_4	Hydrothermal	Visible	Rhodamine B (RhB) MB Methylene orange (MO)	Light intensity = 300 W $\lambda \geq 400$ nm Catalyst dose = 5 mg/mL Irradiance time = 1.5 h for MB and 3 h for MO and RhB	For MB = 66.5% MO = 89.7% RhB = 85.4%	Xu *et al.* (2013)
CNT + P-TiO_2	Hydrothermal	UV and visible	MO	Mass ratio of CNT: P-TiO_2 = 5:100 Light intensity for UV = 250 W and for visible = 400 W Irradiation time = 80 min	~100%	Wang and Zhou (2011)
CNT + g-C_3N_4 + $BiVO_4$	Wet impregnation	Simulated sunlight	Phenolic compound	Irradiation time = 2 h Pseudo first-order kinetics Temkin adsorption model Phenol concentration = 10 mg/L Light intensity = 500 W	80.6%	Samsudin *et al.* (2019)
CNT + TiO_2 + Al_2O_3	Hydrothermal	UV	Metamifop herbicide	Catalyst dose = 10 mg Metamifop herbicide concentration = 5 ppm	84.54%	Yusoff *et al.* (2021)

(*Continued*)

Table 14.1 Effect of CNT as a photocatalyst in wastewater treatment. (*Continued*)

Photocatalyst	Synthesis Method	Light Source	Target Pollutant	Operating Parameters	Removal Efficiency	Reference
CNT + TiO_2 + PAN polymer	Electro-spinning	UV	Phenol	pH = 5 Irradiation time = 7 min Light intensity = 100 W Catalyst dose = 20 mg Phenol concentration = 10 ppm Recycle and reuse = 3 cycles	99.2%	Mohamed *et al.* (2020)
CuO + CNT	Chemical method	UV	Direct red (DR) 31 dye Reactive red (RR) 120 dye	Light intensity = 9 W First-order kinetics Reaction rate 0.0105 and 0.0137/min for RR120 and DR31 Catalyst dose = 5 mg	87% for RR 120 89% for DR 31	Mahmoodi *et al.* (2016)
Ti^{3+} + TiO_2 + SWCNT	Two-step chemical precipitation and calcination	Visible	MB	Irradiation time = 25 min Reaction rate = 0.0083/min	83%	Akter *et al.* (2021)
MWCNT + TiO_2 + SiO_2	Sol–gel	UV	Carbamazepine (CBZ) and Bisphenol A (BPA)	Pseudo first-order kinetics Rate constant = 0.0131–0.0743/min for CBZ and 0.0827–0.1751/min for BPA Irradiation time = 20 min Catalyst concentration = 500 mg/L	50%	Czech and Buda (2015)
MWCNT + Ag-ZnO	Precipitation method	Visible	Congo red (CR)	Catalyst concentration = 150 mg/L Light intensity = 40 W pH = 6 Congo red concentration = 15 mg/L First-order kinetics Rate constant = 0.0023/min	99%	Yibeltal *et al.* (2020)

*Source:*Reproduced with copyright from Mishra and Sundaram (2023).

Table 14.2 Effect of CNT as an adsorbent in wastewater treatment.

Adsorbent	Synthesis Method	Target Pollutant	Operating Parameters	Removal Efficiency/ Adsorption Capacity	Reference
SWCNT	Aryl diazonium salt chemistry and cross-linking processes	1-Pyrene butyric acid, diquat dibromide, and MB	High cross-linking degree with thickness = 50 μm Pollutant concentration = 15 μg/mL	50% 28% 83%	Barrejón *et al.* (2019)
MWCNT	Commercially available	CR, Ponceau 4R (PR) dyes	pH = 6 Temperature = 25°C Dye concentration = 200 mg/L Dose = 1 g/L	PR = 67.9 mg/g CR = 256 mg/g	Ferreira *et al.* (2017)
MWCNT	Commercially available	CR	Contact time = 1 h pH = 11 Temperature = Endothermic Initial concentration = 200 ppm Adsorbent dose = 50 mg	92%	Zare *et al.* (2015)
MWCNT	Catalytic chemical vapor deposition (CVD)	Yellow 81, red 159, and blue 116 dye	Initial concentration of dye = 100 mg/L CNT dose = 60 mg Kinetics = pseudo second order Isotherm model Blue 16 = Temkin model Red 159 = Langmuir and Freundlich Yellow 81 = Freundlich and Temkin	33 mg/g of CNT	Vuono *et al.* (2017)
MWCNT	Catalytic CVD	MB and MO	Contact time = 1 h Catalyst dose = 20 mg Initial concentration of dye = 10 mg/L Temperature = 298 K pH = 6 Kinetics = pseudo second order	MB = 6.96 mg/g MO = 5.464 mg/g	Robati *et al.* (2016)
Chitosjan + SiO_2 + MWCNT	Gelation method	Reactive blue 19 (RB19) and direct blue 71 (DB 71)	pH = 6.8 and 2 for DB71 and RB 19 respectively Isotherm model = Langmuir Kinetic = pseudo second order $R^2 = 0.996$ and 0.998 for DB71 and RB19	DB71 = 61.4 mg/g RB19 = 97.1 mg/g	Abbasi (2017)

(Continued)

Table 14.2 Effect of CNT as an adsorbent in wastewater treatment. (*Continued*)

Adsorbent	Synthesis Method	Target Pollutant	Operating Parameters	Removal Efficiency/ Adsorption Capacity	Reference
	–	Disperse red 1 (DR 1), acid blue 113 (AB 113), MB, and MO	Kinetics = pseudo second order Isotherm = Langmuir model Initial concentration of dye = 20 mg/L Temperature = 25°C Dose (mg/g) = 19.4 for DR1 and MB = 18.4 for AB113 = 19 for MO pH = 8, 7, 6, and 7 for MB, AB113, MO, and DR1, respectively Contact time = 15 min for MB, MO, AB113 and 10 min for DR1, respectively	DR1 = 500 mg/g MB = 91 mg/g MO = 96 mg/g AB113 = 172 mg/g	Mohammadi and Veisi (2018)
Fe_3O_4/acrylic acid/*N*-isopropyl acrylamide/ MWCNTs	–	MB, RhB, and crystal violet (CV)	Kinetics = pseudo second order Isotherm = Langmuir Initial concentration of dye = 50 mg/L Dose of adsorbent = 50 mg pH = 8 Temperature = 25°C	CV = 287 mg/g MB = 302 mg/g RhB = 231 mg/g	Hosseinzadeh *et al.* (2018)
Gelatin + iron oxide + MWCNT	Co-precipitation and emulsification	MB, direct red 80 (DR 80)	Isotherm = Freundlich model Kinetic = pseudo second order Temperature = 65°C Time = 6 h Initial concentration = 500 mg/g	DR81 = 96.1% MB = 76.3%	Saber-Samandari *et al.* (2017)
Microwave-induced MWCNT		Cu(II)	Contact time = 35 min Dose of adsorbent = 100 mg pH = 5.5 Agitation speed = 160 r/min Isotherm = Freundlich and Langmuir Kinetics = pseudo second order	99.9%	Mubarak *et al.* (2016)

MWCNT	Tubular microwave CVD	Pb(II)	pH = 5 Initial concentration of Pb(II) = 10 mg/L Agitation speed = 2.8 r/s Dose of adsorbent = 100 mg Contact time = 22.5 min Isotherm = Langmuir and Freundlich Kinetics = pseudo second order	99.9%	Mubarak *et al.* (2015)
Functionalized MWCNT	–	Cu(II)	Contact time = 1 h Dose of adsorbent = 10 mg/L Initial concentration = 20 mg pH = 3 Isotherm = Langmuir Kinetics = pseudo second order	93%	Gupta *et al.* (2017)
CNT + waste cooking palm oil + Fe	Thermal CVD	Zn (II), Cu (II), and Fe (II)	Dose of adsorbent = 1800 mg/L pH = 7 Initial concentration of heavy metal = 100 mg/L Isotherm model = Langmuir for Cu (II)	99.2–99.9%	Abu Bakar *et al.* (2021)
MoS_2 + thiol-functionalized MWCNT	Tubular microwave CVD	Pb(II)Cd (II)	Isotherm = Freundlich Kinetics = pseudo second order Dose of adsorbent = 2 mg/mL pH = 6 Contact time = 1 h	Cd(II) = 66.6 mg/gPb (II) = 90 mg/g	Gusain *et al.* (2019)
MWCNT-COOH and MWCNT-OH	Catalytic CVD	Cr(VI)	Initial concentration of Cr(VI) = 5–60 mg/L pH = 5 Dose of adsorbent = 25 mg Kinetic = intra-particle diffusion model and pseudo second order	8.09 mg/g by MWCNT-COOH 7.85 mg/g by MWCNT-OH	Zhang *et al.* (2018)

*Source:*Reproduced with copyright from Mishra and Sundaram (2023).

Table 14.3 CNT in membrane and filtration for wastewater treatment.

Membrane/Filter Type	Material	Synthesis Method	Membrane Performance	Reference
VACNT	CNT + polyethersulfone (PES)	CVD	Used for ultrafiltration (UF) Water transportation speed = 100 L/m^2.h at 60 Psi 3 times faster than random oriented CNT + PES 10 times faster than PES membrane	Li *et al.* (2014)
VACNT	CNT + epoxy	Water-assisted thermal CVD	3 times higher water flux than UF2 log Less bacterial concentration in VCNT (4×10^5 CFU/mL) as compared to UF (8×10^7 CFU/mL) Permeate flux reduction after 600 min = 67% for VCNT and 55% for UF	Baek *et al.* (2014)
VACNT	CNT + stainless-steel mesh	Thermal CVD	Separate water layer and diesel and even surfactant-stabilized emulsions High hydrophobicity and oleophobicity	Lee and Baik (2010)
SWCNT	CNT + poly vinylidene fluoride	Vacuum filtration	Complete removal of viral pathogens and bacteria 5×10^5 *Escherichia coli* cells were removed Cell inactivation = 79% after contact time of 20 min Metabolically active *E. coli* = 6% as compared to over 70% in control filter Damage *E. coli* cell membrane	Brady-Estévez *et al.* (2008)
VACNT	CNT + poly tetrafluoro ethylene + Si substrate	Water-assisted CVD	Millimeter-thick UF membrane Water permeability = 30 000/Lm^2/h/bar Inhibit bacterial growth and resist biofilm formation	Lee *et al.* (2015)

VACNT	MWCNT + Fe + Al_2O_3 + Si	Thermal CVD	The proportion of irreversible fouling to total resistance for UF and VCNT membranes were 24% and 69%, respectively, while the proportion of reversible fouling resistance was 38% and 5%, respectively BSA (bovine serum albumin) removal improved from 71% to 90% after surface modification by graft-polymerization of methacrylic acid	Park *et al.* (2014)
Vertically aligned double-wall CNT	Double-wall CNT + Si wafer substrate	CVD	NaCl rejection = 41.4% Water flux = 1.31×10^{-3} to 62.7×10^{-3} $L/cm^2/day/MPa$	Matsumoto *et al.* (2017)
CNT bucky paper	CNT	CVD, UV/ozone treatment and alkoxysilanization	Salt rejection ≥95% Life span improved by 50%	Dumée *et al.* (2011)
VACNT	MCNT + polydimethylsiloxane + Si wafer	CVD	Salt rejection = 96.5% at operational pressure of 2 bar	Trivedi and Alameh (2016)
Graphene oxide coated VACNT (GO-VACNT) and polyamide coated VA-CNT (PC-VACNT)	CNT + epoxy	–	Salt rejection = 45% and 65% for GO-VCNT and PO-VCNT at operational pressure of 15.5 bar, respectively	Baek *et al.* (2016)
Polyamide/outer-wall VA-CNT	CNT + epoxy	Interfacial polymerization	Reverse osmosis membrane Flux = $128.6/Lm^2/h$ Salt rejection = 98.3% at operational pressure of 15.5 bar	Li *et al.* (2019)

*Source:*Reproduced with copyright from Mishra and Sundaram (2023).

to wastewater treatment, the concept of water is modified by modifying the organic oxygen demand and the material oxygen demand of the water. The potential risks to human health posed by using nanotechnology to purify water are one of the factors limiting the case's scope. As nanotechnology retains substantial water additions and raises the degree of poisonousness in the water, it should be used only for cleaning wastewater. The effect of CNTs on living species is an uncharted topic, despite the fact that several literatures declare and support their usage in wastewater treatment. Several studies have demonstrated CNTs' cytotoxicity toward mammalian cells. Mice were used to investigate the impact of inhaled MWCNTs on their biological systems. Mice were given MWCNT doses of 0.3 and 1 ppm where the reduction of immunity was observed at 1 ppm. CNTs are more hazardous than quartz and asbestos on mammalian cells. Several studies have demonstrated that agglomerated CNTs are more hazardous than their dispersed counterparts. Nevertheless, CNT agglomeration can be prevented by modifying the CNTs' surfaces. Based on their research comparing raw CNTs to surface-modified CNTs, it was concluded that the latter is having higher solubility. To fully understand the impacts and repercussions of utilizing CNP in wastewater treatment, additional, in-depth preclinical investigations are required. Thin film photocatalysts for the purification of wastewater have only been the subject of a small number of research projects. However, there is a paucity of data on the characteristics of photo-active nanomaterials regarding the toxicity levels of treated water sample containing spent catalysts, standard processes of toxicity tests, reusing and recirculation of spent catalysts, safety of treatment impacts, and economic assessment (Rueda-Marquez *et al.*, 2020). Due of nanoparticles' adaptability, interest in them has been generated from a wide range of sectors and individuals. Due to a lack of qualified individuals, Labfirm Fusion is unable to serve as a bridge across different industries and technologies. The water and sewage treatment industries have benefited greatly from this line of research. In addition to these sectors, others, such as those involved in healthcare, electrical instruments, building materials, and consumer goods, also reap substantial benefits. The novelty of nanotechnology means that information associated with the subject remains hidden to many water partners, making their work difficult. Manufactured nanoparticles can be used in the practical purification and disinfection of water and wastewater. Therefore, more effort must be devoted to enhancing this breakthrough. The government, along with private sectors, must coordinate, be pro-active, and include skills, resources, policies, and standard operating procedures to increase the safe application of nanoremediation. Nanotechnology needs to be widely adopted for economic, social, and strategic reasons, and there is a sizable market for this. Effective methods for removing nanoparticles from water are needed to mitigate or eliminate their harmful effects on humans and the environment.

14.7 CONCLUSIONS

Despite water's importance in every aspect of human life, it is becoming increasingly hard to come by in many places of the world. Challenges in providing safe drinkable water can be mitigated through the use of renewable resources to provide ultrafine freshwater, as well as through the conservation and protection of water bodies from contamination. Nanotechnology and its potential uses have been identified as promising avenues for addressing water scarcity and quality. Because of their huge surface areas and size- and shape-dependent catalytic capabilities, nanomaterials are the subject of a significant amount of research and investigation into their potential use in applications such as membrane separations, catalysis, and adsorption. Although it is expensive, membrane technology is increasingly being seen as the best option for purifying water. Nanoparticle-functionalized membranes have emerged as a promising new alternative to the conventional approaches. The versatility of today's technologies, such as membrane technology, nanotechnology, and biochar adsorption, also permits the creation of the most practical applications possible. While constructed biochar may have certain uses, the most crucial consideration when deciding on a wastewater treatment strategy is how to properly dispose of the sludge that is generated during the treatment process. Therefore, an effective and discharge-free solution is urgently needed to address the most challenging problem facing sustainable development. There are more upsides than downsides to employing nanostructures in wastewater treatment, making them a potentially beneficial alternative to the many existing treatment approaches. The water treatment procedure benefits greatly from the use of biochar made from Municipal Solid Waste (MSW). The low specific surface area of biochar made from mixed municipal solid waste could be raised through clay alteration. Biochar made from properly sorted MSW outperforms adsorption and advanced oxidation processes, respectively, in terms of efficiency and stability in the removal of pollutants. Biochar made from food waste in municipal solid waste is very good at cleaning the air through adsorption and oxidation processes. In order to get the most use out of the materials contained in municipal solid waste, it is possible to separate out things such as fruit peels, bones, and shells. Bone and shell biochar is extremely effective at removing organic pollutants thanks to its high adsorption capacity and catalytic activity. Biochar made from peels is very effective at activating persulfate. Biochar made from recycled paper is also effective for the adsorption removal of heavy metals. Biochar's adsorption capacity and catalytic activity inside oxidation systems were enhanced by modifying its surface structure and chemical composition utilizing various preparation processes. In order to enhance the adsorption system's ability to remove contaminants, an alkaline treatment was performed to the biochar that was produced from mixed municipal solid waste. Biochar produced by pyrolysis and acid treatment can be used to eliminate organic pollutants in oxidation systems. Bone and eggshells were used to create this biochar. To further facilitate the removal of pollutants via adsorption and oxidation processes, bone is frequently pre-carbonized prior to the formation of biochar via the process of pyrolysis. CNTs outperform traditional water and wastewater treatment methods in several key areas. Improved adsorption capacity, greater selectivity, and a rapid response time are all hallmarks of their performance as an efficient adsorbent. Filters made from CNTs are also effective in sterilizing the air. Water filtration and desalination are two more useful applications among them. Incorporating CNT into the membrane improves its water permeability, strength, and antifouling properties. Two major obstacles are lowering the cost of generating CNTs and increasing their efficiency. However, CNTs almost see only academic and laboratory applications. The feasibility of their usage in industrial and large-scale applications, as well as the development of low-cost, non-toxic methods of synthesis, warrants further investigation.

REFERENCES

Abbasi M. (2017). Synthesis and characterization of magnetic nanocomposite of chitosan/SiO_2/carbon nanotubes and its application for dyes removal. *Journal of Cleaner Production*, **145**, 105–113, https://doi.org/10.1016/j.jclepro.2017.01.046

Abu Bakar S., Jusoh N., Mohamed A., Muqoyyanah M., Othman M. H. D., Mamat M. H., Ahmad M. K., Mohamed M. A., Azlan M. N., Hashim N., Birowosuto M. D. and Soga T. (2021). Carbon nanotubes from waste cooking palm oil as adsorbent materials for the adsorption of heavy metal ions. *Environmental Science and Pollution Research*, **28**(46), 65171–65187.

Adegoke A. A., Amoah I. D., Stenström T. A., Verbyla M. E. and Mihelcic J. R. (2018). Epidemiological evidence and health risks associated with agricultural reuse of partially treated and untreated wastewater: a review. *Frontiers in Public Health*, **6**, 337, https://doi.org/10.3389/fpubh.2018.00337

Ahmad A. and Hameed B. (2010). Fixed-bed adsorption of reactive azo dye onto granular activated carbon prepared from waste. *Journal of Hazardous Materials*, **175**, 298–303.

Ahmad M., Rajapaksha A. U., Lim J. E., Zhang M., Bolan N., Mohan D., Vithanage M., Lee S. S. and Ok Y. S. (2014). Biochar as a sorbent for contaminant management in soil and water: a review. *Chemosphere*, **99**, 19–33.

Akter J., Hanif M., Islam M. A., Sapkota K. and Hahn J. (2021). Selective growth of Ti^{3+}/TiO_2/CNT and Ti^{3+}/TiO_2/C nanocomposite photocatalysts for enhanced visible-light utilization to degrade organic pollutants by lowering the bandgap of TiO2, https://doi.org/10.21203/rs.3.rs-199530/v1

Al-Mamun M. R., Kader S., Islam M. S., Khan M. Z. H. (2019). Photocatalytic activity improvement and application of UV-TiO_2 photocatalysis in textile wastewater treatment: A review. *Journal of Environmental Chemical Engineering*, **7**(1032), 1–17.

Ambaye T., Vaccari M., van Hullebusch E. D., Amrane A. and Rtimi S. (2021). Mechanisms and adsorption capacities of biochar for the removal of organic and inorganic pollutants from industrial wastewater. *International Journal of Environmental Science and Technology*, **18**, 3273–3294.

Baek Y., Kim C., Seo D. K., Kim T., Lee J. S., Kim Y. H., Ahn K. H., Bae S. S., Lee S. C., Lim J., Lee K. and Yoon J. (2014). High performance and antifouling vertically aligned carbon nanotube membrane for water purification. *Journal of Membrane Science*, **460**, 171–177.

Baek Y., Seo D. K., Choi J. H., Lee B., Kim Y. H., Park S. M., Jung J., Lee S. and Yoon J. (2016). Improvement of vertically aligned carbon nanotube membranes: desalination potential, flux enhancement, and scale-up. *Desalination and Water Treatment*, **57**(58), 28133–28140.

Barrejón M., Syrgiannis Z., Burian M., Bosi S., Montini T., Fornasiero P., Amenitsch H. and Prato M. (2019). Cross-linked carbon nanotube adsorbents for water treatment: tuning the sorption capacity through chemical functionalization. *ACS Applied Materials & Interfaces*, **11**(13), 12920–12930.

Bharti S. K. and Kumar N. (2018). Kinetic study of lead (Pb 2+) removal from battery manufacturing wastewater using bagasse biochar as biosorbent. *Applied Water Science*, **8**, 1–13.

Bradder P., Ling S. K., Wang S. and Liu S. (2011). Dye adsorption on layered graphite oxide. *Journal of Chemical & Engineering Data*, **56**, 138–141.

Brady-Estévez A. S., Kang S. and Elimelech M. (2008). A single-walled-carbon-nanotube filter for removal of viral and bacterial pathogens. *Small*, **4**(4), 481–484, https://doi.org/10.1002/smll.200700863

Crane R. A. and Scott T. B. (2012). Nanoscale zero-valent iron: future prospects for an emerging water treatment technology. *Journal of Hazardous Materials*, **211**, 112–125.

Czech B. and Buda W. (2015). Photocatalytic treatment of pharmaceutical wastewater using new multiwall-carbon nanotubes/TiO_2/SiO_2 nanocomposites. *Environmental Research*, **137**, 176–184, https://doi.org/10.1016/j.envres.2014.12.006

Deng X., Lü L., Li H. and Luo F. (2010). The adsorption properties of Pb (II) and Cd (II) on functionalized graphene prepared by electrolysis method. *Journal of Hazardous Materials*, **183**, 923–930.

Dong L.-X., Huang X.-C., Wang Z., Yang Z., Wang X.-M. and Tang C. Y. (2016). A thin-film nanocomposite nanofiltration membrane prepared on a support with in situ embedded zeolite nanoparticles. *Separation and Purification Technology*, **166**, 230–239.

Dumée L., Germain V., Sears K., Schütz J., Finn N., Duke M., Cerneaux S., Cornu D. and Gray S. (2011). Enhanced durability and hydrophobicity of carbon nanotube bucky paper membranes in membrane distillation. *Journal of Membrane Science*, **376**(1–2), 241–246.

Fagbohungbe M. O., Herbert B. M., Hurst L., Ibeto C. N., Li H., Usmani S. Q. and Semple K. T. (2017). The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion. *Waste Management*, **61**, 236–249.

Feizpoor S., Habibi-Yangjeh A. and Yubuta K. (2018). Integration of carbon dots and polyaniline with TiO_2 nanoparticles: Substantially enhanced photocatalytic activity to removal various pollutants under visible light. *Journal of Photochemistry and Photobiology A: Chemistry*, **367**, 94–104.

Ferreira G. M. D., Ferreira G. M. D., Hespanhol M. C., de Paula Rezende J., dos Santos Pires A. C., Gurgel L. V. A. and da Silva L. H. M. (2017). Adsorption of red azo dyes on multi-walled carbon nanotubes and activated carbon: A thermodynamic study. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **529**, 531–540, https://doi.org/10.1016/j.colsurfa.2017.06.021

Gupta V. K., Agarwal S., Bharti A. K. and Sadegh H. (2017). Adsorption mechanism of functionalized multi-walled carbon nanotubes for advanced Cu (II) removal. *Journal of Molecular Liquids*, **230**, 667–673, https://doi.org/10.1016/j.molliq.2017.01.083

Gusain R., Kumar N., Fosso-Kankeu E. and Ray S. S. (2019). Efficient removal of Pb(II) and Cd(II) from industrial mine water by a hierarchical MoS2/SH-MWCNT nanocomposite. *ACS Omega*, **4**(9), 13922–13935.

Haque A. N. M. A., Remadevi R., Rojas O. J., Wang X. and Naebe M. (2020). Kinetics and equilibrium adsorption of methylene blue onto cotton gin trash bioadsorbents. *Cellulose*, **27**, 6485–6504.

Hara T. O. and Singh B. (2021). Electrochemical biosensors for detection of pesticides and heavy metal toxicants in water. *Recent Trends in Program*, **1**(3), 462–478, https://doi.org/10.1021/acsestwater.0c00125

Higashikawa F. S., Conz R. F., Colzato M., Cerri C. E. P. and Alleoni L. R. F. (2016). Effects of feedstock type and slow pyrolysis temperature in the production of biochars on the removal of cadmium and nickel from water. *Journal of Cleaner Production*, **137**, 965–972.

Hosseinzadeh S., Hosseinzadeh H., Pashaei S. and Khodaparast Z. (2018). Synthesis of magnetic functionalized MWCNT nanocomposite through surface RAFT co-polymerization of acrylic acid and N-isopropyl acrylamide for removal of cationic dyes from aqueous solutions. *Ecotoxicology and Environmental Safety*, **161**, 34–44, https://doi.org/10.1016/j.ecoenv.2018.05.063

Huang Z.-H., Zheng X., Lv W., Wang M., Yang Q.-H. and Kang F. (2011). Adsorption of lead (II) ions from aqueous solution on low-temperature exfoliated graphene nanosheets. *Langmuir*, **27**, 7558–7562.

Jeon I.-Y., Chang D. W., Kumar N. A. and Baek J.-B. (2011). Functionalization of carbon nanotubes. In: Carbon Nanotubes-Polymer Nanocomposites, InTech, United Kingdom, ISBN 978-953-307-498-6, pp. 91–110, https://doi.org/10.5772/18396

Jeong B.-H., Hoek E. M., Yan Y., Subramani A., Huang X., Hurwitz G., Ghosh A. K. and Jawor A. (2007). Interfacial polymerization of thin film nanocomposites: a new concept for reverse osmosis membranes. *Journal of Membrane Science*, **294**, 1–7.

Jiang Q. L., Tian H. L. and Zhang K. S. (2019). Enhanced performance of poly(m-phenylene isophthalamide) (PMIA) composite hollow fiber ultrafiltration membranes by O-MoS2 nanosheets modification. *Desalination and Water Treatment*, **166**, 245–258.

Jiang Q., Wang Y., Li Y., Luo J. and Xiong J. (2023). Nanocomposite substrate-supported nanofiltration membrane for efficient treatment of rare earth wastewater. *Results in Engineering*, **18**, 101040, https://doi.org/10.1016/j.rineng.2023.101040

Joshi S., Garg V. K. and Kataria N. (2019). Applications of Fe_3O_4 Ac nanoparticles for removal of dyes in simulated wastewater. *Chemosphere*, **236**, Article 124280, 1–11.

Kaetzl K., Lübken M., Uzun G., Gehring T., Nettmann E., Stenchly K. and Wichern M. (2019). On-farm wastewater treatment using biochar from local agroresidues reduces pathogens from irrigation water for safer food production in developing countries. *Science of the Total Environment*, **682**, 601–610.

Karim A. A., Kumar M., Singh S. K., Panda C. R. and Mishra B. K. (2017). Potassium enriched biochar production by thermal plasma processing of banana peduncle for soil application. *Journal of Analytical and Applied Pyrolysis*, **123**, 165–172.

Kausar A. (2016). Properties of polyacrylamide and functional multi-walled carbon nanotubes. *American Journal of Nanoscience Nanotechnology Research*, **4**(1), 1–8.

Krishnamurthy G. and Agarwal S. (2013). Optimization of reaction conditions for high yield synthesis of carbon nanotube bundles by low-temperature solvothermal process and study of their H2 storage capacity. *Bulletin of the Korean Chemical Society*, **34**, 3046–3054.

Kumar S., Ahlawat W., Bhanjana G., Heydarifard S., Nazhad M. M. and Dilbaghi N. (2014). Nanotechnology-based water treatment strategies. *Journal of Nanoscience and Nanotechnology*, **14**, 1838–1858.

Kuo C.-Y., Wu C.-H. and Wu J.-Y. (2008). Adsorption of direct dyes from aqueous solutions by carbon nanotubes: determination of equilibrium, kinetics and thermodynamics parameters. *Journal of Colloid and Interface Science*, **327**, 308–315.

Lai G. S., Lau W. J., Goh P. S., Ismail A. F., Yusof N. and Tan Y. H. (2016). Graphene oxide incorporated thin film nanocomposite nanofiltration membrane for enhanced salt removal performance. *Desalination*, **387**, 14–24.

Lee C. and Baik S. (2010). Vertically-aligned carbon nano-tube membrane filters with superhydrophobicity and superoleophilicity. *Carbon*, **48**(8), 2192–2197, https://doi.org/10.1016/j.carbon.2010.02.020

Lee B., Baek Y., Lee M., Jeong D. H., Lee H. H., Yoon J. and Kim Y. H. (2015). A carbon nanotube wall membrane for water treatment. *Nature Communications*, **6**(1), 1–7, https://doi.org/10.1038/ncomms8109

Li S., Liao G., Liu Z., Pan Y., Wu Q., Weng Y., Zhang X., Yang Z. and Tsui O. K. C. (2014). Enhanced water flux in vertically aligned carbon nanotube arrays and polyethersulfone composite membranes. *Journal of Materials Chemistry A*, **2**(31), 12171–12176.

Li K., Lee B. and Kim Y. (2019). High-performance reverse osmosis membrane with carbon nanotube support layer. *Journal of Membrane Science*, **592**, 117358.

Li X., Wang Z., Han X. L., Liu Y. Y., Wang C., Yan F. Z. and Wang J. X. (2021). Regulating the interfacial polymerization process toward high-performance polyamide thin-film composite reverse osmosis and nanofiltration membranes: a review. *Journal of Membrane Science*, **640**, Article 119765, 1–24.

Liao Z. P., Zhu J. Y., Li X. and Van der Bruggen B. (2021). Regulating composition and structure of nanofillers in thin film nanocomposite (TFN) membranes for enhanced separation performance: a critical review. *Separation and Purification Technology*, **266**, Article 118567, 1–21.

Lin Y. T., Kao F. Y., Chen S. H., Wey M. Y. and Tseng H. H. (2020). A facile approach from waste to resource: reclaimed rubber-derived membrane for dye removal. *Journal of the Taiwan Institute of Chemical Engineers*, **112**, 286–295.

Lind M. L., Jeong B.-H., Subramani A., Huang X. and Hoek E. M. (2009). Effect of mobile cation on zeolite-polyamide thin film nanocomposite membranes. *Journal of Materials Research*, **24**, 1624–1631.

Liu X., Wang M., Zhang S. and Pan B. (2013). Application potential of carbon nanotubes in water treatment: A review. *Journal of Environmental Sciences*, **25**(7), 1263–1280, https://doi.org/10.1016/S1001-0742(12)60161-2

Low S. S., Chen Z., Li Y., Lu Y. and Liu Q. (2021a). Design principle in biosensing: critical analysis based on graphitic carbon nitride (G-C3N4) photoelectrochemical biosensor. *TrAC Trends in Analytical Chemistry*, **145**, 116454. ISSN 0165-9936, https://doi.org/10.1016/j.trac.2021.116454

Low S. S., Lim C. N., Yew M., Chai W. S., Low L. E., Manickam S., Tey B. T. and Show P. L. (2021b). Recent ultrasound advancements for the manipulation of nanobiomaterials and nanoformulations for drug delivery. *Ultrasonics Sonochemistry*, **80**, 105805. ISSN 1350-4177, https://doi.org/10.1016/j.ultsonch.2021.105805

Low S. S., Yew M., Lim C. N., Chai W. S., Low L. E., Manickam S., Tey B. T. and Show P. L. (2022). Sonoproduction of nanobiomaterials – a critical review. *Ultrasonics Sonochemistry*, **82**, 105887. ISSN 1350-4177, https://doi.org/10.1016/j.ultsonch.2021.105887

Machado F. M., Bergmann C. P., Fernandes T. H. M., Lima E. C., Royer B., Calvete T. and Fagan S. B. (2011). Adsorption of reactive red M-2BE dye from water solutions by multi-walled carbon nanotubes and activated carbon. *Journal of Hazardous Materials*, **192**(3), 1122–1131.

Mahmoodi N. M., Rezaei P., Ghotbei C. and Kazemeini M. (2016). Copper oxide-carbon nanotube (CuO/CNT) nanocomposite: synthesis and photocatalytic dye degradation from colored textile wastewater. *Fibers and Polymers*, **17**(11), 1842–1848, https://doi.org/10.1007/s12221-016-6645-y

Matsumoto H., Tsuruoka S., Hayashi Y., Abe K., Hata K., Zhang S., Saito Y., Aiba M., Tokunaga T., Iijima T., Hayashi T., Inoue H. and Amaratunga G. A. J. (2017). Water transport phenomena through membranes consisting of vertically-aligned double-walled carbon nanotube array. *Carbon*, **120**, 358–365.

Meng H., Nie C., Li W., Duan X., Lai B., Ao Z., Wang S. and An T. (2020). Insight into the effect of lignocellulosic biomass source on the performance of biochar as persulfate activator for aqueous organic pollutants remediation: epicarp and mesocarp of citrus peels as examples. *Journal of Hazardous Materials*, **399**, 123043, https://doi.org/10.1016/j.jhazmat.2020.123043

Meshko V., Markovska L., Mincheva M. and Rodrigues A. (2001). Adsorption of basic dyes on granular acivated carbon and natural zeolite. *Water Research*, **35**, 3357–3366.

Mishra S. and Sundaram B. (2023). Efficacy and challenges of carbon nanotube in wastewater and water treatment. *Environmental Nanotechnology, Monitoring & Management*, **19**, 100764, https://doi.org/10.1016/j.enmm.2022.100764

Mohamed A., Yousef S., Nasser W. S., Osman T. A., Knebel A., Sánchez E. P. V. and Hashem T. (2020). Rapid photocatalytic degradation of phenol from water using composite nanofibers under UV. *Environmental Sciences, Europe*, **32**(1), 1–8, https://doi.org/10.1186/s12302-020-00436-0

Mohammadi A. and Veisi P. (2018). High adsorption performance of β-cyclodextrin-functionalized multi-walled carbon nanotubes for the removal of organic dyes from water and industrial wastewater. *Journal of Environmental Chemical Engineering*, **6**(4), 4634–4643, https://doi.org/10.1016/j.jece.2018.07.002

Mohanty P., Nanda S., Pant K. K., Naik S., Kozinski J. A. and Dalai A. K. (2013). Evaluation of the physiochemical development of biochars obtained from pyrolysis of wheat straw, Timothy grass and pinewood: effects of heating rate. *Journal of Analytical and Applied Pyrolysis*, **104**, 485–493.

Mubarak N. M., Sahu J. N., Abdullah E. C., Jayakumar N. S. and Ganesan P. (2015). Novel microwave-assisted multiwall carbon nanotubes enhancing Cu (II) adsorption capacity in water. *Journal of the Taiwan Institute of Chemical Engineers*, **53**, 140–152, https://doi.org/10.1016/j.jtice.2015.02.016

Mubarak N. M., Sahu J. N., Abdullah E. C. and Jayakumar N. S. (2016). Rapid adsorption of toxic Pb(II) ions from aqueous solution using multiwall carbon nanotubes synthesized by microwave chemical vapor deposition technique. *Journal of Environmental Sciences*, **45**, 143–155, https://doi.org/10.1016/j.jes.2015.12.025

Mubaraka N., Daniela S., Khalid M. and Tana J. (2012). Comparative study of functionalize and non-functionalized carbon nanotube for removal of copper from polluted water. *International Journal of Chemical and Environmental Engineering*, **3**, 314–317.

Ndoun M. C., Knopf A., Preisendanz H. E., Vozenilek N., Elliott H. A., Mashtare M. L., Velegol S., Veith T. L. and Williams C. F. (2023). Fixed bed column experiments using cotton gin waste and walnut shells-derived biochar as low-cost solutions to removing pharmaceuticals from aqueous solutions. *Chemosphere*, **330**, 138591, 1–9, https://doi.org/10.1016/j.chemosphere.2023.138591

Park S.-M., Jung J., Lee S., Baek Y., Yoon J., Seo D. K. and Kim Y. H. (2014). Fouling and rejection behavior of carbon nanotube membranes. *Desalination*, **343**, 180–186, https://doi.org/10.1016/j.desal.2013.10.005

Park J.-H., Ok Y. S., Kim S.-H., Cho J.-S., Heo J.-S., Delaune R. D. and Seo D.-C. (2016). Competitive adsorption of heavy metals onto sesame straw biochar in aqueous solutions. *Chemosphere*, **142**, 77–83.

Perez-Mercado L. F., Lalander C., Joel A., Ottoson J., Dalahmeh S. and Vinnerås B. (2019). Biochar filters as an on-farm treatment to reduce pathogens when irrigating with wastewater-polluted sources. *Journal of Environmental Management*, **248**, 109295.

Rajapaksha A. U., Vithanage M., Ahmad M., Seo D.-C., Cho J.-S., Lee S.-E., Lee S. S. and Ok Y. S. (2015). Enhanced sulfamethazine removal by steam-activated invasive plant-derived biochar. *Journal of Hazardous Materials*, **290**, 43–50.

Rao G. P., Lu C. and Su F. (2007). Sorption of divalent metal ions from aqueous solution by carbon nanotubes: a review. *Separation and Purification Technology*, **58**, 224–231.

Ren D., Colosi L. M. and Smith J. A. (2013). Evaluating the sustainability of ceramic filters for point-of-use drinking water treatment. *Environmental Science & Technology*, **47**(19), 11206–11213, https://doi.org/10.1021/es4026084

Robati D., Bagheriyan S., Rajabi M., Moradi O. and Peyghan A. A. (2016). Effect of electrostatic interaction on the methylene blue and methyl orange adsorption by the pristine and functionalized carbon nanotubes. *Physica E: Low-Dimensional Systems and Nanostructures*, **83**, 1–6, https://doi.org/10.1016/j.physe.2016.04.005

Rueda-Marquez J. J., Levchuk I., Fernández Ibañez P. and Sillanpää M. (2020). A critical review on application of photocatalysis for toxicity reduction of real wastewaters. *Journal of Cleaner Production*, **258**, 120694, https://doi.org/10.1016/j.jclepro.2020.120694

Saber-Samandari S., Saber-Samandari S., Joneidi-Yekta H. and Mohseni M. (2017). Adsorption of anionic and cationic dyes from aqueous solution using gelatin-based magnetic nanocomposite beads comprising carboxylic acid functionalized carbon nanotube. *Chemical Engineering Journal*, **308**, 1133–1144, https://doi.org/10.1016/j.cej.2016.10.017

Samsudin M. F. R., Bacho N., Sufian S. and Ng Y. H. (2019). Photocatalytic degradation of phenol wastewater over Z-scheme g-C3N4/CNT/BiVO4 heterostructure photocatalyst under solar light irradiation. *Journal of Molecular Liquids*, **277**, 977–988.

Shang J., Zong M., Yu Y., Kong X., Du Q. and Liao Q. (2017). Removal of chromium (VI) from water using nanoscale zerovalent iron particles supported on herb-residue biochar. *Journal of Environmental Management*, **197**, 331–337.

Tan X.-F., Zhu S.-S., Wang R.-p., Chen Y.-D., Show P.-L., Zhang F.-F. and Ho S.-H. (2021). Role of biochar surface characteristics in the adsorption of aromatic compounds: pore structure and functional groups. *Chinese Chemical Letters*, **32**(10), 2939–2946. ISSN 1001-8417, https://doi.org/10.1016/j.cclet.2021.04.059

Tang B., Shan L., Liang S. and Zhou J. (2019). Issues and opportunities facing aqueous zinc-ion batteries. *Energy & Environmental Science*, **12**, 3288–3304.

Trivedi S. and Alameh K. (2016). Effect of vertically aligned carbon nanotube density on the water flux and salt rejection in desalination membranes. *SpringerPlus*, **5**(1), 1–13, https://doi.org/10.1186/s40064-016-2783-3

Vunain E., Mishra A. and Mamba B. (2016). Dendrimers, mesoporous silicas and chitosan-based nanosorbents for the removal of heavy-metal ions: a review. *International Journal of Biological Macromolecules*, **86**, 570–586.

Vuono D., Catizzone E., Aloise A., Policicchio A., Agostino R., Migliori M. and Giordano G. (2017). Study of adsorption behavior of multi-walled carbon nanotubes towards dyes applied in textile applications. *Advanced Science Letters*, **23**, 5851–5854, https://doi.org/10.1166/asl.2017.9048

Wang S. and Zhou S. (2011). Photodegradation of methyl orange by photocatalyst of CNTs/P-TiO2 under UV and visible-light irradiation. *Journal of Hazardous Materials*, **185**(1), 77–85, https://doi.org/10.1016/j.jhazmat.2010.08.125

Westerhoff P., Hritvoski K. M., Lopes L. and Sergi Garcia S. (2018). Electrolytic reduction of nitrate fundamental to full scale water treatment. *Applied Catalysis B: Environment*, **236**, 546–568.

Wongaree M., Chiarakorn S. and Chuangchote S. (2015). Photocatalytic improvement under visible light in TiO_2 nanoparticles by carbon nanotube incorporation. *Journal of Nanomaterials*, **16**(1), 295, 1–10, https://doi.org/10.1155/2015/689306

Xu Y., Hui X., Wang L., Yan J., Li H., Song Y., Huang L. and Cai G. (2013). The CNT modified white C_3N_4 composite photocatalyst with enhanced visible-light response photoactivity. *Dalton Transactions (Cambridge, England: 2003)*, **42**, 7604, https://doi.org/10.1039/c3dt32871f

Yang S. S., Tian H. L., Hill M. R. and Zhang K. S. (2021). Effect and regulation mechanism of oxidation degrees on the O-MoS2 structure and separation performance of nanofiltration membrane. *Journal of Membrane Science*, **635**, Article 119468, 1–12.

Yao D., Chen Z., Zhao K., Yang Q. and Zhang W. (2013). Limitation and challenges faced to the researches on the environmental risk of nanotechnology. *Procedia Environmental Sciences*, **18**, 149–156, https://doi.org/10.1016/j.proenv.2013.04.020

Yibeltal A. W., Beyene B. B., Admassie S. and Taddesse A. M. (2020). MWCNTs/Ag-ZnO nanocomposite for efficient photocatalytic degradation of Congo red. *Bulletin of the Chemical Society of Ethiopia*, **34**(1), 55–66.

Yu H., Zou W., Chen J., Chen H., Yu Z., Huang J., Tang H., Wei X. and Gao B. (2019). Biochar amendment improves crop production in problem soils: a review. *Journal of Environmental Management*, **232**, 8–21.

Yusoff N., Lim P., Azmi N., Yusoff M. and Muhammad M. (2021). Effect of photocatalyst dosage and air loading in photocatalytic degradation of metamifop. *IOP Conference Series: Earth and Environmental Science*, **842**, Article 012061, 1–8, https://doi.org/10.1088/1755-1315/842/1/012061

Zare K., Sadegh H., Shahryari-hoshekandi R., Maazinejad B., Ali V., Tyagi I., Agarwal S. and Gupta V. K. (2015). Enhanced removal of toxic Congo red dye using multi-walled carbon nanotubes: kinetic, equilibrium studies and its comparison with other adsorbents. *Journal of Molecular Liquids*, **212**, 266–271.

Zazycki M. A., Godinho M., Perondi D., Foletto E. L., Collazzo G. C. and Dotto G. L. (2018). New biochar from pecan nutshells as an alternative adsorbent for removing reactive red 141 from aqueous solutions. *Journal of Cleaner Production*, **171**, 57–65.

Zhang Y., Yang J., Zhong L. and Liu L. (2018). Effect of multi-wall carbon nanotubes on Cr(VI) reduction by citric acid: implications for their use in soil remediation. *Environmental Science and Pollution Research*, **25**(24), 23791–23798, https://doi.org/10.1007/s11356-018-2438-8

Zhang M., Xu L., Qi C. and Zhang M. (2019). Highly effective removal of tetracycline from water by hierarchical porous carbon: batch and column adsorption. *Industrial & Engineering Chemistry Research*, **58**(43), 20036–20046.

Zhao N., Yang X., Zhang J., Zhu L. and Lv Y. (2017). Adsorption mechanisms of dodecylbenzene sulfonic acid by corn straw and poplar leaf biochars. *Materials*, **10**, 1119.

Zhao B., Guo Z. Q., Wang H. L., Wang L., Qian Y. R., Long X. L., Ma C., Zhang Z. H., Li J. J. and Zhang H. W. (2021). Enhanced water permeance of a polyamide thin-film composite nanofiltration membrane with a metal-organic framework interlayer. *Journal of Membrane Science*, **625**, Article 119154, 1–9.

Zhou J., Liu Y., Han Y., Jing F. and Chen J. (2019). Bone-derived biochar and magnetic biochar for effective removal of fluoride in groundwater: effects of synthesis method and coexisting chromium. *Water Environment Research*, **91**(7), 588–597, https://doi.org/10.1002/wer.1068

doi: 10.2166/9781789063783_0283

Chapter 15

Fungi: a veritable tool for refractory pollutant remediation

Shivangi Mudaliar[1], *Komal Agrawal*[2] *and Pradeep Verma*[1]*

[1]Bioprocess and Bioenergy Laboratory (BPEL), Department of Microbiology, Central University of Rajasthan, Bandarsindri, Kishangarh, Ajmer, Rajasthan 305817, India
[2]Department of Microbiology, School of Bio Engineering and Biosciences, Lovely Professional University, Phagwara, Punjab 144411, India
**Corresponding author: pradeepverma@curaj.ac.in; vermaprad@yahoo.com*

ABSTRACT

The rapidly escalating population, urbanization, and industrial development are intensifying problems related to pollution. Soil and water pollution affect the health of organisms and the environment. Remarkable results in environmental cleaning have been obtained through the practice of bioremediation. Bioremediation is a sustainable and inexpensive method for wastewater treatment. Currently, the focus is on having clean technologies that generate less waste, with maximum output. Mycoremediation has several advantages over other bioremediation tools. Fungi are abundantly found in the environment and offer a non-toxic, zero maintenance, recyclable, less invasive, and secure approach for the treatment of wastewater. Fungus and their enzyme systems have been well explored for the degradation of toxic wastes and remediation of polluted sites. The extracellular enzyme system of fungus secretes various enzymes that break down the complex substances into simpler forms. Additionally, it is an in-situ remediation approach that exploits the capability of the fungus to reuse. This chapter deals with the idea of mycoremediation and its mechanism. The role of fungal cell walls in the biosorption, bioaccumulation, and biomineralization of heavy metals has also been discussed.

Keywords: bioremediation, mycoremediation, fungi, in situ, pollutants

15.1 INTRODUCTION

Anthropological and industrial actions, such as refinery, chemical industry, pharmaceutical industry, leather industry, heavy metals, dyes, mining, agrochemicals, detergents, food industry, and so on are progressively polluting the available source of potable water. According to the World Health Organization (WHO), over 30% of all human illnesses and 40% of all morbidity worldwide are due to contaminated water (Negi & Das, 2023). Wastewater must first go through the treatment process before it is disposed of into rivers (Akhtar & Mannan, 2020). Precipitation, electrocoagulation, adsorption, zerovalent iron, ion exchange, and membrane separation are some of the physical processes used in the treatment of wastewater

(GilPavas *et al.*, 2020). On the contrary, methods have numerous downsides, including high costs, high energy requirements, and the production of hazardous components as a result of the use of chemicals (Crini & Lichtfouse, 2019). Therefore, biological methods are favored over physicochemical procedures because biological methods are cost-effective and non-toxic to the ecosystem. Various microorganisms have been widely used in the industrial sector for wastewater treatment because they are inexpensive, use less energy, and yield useful products that can be utilized in the generation of energy as well as other processes (Grelska & Noszczyńska, 2020; Rajta *et al.*, 2020). Contrarily, fungi have attracted much less attention than bacteria for wastewater treatment. Fungi can release a variety of reductive enzymes that transform wastewater organics into valuable fungal proteins and biochemicals (Grelska & Noszczyńska, 2020). The biosorption technique, which can be used, depends on proteins found on the fungal surface and other compounds to filter out hazardous metallic elements and other inorganic materials from wastewater (Dang *et al.*, 2018). In this chapter, the focus is on the efficacy of using fungus in the treatment of wastewater. These organisms are capable of generating a huge number of reductive proteins and enzymes. The hydrolysis of a variety of environmental contaminants, including dyes, pharmaceuticals, heavy metals, trace organic contaminants, and endocrine disruptors, is carried out by a variety of fungal strains.

15.2 MYCOREMEDIATION: A SUSTAINABLE ALTERNATIVE

One of the aspects of bioremediation is mycoremediation where fungi are being used to remove pollutants sustainably. The first reported fungus that was able to degrade pollutants was *Phanerochaete chrysosporium* (Bumpus & Aust, 1984). Fungi can store the contaminants in their tissue. The intricate cell wall structure improves their potential of binding different metals through various functional groups and helps in metal sequestration. Ascomycota and Basidiomycota are the commonly found phyla of fungi in heavy metal-polluted sites (Hyde *et al.*, 2019). Moreover, they can target a broad range of organic and inorganic pollutants as they possess many different intracellular and extracellular enzymes with low substrate specificity (Bhadouria *et al.*, 2020). The properties of fungi such as their enzyme system, ability to overcome certain environmental conditions, and saprophytic nature help them in the utilization of waste compounds as carbon and energy sources. Diversified metabolic capabilities have made fungi an excellent selection for the bioremediation process (Deshmukh *et al.*, 2016; Noman *et al.*, 2019). Bioremediation of heavy metals and other toxic compounds using fungi generally occurs through biosorption, biotransformation, bioaccumulation, precipitation, and sequestration (Kumar *et al.*, 2021a). Besides, the enzymatic approaches are being used for several years and the commonly released enzyme by fungi is oxidoreductase. Furthermore, ligninolytic fungi, also offer the conversion of harmful chemicals without the need of transporting contaminants across cell membranes to meet cytosolic enzymes (Park & Choi, 2020). The mycelium of fungi has filtering abilities that target specific pollutant compounds. Mycelium stimulates microbial and enzymatic activity and thus, less toxins are produced. Not only in organic pollutants or heavy metal compounds but mycoremediation has also been applied for the removal of oil spills (Roshandel *et al.*, 2021) in soil or water. The intra- and extracellular enzymes and their metabolic mechanism have proven to be efficient in the removal of pollutants from water. This approach is worthwhile and an eco-friendly alternative for the exclusion of recalcitrant pollutants (Park & Choi, 2020).

15.3 MECHANISM FOR MYCOREMEDIATION OF REFRACTORY POLLUTANTS

A sustainable way to remediate pollutants is bioremediation and fungi seem to be a good agent for this, especially in treating heavy metal-polluted water. The high metal adsorption and accumulation ability of fungi could be used. The major pollutants in wastewater are heavy metals. Fungi can change or reduce the toxicity of metal through changes in pH, bioaccumulation, and biosorption. The most important biomechanisms used by fungi for heavy metal and other pollutant degradation are metabolically passive (bioadsorption) and metabolically active (bioaccumulation, biomineralization, biotransformation, and bioprecipitation) process (Dutta & Hyder, 2017; Kumar & Dwivedi, 2021b).

15.3.1 Biosorption

The presence of different functional groups on the wall of fungi makes it an important source of biosorbent. In a metabolically independent manner, metal ions bind to negatively charged free groups in different biopolymers. It is also known as mycoadsorption in the context of fungi and occurs on the surface of the biomass. The cell wall composition of fungi consists of 80–90% of polysaccharides, lipids, and proteins with various functional groups which help in the sorption of recalcitrant pollutants (Dhankhar & Hooda, 2011). The binding of metal occurs in two steps (1) interaction of metal and chemical groups of the cell wall, (2) mineral deposition of a large number of metals. The cell wall of fungus is the foremost thing that interacts with the metal ions (Negi & Das, 2023).

Biosorption can be categorized into various headings according to the dependence on the metabolism–metabolism-dependent and -independent process. Besides, the site from where the metal is removed, on that basis it can be classified as (a) cell surface sorption (occurs on the surface of fungi by physicochemical interaction, it is reversible) (b) intracellular accumulation (transportation of metals across the membrane occurs in viable cells only) (c) extracellular accumulation (uptake of metal takes place on the surface and the solution) (Dhankhar & Hooda, 2011) (Figure 15.1).

Biosorption is widely used for heavy metal removal due to its low cost, eco-friendly nature, easy handling, removing the smallest amount of toxic compounds, and high biomass production (Sharma *et al.*, 2018). The fungi used for biosorption are mostly non-pathogenic. The procedure is affected by several factors such as pH, inoculum amount, temperature (to a certain extent), and metal ion used. Amidst all other mechanisms for metal remediation, biosorption has come out as a promising tool as compared to the existing expensive methods. Mathur *et al.* (2018) evaluated the performance of *Aspergillus lentulus* and *Aspergillus fumigatus* for the decolorization of dye using bioaccumulation and biosorption and found that the biosorption followed by bioaccumulation was successfully removing reactive remazol red dye with 76% removal rate (Mathur *et al.*, 2018). Sawicka (2019), with the help of *Aspergillus* sp. and *Saccharomyces* sp. removed Pt (IV) and Pd (II). They found that the maximum competence of Pt (IV) on yeast and fungi was attained at acidic pH (pH 2.0) and pH 2.5–3.5 for Pd (II). *Aspergillus* sp. shows a higher adsorption capability of 5.49 mg/g for Pt (IV) and 4.28 mg/g for Pd (II). The study showed that fungi can eradicate the ions of metals from polluted water (Sawicka, 2019).

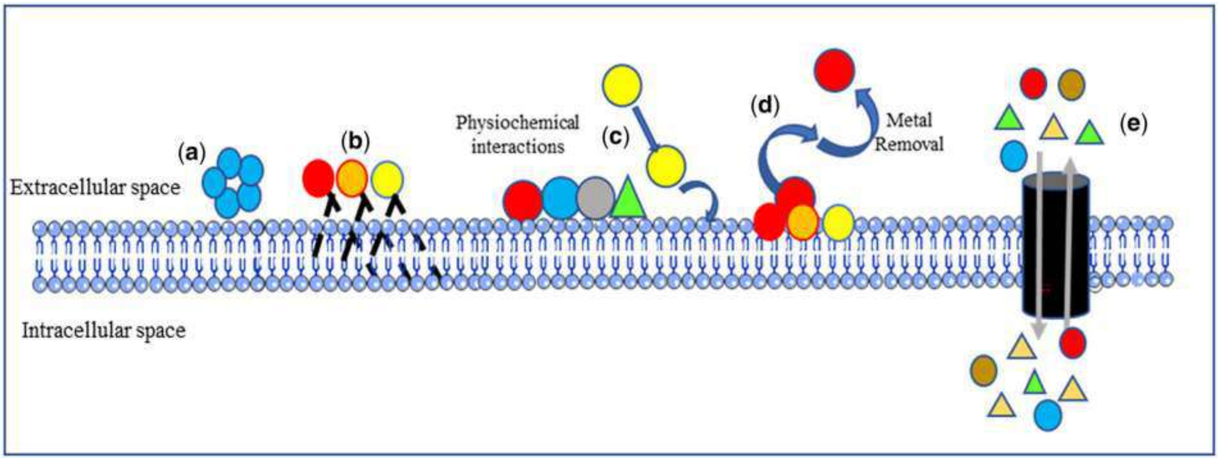


Figure 15.1 Pictorial representation of various categories of biosorption in the fungus cell wall: (a) mineral deposition, (b) metal and chemical interaction, (c) cell surface sorption, (d) extracellular accumulation, and (e) intracellular accumulation.

15.3.2 Bioaccumulation

Bioaccumulation is a metabolically energy-driven procedure where microbes with the help of a translocation system uptakes the heavy metal into their intracellular space. Once inside the intracellular space, the heavy metals can be sequestered by proteins and peptide ligands (Mishra & Malik, 2013). In the process of bioaccumulation, physical or chemical damage to the cell wall and lipid membrane is needed to acquire the heavy metals, thus the cells cannot be recycled. The process of bioaccumulation is followed by biosorption on the cell wall then the compounds are transported to intracellular space by transformation with the help of enzymes and ATP. With the use of energy, metals are transported through the cell membrane inside the cell. Bioaccumulation depends on the biological and chemical properties of the cell, internal structure, genetic adaptation of the organism, availability of metal, changes in the metal and toxicity (Vašinková *et al.*, 2021). Vašinková *et al.* (2021) compared the ability of *Aspergillus* genus from the affected site of Ostramo Lagoons (Ostrava, Czech Republic). The experimentation was done on six isolated fungal species. The media was supplemented with Cu (II), Ni (II), and Cr (III) and were separately added with spores of these fungi. It was found that the accumulation capacity of *A. ochraceus* exhibited the highest efficiency of 57.42, 56.88, and 37.73% for Cu (II), Zn (II), and Cr (III), respectively (Figure 15.2).

15.3.3 Biotransformation

In biotransformation, enzymes isolated from microorganisms are used for the conversion of toxic compounds. It is a harmless approach that produces nontoxic products, and leads to the almost complete removal of toxic compounds and is thus recognized as a better mechanism to degrade contaminants. Different microorganisms such as bacteria, plants, and fungi isolated have been used for biotransformation. The biotransformation converts toxic compounds into nontoxic by various conversion methods such as hydroxylation (addition of hydroxyl group to the molecule in the presence of hydroxylases which often increases the polarity and reduces toxicity), oxidation–reduction (oxidation of hydroxyl groups and reduction of carbonyl groups), de-epoxidation (helps in opening the epoxide ring which ultimately reduces

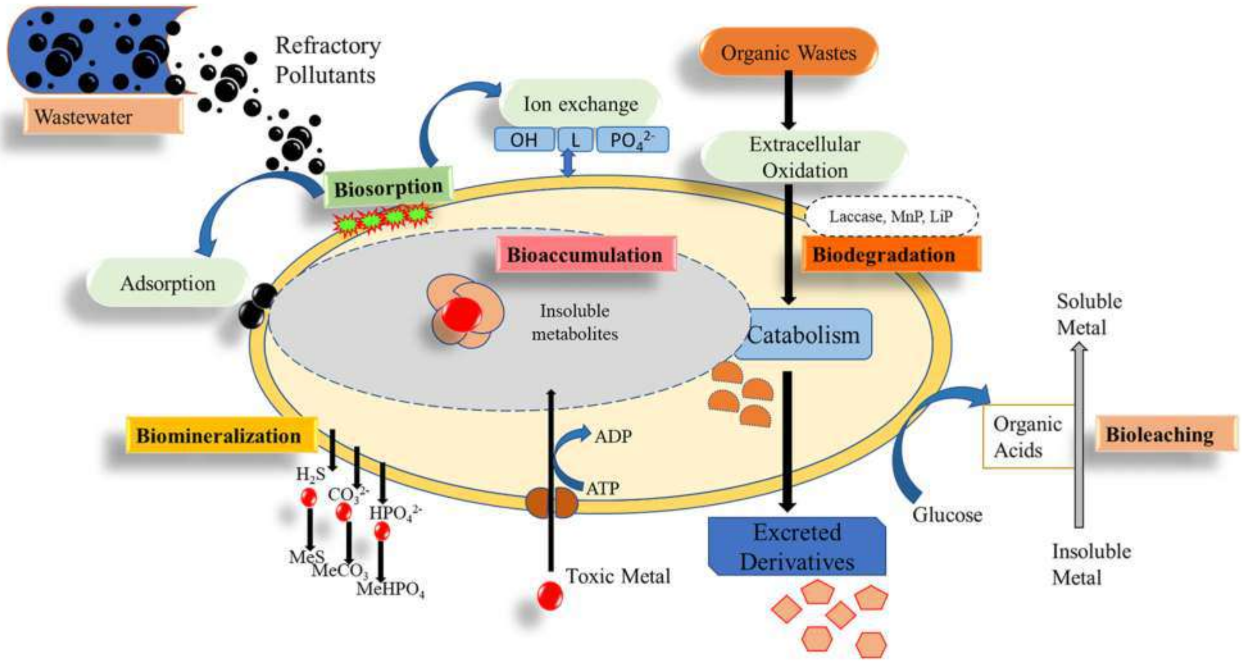


Figure 15.2 Various paths for the degradation of refractory pollutants through different biological mechanisms in fungi.

the toxicity of the compound), hydrogenation (reduction of C = C double bond by addition of hydrogen), glucuronidation/glycosylation (the process in which glucuronic acid or glucose is covalently attached to hydroxyl group), deamination (removal of amino group), demethylation (removal of methyl group), and sulfation (sulfotransferase-catalyzed conjugation of a sulfo group on a hydroxyl group) (Li *et al.*, 2020). About 14 major transformed products were identified in experiments from metoprolol biodegradation with the use of three fungi. Mainly hydroxylation, oxidation, and *o*-dealkylation mechanism were used and TP238, TP282A, TP284, TP300, TP316, and TP134 were the major compounds obtained. It was found that the maximum competence was attained through *Ganoderma lucidum* up to 51 and 77% for metoprolol and metoprolol acid, respectively (Jaén-Gil *et al.*, 2019). Also, in some reports biotransformation is done using biocatalysts. García-Morales *et al.* (2018), reported that laccase enzyme released by *Pycnoporus sanguineus* CS43 can efficiently transform pharmaceutically toxic pollutants acetaminophen (ACE) and diclofenac (DCF). ACE and DCF showed 90 and 50% of transformation, respectively, by free and immobilized laccase detoxification through biotransformation, which could be an important and sustainable approach for bioremediation (García-Morales *et al.*, 2018).

15.3.4 Biomineralization

Biomineralization is the process where microorganisms are used for the conversion of biomacromolecules into inorganic minerals. It differs from conventional mineralization using the participation of cells, metabolite, and organic matrix. Biomineralization has a significant role in the elimination of heavy metals from polluted location (Zhao *et al.*, 2020). The participation of fungi in this method is very well established. The process can be active or passive. Different types of biomineralization identified are: (a) biologically controlled mineralization (biologically controlled growth and morphology), (b) biologically induced mineralization (induces changes in the environment), (c) biologically influenced mineralization (biological matrix starts mineral nucleation) (Planý *et al.*, 2021).

15.4 ROLE OF FUNGAL ENZYMES IN MYCOREMEDIATION

The self-purification ability of ecosystems is greatly enhanced by the ability of the fungus to break down a wide variety of natural substances, and these abilities can be utilized to create ecological biotechnologies. Fungi are natural decomposers of the ecosystem as they release several enzymes naturally. These enzymes such as catalases, lipases, laccases, peroxidases, xylanases, and proteases are being used for bioremediation. Fungal enzymes are capable of degrading organic as well as inorganic compounds. Fungi produce ligninolytic enzymes that can hydrolyze lignin and are capable to break the toxic compounds. The enzyme can hydrolyze the chromophore structure of reactive dyes such as azo dyes (Sheam *et al.*, 2021), can transform heavy metals and metalloids (Kumar & Dwivedi, 2020), and can effectively degrade persistent organic pollutants (Šrédlová *et al.*, 2019) (Table 15.1).

15.4.1 Laccase

Laccases utilize molecular oxygen as a co-substrate in the reaction in addition to copper as a co-factor (Viswanath *et al.*, 2014). These oxidoreductases oxidize diphenols and other similar chemicals by accepting electrons from molecular oxygen. Most phenolic and non-phenolic compounds can be oxidized by laccases; the fungus *Trametes versicolor* has an activity that is 20 times greater than that of other species. The lignin is broken down by fungal laccases (Agrawal *et al.*, 2019; Agrawal & Verma, 2020b), which also remove any potentially dangerous phenols that are released. Additionally, dihydroxy naphthalene melanins, which are dark-colored polymers produced by species unaffected by ecological pressure, are thought to be released by fungal laccases. A varied range of applications for fungal laccases exist including pulp bleaching detoxification and effluent decolorization (Agrawal and Verma 2019a, 2019b, 2020a), phenolic removal in wines, organic synthesis biosensors, hindering dye transfer functions in washing powders and detergents, and the synthesis of composite medical compounds, a number of them are patented (Senthivelan *et al.*, 2016). Laccases have low substrate specificity that

Table 15.1 Fungal strains in the treatment of different wastewater contaminants using various approaches.

	Microbial Agents	Mechanism of Removal	Wastewater Chemical	Conditions optimization	pH	Temp. (°C)	Decolorization Efficiency (%)	References
Dyes	*Pleurotus ostreatus* HAUCC 162	Decolourization	Malachite Green	100 mg/L, 24 h	6	25	91.5	Zhuo *et al.* (2019)
	Pleurotus pulmonarius		Malachite green	100 mg/L, 36 h	–	–	68.6	Lallawmsanga Leo *et al.* (2019)
	Aspergillus iizukae 605EAN		Remazol Brilliant Blue R	100 mg/L, 5 days	7	–	90.43	Noman *et al.* (2020)
	Cyberlindnera fabianii		AR14	50 mg/L, 12 h	5	–	97	Danouche *et al.* (2021)
	Mucor hiemalis	Detoxification	Textile effluent	100 mg/L, 60 h	–	35	91.35	Molla and Khan (2018)
	Trametes versicolor	Biodegradation	Reactive blue 19	200 mg/L, 210 min	4	50	85	Dauda and Erkurt (2020)
	Aspergillus salinarus		Reactive Red HE7B	50 mg/L, 96 h	–	30	97.41	Sheam *et al.* (2021)
	Gloeophyllum trabeum		Methyl orange	75 mg/L, 28 days	–	30	46.67	Purnomo *et al.* (2020)
	Pichia kudriavzevii CR-Y103	Biodegradation and detoxification	Reactive orange 16	1 g/L,160 rpm, 24 h	6	30	100	Rosu *et al.* (2018)
Metals	*Paecilomyces* sp.	Biosorption	Co (II)	5 g, 100 rpm	5	28	100	Cárdenas González *et al.* (2019)
	Talaromyces sp. KM-31		Arsenic	1000 mg/L	5.3	28	>70	Nam *et al.* (2019)
	Penicillium polonicum		Lead (II)	4 mmol/L, 180 rpm	–	30	90.3	Xu *et al.* (2020b)
	Aspergillus flavus CR500	Bio reduction	Hexavalent chromium	50 mg/L	5–9	20–40	99	Kumar and Dwivedi (2019)
	Trichoderma lixii CR700	Bioaccumulation	Copper	10 mg/L, 120 h	5–8	–	84.6	Kumar and Dwivedi (2021a)
	Trichoderma fasciculatum and Trichoderma longibrachiatum	Bioadsorption	Cadmium	20 mg/L, 120 h	5	30	67.10 76.25	Kumar *et al.* (2020)
	Rhizopus oryzae		Lead (II) Cadmium (II)	200 rpm	6	–	94.25 93.38	Naeimi *et al.* (2018)

Agrochemicals	*Mucor racemosus* DDF	Biodegradation	Dieldrin	10 days	–	25	90	Kataoka (2018)
	Trametes versicolor		Diuron Bentazon	0.25 mL/g, 27 days	4.5	25	93 90	Beltrán-Flores *et al.* (2021)
	Rhizopus nodus *Aspergillus fumigatus* *Penicillium citreonigum*		Diazinon	35 mg/L, 21 days	–	28	91.1 76.4 72.2	Jahin *et al.* (2020)
	Trametes hirsuta MTCC-1171		Chlorpyrifos	150 mg/L, 150 rpm	6	30	>95	Patil *et al.* (2021)
Organic compounds	*Serratia liquefaciens*	Biotransformation	Kraft lignin	1000 mg/L, 120 rpm	–	30	65	Singh *et al.* (2019)
	Phlebia acanthocystis TMIC34875	Biodegradation	Pentachlorophenol	–	–	–	100	Xiao and Kondo (2020)
	Pleurotus sajor-caju LBM 105		Polychlorine biphenyl	217 mg/L		–	97.7	Sadañoski *et al.* (2020)
	Aspergillus ochraceus NRRL 3174		Petroleum	1 g/L, 7 days	5	–	80	Bilen Ozyurek *et al.* (2021)
	Pleurotus ostreatus *Pleurotus eryngii*		Trichloroethylene	140 mg/L	–	–	100	Mayans *et al.* (2021)
	Fusarium sp.		PAH	–	–	–	>80	Gupta *et al.* (2021)

lets them oxidize a variety of xenobiotic substances, such as chlorinated phenolics (Deng *et al.*, 2022), pesticides (Zeng *et al.*, 2017), and polycyclic aromatic hydrocarbons (Xu *et al.*, 2020a). Laccases have also been found to degrade polycyclic aromatic hydrocarbons, which are created by fossil fuels and natural oil sources. Laccase derived from Coriolopsis gallica UAMH8260 can oxidize carbazole, n-ethyl carbazole, fluorine, and dibenzothiophene (Bressler *et al.*, 2000). Laccases produced by fungi have various benefits that make them particularly interesting while using biotechnology to clean industrial wastewater. They can be used to decontaminate a number of agricultural goods, including coffee pulp, olive mill wastes, and bleaching kraft pulp, as per their adaptable substrate specificity. Both of these processes are possible. Laccase synthesis in fungi is nitrogen sensitive. Recombinants and nitrogen levels have a close relationship. Homologous or heterologous methods can be used to generate it (Viswanath *et al.*, 2014).

15.4.2 Catalase

It is a broadly dispersed catalyst that can split hydrogen peroxide into oxygen and water. It is a component of oxygen-dependent organisms and has a protective role against the adverse effects of reactive oxygen species (ROS), such as hydroxyl radicals and super-oxide anions (Vara & Karnena, 2020). Mono or bifunctional catalase enzymes are produced by fungi to combat the toxicity of ROS, which breaks down macromolecules in biological systems. ROS is broken down into harmless products by these enzymes. It has been demonstrated that the occurrence of heavy metals, including copper, cadmium, lead, and zinc causes bacteria to produce more ROS. In the consortium of *Aspergillus niger*, *Penicillium* sp., and *Rhizopus* sp. the catalase activity was found to be high when exposed to heavy metals including Pb and Cu at a concentration of 50 mg/L. It has been shown by Lin *et al.* (2009), that to precisely evaluate the remediation potential of fungi catalase activity may be utilized, and as the amount of oil increased in polluted environments this activity declines. The usage of catalase is an efficient technique for providing fungi with the tolerance abilities essential for the removal of contaminants from the ecosystem. As a result, fungal enzymes are essential elements in the process of bioremediation of areas polluted with metals (Negi & Das, 2023).

15.4.3 Peroxidase

Peroxidases are oxidoreductases that catalyze a variety of reactions such as the oxidation of numerous organic and inorganic molecules as well as the reduction of peroxides like hydrogen peroxide. These heme proteins have the protoporphyrin IX iron (III) prosthetic group. Three distinct categories of peroxidases can be identified based on their corresponding sources and activities: lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP). LiP and MnP are two heme peroxidases that are dependent on the presence of hydrogen peroxidase and manganese to carry out their functions (Sellami *et al.*, 2022). This enzyme plays a role in the breakdown of potentially hazardous compounds (Zhou *et al.*, 2013). The VP enzymes have wide substrate specificity and can oxidize both phenolic and non-phenolic substances as they have three active oxidation sites. Initially, a diffusible mediator converts manganese (II) to manganese (III), followed by a heme-dependent binding pocket with a low redox potential, and finally, an electron transfer pathway connecting the surface-reactive tryptophan radical to the heme with a high redox potential (Barber-Zucker *et al.*, 2022). LiP and MnP have been the focus of significant research because of their ability to hydrolyze a wide range of industrial wastes, such as a complex and diverse mix of organic and hazardous chemicals.

15.5 DEGRADATION OF POLLUTANTS USING MYCOREMEDIATION

Fungi have shown some outbreaking outcomes in providing redressal for various refractory pollutants through biosorption and bioaccumulation procedures. The previous proofs or details of the degradation of refractory pollutants have been discussed in the below-mentioned sections (Table 15.2).

Table 15.2 Fungal strains and enzymes in bioremediation of refractory pollutants.

Fungal Strains	Type of Pollutants	Pollutants	Enzymes	Optimized Conditions	Degradation Efficiency (%)	References
Trametes versicolor	PAH	Chrysene Benzo pyrene	LAC	30 °C, 7 days	87.9 38.8	Vipotnik *et al.* (2022)
Phialophora fastigiata		Naphthalene	LAC	20 °C, pH 3	38	Simanjorang and Subowo (2018)
Lasiodiplodia theobromae		Benzo pyrene	LiP, LAC	–	–	Cao *et al.* (2020)
Coriolopsis byrsina		Phenanthrene	LiP, LAC, MnP	50 mg/L, 25°C, pH 6	99.90	Agrawal *et al.* (2018)
Byssochlamys spectabilis and *Aspergillus fumigates*		Chlorpyrifos	LAC	20 mg/L, 28°C, 150 rpm	98.4	Kumar *et al.* (2021b)
Trametes versicolor	Polar pesticide	Malathion	Cytochrome P450	25°C, 135 rpm, 7 days	100	Hu *et al.* (2022)
Coriolopsis gallica	Phenolics	Phenol *p*-Chlorophenol Bisphenol	LAC	60°C, pH 6–8	90.78 93.26 99.66	Cen *et al.* (2022)
Trichoderma citrinoviride AJAC3		17 β-estradiol	MnP, LiP	200 mg/L, 28°C, 120 rpm	99.6	Chatterjee and Abraham (2019)
Ganoderma applanatum and *Laetiporus sulphureus*	NSAID	Celecoxib Diclofenac ibuprofen	MnP, LiP, LAC	–	98 96 95	Bankole *et al.* (2020)
Pleurotus ostreatus	Triclosan	Triclosan	LAC MnP	–	90	Maadani Mallak *et al.* (2020)
Aspergillus arcoverdensis SSSIHL-01	Azo dye	Congo red	LAC LiP MnP	100 mg/L 30°C, 48 h	98.61	Skanda *et al.* (2023)

LAC, laccase, LiP, lignin peroxidase, MnP, manganese peroxidase.

15.5.1 Organic pollutants

The deliberate manufacturing of chemicals used in the industrial process, disease control, and agriculture is responsible for the release of environmentally degrading organic pollutants including dioxins, dichloro diphenyl trichloroethane (DDT), furan, phenol, pyridine, dimethyl phthalate (DMP), and trichloroethylene in the environment. The remaining of these pollutants in the environment is an alarming situation for human as well as wildlife health. Some commonly detected chemicals such as polycyclic aromatic hydrocarbon (PAH) and polychlorine biphenyl (PCB) are carcinogenic, persistent hydrocarbons and can cause harmful effects. Several procedures such as chlorination, ozonation, membrane process, flocculation, solvent extraction, and biological degradation have been developed to treat these harmful pollutants (Uthayakumar *et al.*, 2022). Howbeit, these approaches are quite expensive and can generate secondary pollutants that could be more toxic than the primary ones. And thus, the focus is on biological approaches as they are more sustainable and greener. Bioremediation is used for degradation purpose as it can restore the affected area as well as mineralizes the pollutants (Haripriyan *et al.*, 2022). Various organic pollutants and their degradation using fungi have been discussed in the below sections.

15.5.2 Polycyclic aromatic hydrocarbon

Polycyclic aromatic hydrocarbon is a diverse category of toxic organic compounds having two or more fused rings of benzene. Anthropogenic and natural activities such as pyrolysis of fossil fuels, oil, petroleum, gas, and so on are the chief source of their release into the ecosystem. PAHs are carcinogenic, teratogenic, and mutagenic, that need to be considered for appropriate treatment before release into the natural environment. One such approach is bioremediation, where microorganisms are used for the degradation or mineralization of pollutants. Much literature has been reported regarding the efficiency of bacteria and fungi in the remediation of PAHs. Several fungal species such as *Aspergillus*, *Cunninghamella*, and *Penicillium* are useful in transforming PAHs classified pollutants (Al-Hawash, 2018). Torres-Farradá *et al.* (2019) worked with seven *Ganoderma* strains to check their efficiency for the breakdown of PAHs – naphthalene, fluorene, and phenanthrene. They found that these strains were capable of degrading the PAHs compounds and amongst the different *Ganoderma* strains, UH-M strain exhibited the maximum degradation potential (~100%) for three PAHs. The degradation of PAHs from fungal strains showed enhanced degradation and thus are promising candidates for the bioremediation of refractory pollutants (Torres-Farradá *et al.*, 2019). Agrawal *et al.* (2018) testified that *Ganoderma lucidum* a white-rot fungi (WRF) has shown maximum biodegradation efficiency of phenanthrene (99.65%), and pyrene (99.58%) in mineral broth medium (Agrawal *et al.*, 2018). Hadibarata and Yuniarto (2020) screened 804 isolates on plates containing Poly R-478, RBBR, syringaldehyde and guaiacol. They identified a few strains with positive reactions and showed more than 90% results with indicators. The highest activity of laccase was found in *Coriolopsis caperata* BM-172 (880 U/L, 15–20 days, and 3500–3600 mg/L). The highest degradation rate for pyrene (89.5%), anthracene (85.2%), and phenanthrene (95.6%) was detected with *Pluteus chysophaeus* BM-792 after 25 days of incubation (Hadibarata & Yuniarto, 2020). More experimental studies will be required for the remediation of PAHs from wastewater and soil using fungi and other microorganisms.

15.5.3 Polychlorinated biphenyls

They are synthetically stable chlorinated organic pollutants and one of the most obstinate classes of xenobiotic contaminants. The physiochemical removal techniques are expensive and require a cost-effective approach for the degradation of pollutants. One such approach is bioremediation using different fungus is coming out as an effective tool for the degradation of PCBs. PCBs have low reactivity and can be stable in harsh environments and are not easily degradable. The metabolism by microbial enzymes could be an effective route for the biodegradation of PCBs. Mycoremediation using wood-rot fungi strain has been proven as an efficient approach for the bioremediation of PCBs. Sadanoski *et al.* (2019) showed the efficiency of *Pleurotus sajor-caju* (*P. sajor-caju*) LBM 105 in the remediation of PCBs. It was found that after 35 days of incubation, *P. sajor-caju* LBM 105 was able to eradicate 97.7 and 91.7% of the PCBs mixture and toxicity was reduced in complex and mineral media, respectively. They added PCBs to *P. sajor-caju* LBM 105 media and found that the laccase activity enhanced three-fold on the 28th day. By cultivating them in an N-limited medium they found that mycoremediation of PCBs is a cost-effective (0.69 $/g/d) method (Sadañoski *et al.*, 2019). Šrédlová *et al.* (2019) worked with the other species of *Pleurotus* and proved their efficiency by the degradation of PCBs. They took spent oyster mushroom substrate as the inoculum and the growth substrate as well. In continuous low flow setup, the highest biodegradation efficiency (87%) was recorded, and subsequently scaled up the volume by 500 L and treated 4000 L of real polluted groundwater containing 0.1–1 μg/L of PCBs. This setup was able to eliminate 82, 80, 65, and 30–50% of di, tri, tetra, and pentachlorinated PCBs congeners, respectively. *P. ostreatus* was able to hydrolyze PCBs in the presence of other fungal species and bacteria (Šrédlová *et al.*, 2019). Fungus ability to degrade aromatic compounds, and growth capability on PCBs was an indicator that they could degrade PCBs. Other than *Pleurotus* sp. there are several other fungi strain such as *Phanerochaete chrysosporium, Trametes versicolor, Coriolopsis polyzona*, and *Grifola frondosa* was found efficient in the degradation of PCBs (Elangovan *et al.*, 2019).

15.5.4 Phenolic compounds

Industries like pulp and paper, tanning, and olive oil mill processing are the major sources of phenolic contaminants in the environment. Phenolic compounds are recalcitrant by nature and need proper treatment for their degradation. Biocatalytic strategies for the biodegradation of phenolic pollutants have gained a lot of attention. Laccases and polyphenol oxidases are a group of enzymes having low substrate specificity, and efficient oxidation potential, and hence it is highly utilized in the biodegradation of phenolic pollutants. Utilization of laccases enzyme can be a better tool for the biodegradation of phenolic pollutants in wastewater. Moreover, they are available abundantly and also have a brilliant capability to oxidize a variety of organic compounds (Cen *et al.*, 2022). Cen *et al.* (2022) studied the efficiency of yellow laccase extracted from WRF *Coriolopsis gallica* (*C. gallica*) NCULAC F1 and checked its degradation efficiency against phenolic compounds. Initially, they observed an increase in enzyme activity to 10 690 U/L. Fe^{3+} and Mn^{2+} highly stimulated the *C. gallica* Lac enzymatic (laccase) activity by 162.56 and 226.05%, respectively. The activity of the enzyme was high when it was exposed to organic reagents and inhibitors. The pollutants selected for the experiment are phenol, *p*-chlorophenol and bisphenol. In the first 60 min, the elimination rates were more than 80% and in 120 min the elimination rates of chlorophenol, phenol, and bisphenol were 93.26, 90.78, 99.66%, respectively (Cen *et al.*, 2022). This study proves how efficiently the fungal enzymes could remove the phenolic compounds from wastewater and thus, could be used in environmental treatment.

15.5.5 1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane

1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) is an organochlorine pesticide that is being used extensively to control insect-borne disease and agricultural pests. They can easily enter the environment through air and water and can enter into the food chain. They get accumulated through the food chain. Though DDT is banned due to its negative impacts on the environment, its residues are still detectable. They have been shown to remain in the ecosystem mainly in the form of DDT, 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane, and 1,1-dichloro-2,2-bis-(4-chlorophenyl) ethylene (DDE). To eliminate DDT in a greener and harmless way, bioremediation is the best option. WRF have been reported for the biotransformation and biodegradation of DDT from water and soil. *Ganoderma lingzhi* is one of the most significant and broadly dispersed WRFs globally and is related to the degradation of a wide variety of woods. Grizca Boelan (2018) reported that *G. lingzhi* BMC9057 is degrading DDT in the PDB medium. They found that on the 7th day of incubation, 53% of DDT was eliminated. Their ability to produce ligninolytic enzymes helps them to degrade pollutants. Also, with fungus, they added 5 and 10 ml of *Bacillus subtilis* and found that the degradation was enhanced to 71.79 and 82.30%. Here the bacteria helped in increasing the solubility of *G. lingzhi* and thus enhanced the overall DDT degradation process. Thus, the use of fungus in the degradation of DDT or other pollutants has proven to be very efficient up to a large extent and with further improvements could be the future alternative for almost every remediation method (Grizca Boelan & Setyo Purnomo, 2018).

15.6 INORGANIC POLLUTANTS

The increasing population and human activities have increased the amount of waste and ultimately resulting in the accumulation of several recalcitrant pollutants. There are various methods to degrade these pollutants but still, the contamination is increasing. The major source of water pollutant is human and natural activities such as sewage, agriculture, chemical waste from industries, volcanic eruptions, and so on. These inorganic pollutants contain many organic metal complexes, different inorganic compounds, mineral acids, cyanides, metals, metals complexes, and so on. Aquatic life is affected by the accumulation of heavy metal ions and is the reason for diverse health effects on the kidney, liver, circulatory system, nervous system, blood, gastrointestinal system, skin, and bones (Gadd, 2010). Generally, inorganic pollutants comprise of cadmium, arsenic, fluorides, mercury, aluminum, chromium, nitrites, nitrates, and lead (Yadav *et al*., 2021). Mostly they are persistent and resistant to degradation. Many physiochemical techniques have been reported for their degradation and removal but they have significant disadvantages and thus needed a more cost-effective and efficient alternative that could not harm to the environment as well (Dhankhar & Hooda, 2011). Out of all methods, bioremediation (mycoremediation) has shown considerable positive effects that fascinate researchers. As opposed to organic pollutants, which can be mineralized into carbon dioxide and water, heavy metals can only be changed into less harmful forms, making heavy metal bioremediation more challenging. The remediation of heavy metal polluted sites has been a matter of concern for decades. The need of the hour is to have a sustainable and eco-friendly process for the recovery and degradation of these pollutants. Mycoremediation can be used for the biodegradation and elimination of metal contaminants from the water or soil. These are cost-effective and also help in the restoration of the site, and seem as a better alternative compared to conventional remediation methods (Sharma *et al*., 2018). Several fungi were reported for the bioremediation of metal. Cárdenas González *et al*. (2019) showed biosorption of Co (II) using fungal biomasses, out of which *Paecilomyces* showed the highest metal removal efficiency, 93% at 24 h of incubation, while *Penicillium* sp. and *Aspergillus niger* (*A. niger*) showed 77.5 and 70% metal removal efficiency at the same time of incubation, respectively. Also, the same fungi showed higher efficiency to remove metals when the concentration of bioadsorbents was increased by 100% for *Paecilomyces* sp. and *Penicillium* sp. and 96.4% for *A. niger* from contaminated water (Cárdenas González *et al*., 2019). Dey *et al*. (2020) studied the potential of *Aspergillus fumigatus* (*A. fumigatus*) for the bioremediation of metal. They found the accumulation efficiency of the fungus with different metal ions and arranged them in an increasing order. They took lindane (pesticide) and a multi-metal mixture that included Cd, Cr, Cu, Ni, Pb, and Zn. The metal removal trend was Cd (100%) > Ni (99%) > Cu (98%) > Pb (91%) > Cr (81%) in the absence of lindane. The removal efficiency depends on the metal ion characteristics and the more electropositive metal ion will be more strongly attracted to the surface and will accumulate more. When they check accumulation in the presence of co-contaminant (multi-metal and lindane-amended medium), it was Zn (98%) > Pb (95%) > Cd (63%) > Ni (46%) > Cu (37%). This decrease could be because of the screening effect by pesticide lindane. This published literature has provided a comprehensive evidence on the strategy that *A. fumigatus* adapted to remove metals (Dey *et al*., 2020). Similarly, in a study, *Trichoderma lixii* CR700 was investigated for its Cu^{2+} removal efficiency. In batch study, CR700 was able to remove 84.6% of Cu^{2+} at the concentration of 10 mg/L in 120 h. The CR700 uses simultaneous sorption and accumulation mechanism in the removal of Cu^{2+} (Kumar & Dwivedi, 2021a). One report showed the *in vitro* bioadsorption of Cd^{2+} ions using *Trichoderma fasciculatum* (*T. fasciculatum*) and *Trichoderma longibrachiatum* (*T. longibrachiatum*) fungal species. Under the optimized condition, *T. fasciculatum* showed a 67.10% removal rate, while *T. longibrachiatum* showed 76.25% removal of Cd^{2+} ions at pH 5, 120 h incubation, and 30°C. The morphological changes were detected by scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR) and found that metal ions are binding efficiently on the surface of the fungus (Kumar *et al*., 2020). This information helps in the optimization of the process and further developments to design remediation systems in the field safely and sustainably.

15.7 CHALLENGES AND PROSPECTS

Studies over the years have demonstrated that waste and harmful pollutants can be successfully remediated by either enhancing the local microbial population or by adding the necessary fungal strains to the soil. To have an accurate understanding of mycoremediation, it is essential to have a comprehensive knowledge of their degradation and detoxification capacity. For a larger-scale application, this technology still needs to be done with a lot of labor and effort. Subsequently, the process is successful, and thus it requires proper funding so that certain products can be developed and made accessible for licensing and commercialization. Scientists believe that the method could be more effective than other approaches because it is faster and inexpensive, although the full potential of fungi environmental cleanup is yet remaining underexploited. This presents a tremendous opportunity for the full use of nature's most potent decomposer in the future. The scientific community has to pay more attention to and put more effort into understanding the fundamental mechanism of fungi in the degradation of toxic compounds. To comprehend this mechanism, one can use contemporary methods like functional and full proteome analyses, which may reveal many genes and proteins that are essential to the mycoremediation process. With the use of this knowledge, it may be possible to model genetically enhanced fungi with a more effective and rapid hydrolyzing capacity. Additionally, there is a continual need to identify and characterize fungal strains with a better capacity to break down and detoxify different organic and inorganic pollutants (Pandey *et al*., 2021).

15.8 CONCLUSION

The elimination and degradation of refractory contaminants through mycoremediation have shown promising activity. Mycoremediation is an eco-friendly and efficient way to remove pollutants from contaminated water. The mycelial structure of fungus has made it advantageous over bacteria and plants in the degradation process. Most of the studies focussed on the enzymatic system for the degradation of contaminants. But still, in the majority of cases, the basic machinery of mycoremediation needs further research and proteomic studies could help in this aspect. This information will help to develop genetically enhanced fungi that can efficiently degrade pollutants. Further studies are required to address the problems related to this method for the improvement of the remediation process. The evidences support the fact that mycoremediation can be a game changer in the removal of refractory pollutants in the coming future.

REFERENCES

Agrawal N., Verma P. and Shahi S. K. (2018). Degradation of polycyclic aromatic hydrocarbons (phenanthrene and pyrene) by the ligninolytic fungi *Ganoderma lucidum* isolated from the hardwood stump. *Bioresources and Bioprocessing*, **5**(1), 11–20, https://doi.org/10.1186/s40643-018-0197-5

Agrawal K. and Verma P. (2019a). Biodegradation of synthetic dye Alizarin Cyanine Green by yellow laccase producing strain Stropharia sp. ITCC-8422. *Biocatalysis and Agricultural Biotechnology*, **21**, 101291, https://doi.org/10.1016/j.bcab.2019.101291

Agrawal K. and Verma P. (2019b). Column bioreactor of immobilized Stropharia sp. ITCC 8422 on natural biomass support of L. cylindrica for biodegradation of anthraquinone violet R. *Bioresource Technology Reports*, **8**, p. 100345, https://doi.org/10.1016/j.biteb.2019.100345

Agrawal K., Bhardwaj N., Kumar B., Chaturvedi V. and Verma P. (2019). Process optimization, purification and characterization of alkaline stable white laccase from Myrothecium verrucaria ITCC-8447 and its application in delignification of agroresidues. *International Journal of Biological Macromolecules*, **125**, 1042–1055, https://doi.org/10.1016/j.ijbiomac.2018.12.108

Agrawal K. and Verma P. (2020a). Potential removal of hazardous wastes using white laccase purified by ATPS–PEG–salt system: an operational study. *Environmental Technology & Innovation*, **17**, p. 100556, https://doi.org/10.1016/j.eti.2019.100556

Agrawal K. and Verma P. (2020b). Production optimization of yellow laccase from Stropharia sp. ITCC 8422 and enzyme-mediated depolymerization and hydrolysis of lignocellulosic biomass for biorefinery application. *Biomass Conversion and Biorefinery*, pp. 1–20, https://doi.org/10.1007/s13399-020-00869-w

Akhtar N. and Mannan M. A. U. (2020). Mycoremediation: an unexplored gold mine. In: New and Future Developments in Microbial Biotechnology and Bioengineering: Recent Advances in Application of Fungi and Fungal Metabolites: Environmental and Industrial Aspects, J. Singh and P. Gehlot (eds). Elsevier BV, Amsterdam, Netherlands, https://doi.org/10.1016/B978-0-12-821007-9.00002-4

Al-Hawash A. B. (2018). Fungal degradation of polycyclic aromatic hydrocarbons. *International Journal of Pure & Applied Bioscience*, **6**(2), 8–24, https://doi.org/10.18782/2320-7051.6302

Bankole P. O., Adekunle A. A., Jeon B. H. and Govindwar S. P. (2020). Novel cobiomass degradation of NSAIDs by two wood rot fungi, *Ganoderma applanatum* and *Laetiporus sulphureus*: ligninolytic enzymes induction, isotherm and kinetic studies. *Ecotoxicology and Environmental Safety*, **203**, 110997, https://doi.org/10.1016/j.ecoenv.2020.110997

Barber-Zucker S., Mindel V., Garcia-Ruiz E., Weinstein J. J., Alcalde M. and Fleishman S. J. (2022). Stable and functionally diverse versatile peroxidases designed directly from sequences. *Journal of the American Chemical Society*, **144**(8), 3564–3571, https://doi.org/10.1021/jacs.1c12433

Beltrán-Flores E., Sarrà M. and Blánquez P. (2021). Pesticide bioremediation by *Trametes versicolor*: application in a fixed-bed reactor, sorption contribution and bioregeneration. *Science of the Total Environment*, **794**, 148386, https://doi.org/10.1016/j.scitotenv.2021.148386

Bhadouria R., Das S., Kumar A., Singh R. and Singh V. K. (2020). Mycoremediation of agrochemicals. In: Agrochemicals Detection, Treatment and Remediation: Pesticides and Chemical Fertilizers, M. N. Vara Prasad (ed.), Butterworth-Heinemann, United Kingdom. LTD, https://doi.org/10.1016/B978-0-08-103017-2.00022-2

Bilen Ozyurek S., Avcioglu N. H. and Seyis Bilkay I. (2021). Mycoremediation potential of *Aspergillus ochraceus* NRRL 3174. *Archives of Microbiology*, **203**(10), 5937–5950, https://doi.org/10.1007/s00203-021-02490-5

Bressler D. C., Fedorak P. M. and Pickard M. A. (2000). Oxidation of carbazole, N-ethylcarbazole, fluorene, and dibenzothiophene by the laccase of *Coriolopsis gallica*. *Biotechnology Letters*, **22**(14), 1119–1125, https://doi.org/10.1023/A:1005633212866

Bumpus J. A. and Aust S. D. (1984). Bumpus_et_al-1987-BioEssays. **6**(4), 166–170.

Cao H., Wang C., Liu H., Jia W. and Sun H. (2020). Enzyme activities during Benzo[a]pyrene degradation by the fungus *Lasiodiplodia theobromae* isolated from a polluted soil. *Scientific Reports*, **10**(1), 1–11, https://doi.org/10.1038/s41598-020-57692-6

Cárdenas González J. F., Rodríguez Pérez A. S., Vargas Morales J. M., Martínez Juárez V. M., Rodríguez I. A., Cuello C. M., Fonseca G. G., Escalera Chávez M. E. and Muñoz Morales A. (2019). Bioremoval of cobalt(ii) from aqueous solution by three different and resistant fungal biomasses. *Bioinorganic Chemistry and Applications*, **2019**, 1–8, https://doi.org/10.1155/2019/8757149

Cen Q., Wu X., Cao L., Lu Y., Lu X., Chen J., Fu G., Liu Y. and Ruan R. (2022). Green production of a yellow laccase by *Coriolopsis gallica* for phenolic pollutants removal. AMB Express, **12**, 96–111, https://doi.org/10.1186/s13568-022-01434-6

Chatterjee A. and Abraham J. (2019). Mycoremediation of 17 β-Estradiol using *Trichoderma citrinoviride* strain AJAC3 along with enzyme studies. *Environmental Progress and Sustainable Energy*, **38**(4), 1–9, https://doi.org/10.1002/ep.13142

Crini G. and Lichtfouse E. (2019). Advantages and disadvantages of techniques used for wastewater treatment. *Environmental Chemistry Letters*, **17**(1), 145–155, https://doi.org/10.1007/s10311-018-0785-9

Dang C., Yang Z., Liu W., Du P., Cui F. and He K. (2018). Role of extracellular polymeric substances in biosorption of Pb^{2+} by a high metal ion tolerant fungal strain *Aspergillus niger* PTN31. *Journal of Environmental Chemical Engineering*, **6**(2), 2733–2742. Elsevier BV, https://doi.org/10.1016/j.jece.2018.04.005

Danouche M., Ferioun M., Bahafid W. and El Ghachtouli N. (2021). Mycoremediation of azo dyes using *Cyberlindnera fabianii* yeast strain: application of designs of experiments for decolorization optimization. *Water Environment Research*, **93**(8), 1402–1416, https://doi.org/10.1002/wer.1499

Dauda M. Y. and Erkurt E. A. (2020). Investigation of reactive Blue 19 biodegradation and byproducts toxicity assessment using crude laccase extract from *Trametes versicolor*. *Journal of Hazardous Materials*, **393**, 121555, https://doi.org/10.1016/j.jhazmat.2019.121555

Deng W., Zhao W. and Yang Y. (2022). Degradation and detoxification of chlorophenols with different structure by LAC-4 laccase purified from white-rot fungus *Ganoderma lucidum*. *International Journal of Environmental Research and Public Health*, **19**(13), 8150–8191, https://doi.org/10.3390/ijerph19138150

Deshmukh R., Khardenavis A. A. and Purohit H. J. (2016). Diverse metabolic capacities of fungi for bioremediation. *Indian Journal of Microbiology*, **56**(3), 247–264, https://doi.org/10.1007/s12088-016-0584-6

Dey P., Malik A., Mishra A., Kumar D., Von Bergen M. and Jehmlich N. (2020). Mechanistic insight to mycoremediation potential of a metal resistant fungal strain for removal of hazardous metals from multimetal pesticide matrix. *Environmental Pollution*, **262**, 114255, https://doi.org/10.1016/j.envpol.2020.114255

Dhankhar R. and Hooda A. (2011). Fungal biosorption – an alternative to meet the challenges of heavy metal pollution in aqueous solutions. *Environmental Technology*, **32**(5), 467–491, https://doi.org/10.1080/09593330.2011.572922

Dutta S. D. and Hyder S. (2017). Mycoremediation – a potential tool for sustainable management. *Journal of Mycopathological Research*, **57**(1), 25–34.

Elangovan S., Pandian S. B. S., Geetha S. J. and Joshi S. J. (2019). Polychlorinated biphenyls (PCBs): environmental fate, challenges and bioremediation. In: Microbial Metabolism of Xenobiotic Compounds, P. K. Arora (ed.), Springer Nature, Singapore, pp. 165–188, https://doi.org/10.1007/978-981-13-7462-3_8

Gadd G. M. (2010). Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology*, **156**(3), 609–643, https://doi.org/10.1099/mic.0.037143-0

García-Morales R., García-García A., Orona-Navar C., Osma J. F., Nigam K. D. P. and Ornelas-Soto N. (2018). Biotransformation of emerging pollutants in groundwater by laccase from *P. sanguineus* CS43 immobilized onto titania nanoparticles. *Journal of Environmental Chemical Engineering*, **6**(1), 710–717, https://doi.org/10.1016/j.jece.2017.12.006

GilPavas E., Dobrosz-Gómez I. and Gómez-García M. Á. (2020). Efficient treatment for textile wastewater through sequential electrocoagulation, electrochemical oxidation and adsorption processes: optimization and toxicity assessment. *Journal of Electroanalytical Chemistry*, **878**, 114578, https://doi.org/10.1016/j.jelechem.2020.114578

Godlewska-Żyłkiewicz B., Sawicka S. and Karpińska J. (2019). Removal of platinum and palladium from wastewater by means of biosorption on fungi Aspergillus sp. and yeast Saccharomyces sp. *Water (Switzerland)*, **11**(7). https://doi.org/10.3390/w11071522

Grelska A. and Noszczyńska M. (2020). White rot fungi can be a promising tool for removal of bisphenol A, bisphenol S, and nonylphenol from wastewater. *Environmental Science and Pollution Research*, **27**(32), 39958–39976, https://doi.org/10.1007/s11356-020-10382-2

Grizca Boelan E. and Setyo Purnomo A. (2018). Abilities of co-cultures of white-rot fungus *Ganoderma lingzhi* and bacteria *Bacillus subtilis* on biodegradation DDT. *Journal of Physics: Conference Series*, **1095**(1), 1–8, https://doi.org/10.1088/1742-6596/1095/1/012015

Gupta S., Fernandes S., Gupta H. and Rathod S. (2021). Assessment of mycoremediation potential of *Fusarium* spp. on polycyclic aromatic hydrocarbon in Western India. *International Journal of Scientific Research in Science and Technology, Mill*, **8**, 509–515, https://doi.org/10.32628/ijsrst218571

Hadibarata T. and Yuniarto A. (2020). Biodegradation of polycyclic aromatic hydrocarbons by high-laccase Basidiomycetes fungi isolated from tropical forest of Borneo. *Biocatalysis and Agricultural Biotechnology*, **28**, 101717, https://doi.org/10.1016/j.bcab.2020.101717

Haripriyan U., Gopinath K. P., Arun J. and Govarthanan M. (2022). Bioremediation of organic pollutants: a mini review on current and critical strategies for wastewater treatment. *Archives of Microbiology*, **204**(5), 1–9, https://doi.org/10.1007/s00203-022-02907-9

Hu K., Barbieri M. V., López-García E., Postigo C., López de Alda M., Caminal G. and Sarrà M. (2022). Fungal degradation of selected medium to highly polar pesticides by *Trametes versicolor*: kinetics, biodegradation pathways, and ecotoxicity of treated waters. *Analytical and Bioanalytical Chemistry*, **414**(1), 439–449, https://doi.org/10.1007/s00216-021-03267-x

Hyde K. D., Xu J., Rapior S., Jeewon R., Lumyong S., Niego A. G. T., Abeywickrama P. D., Aluthmuhandiram J. V. S., Brahamanage R. S., Brooks S., Chaiyasen A., Chethana K. W. T., Chomnunti P., Chepkirui C., Chuankid B., de Silva N. I., Doilom M., Faulds C., Gentekaki E., … Stadler M. (2019). The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity*, **97**(1), 1–136, https://doi.org/10.1007/s13225-019-00430-9

Jaén-Gil A., Castellet-Rovira F., Llorca M., Villagrasa M., Sarrà M., Rodríguez-Mozaz S. and Barceló D. (2019). Fungal treatment of metoprolol and its recalcitrant metabolite metoprolol acid in hospital wastewater: biotransformation, sorption and ecotoxicological impact. *Water Research*, **152**, 171–180, https://doi.org/10.1016/j.watres.2018.12.054

Jahin H. S., Gaber S. E. and Hussain M. T. (2020). Bioremediation of diazinon pesticide from aqueous solution by fungal-strains isolated from wastewater. *World Journal of Chemistry*, **15**(1), 15–23, https://doi.org/10.5829/idosi.wjc.2020.15.23

Kataoka R. (2018). Biodegradability and biodegradation pathways of chlorinated cyclodiene insecticides by soil fungi. *Journal of Pesticide Science*, **43**(4), 314–320, https://doi.org/10.1584/jpestics.J18-03

Kumar V. and Dwivedi S. K. (2019). Hexavalent chromium reduction ability and bioremediation potential of *Aspergillus flavus* CR500 isolated from electroplating wastewater. *Chemosphere*, **237**, 124567, https://doi.org/10.1016/j.chemosphere.2019.124567

Kumar V. and Dwivedi S. K. (2020). Multimetal tolerant fungus *Aspergillus flavus* CR500 with remarkable stress response, simultaneous multiple metal/loid removal ability and bioremediation potential of wastewater. *Environmental Technology and Innovation*, **20**, 101075, https://doi.org/10.1016/j.eti.2020.101075

Kumar V. and Dwivedi S. K. (2021a). Bioremediation mechanism and potential of copper by actively growing fungus *Trichoderma lixii* CR700 isolated from electroplating wastewater. *Journal of Environmental Management*, **277**, 111370, https://doi.org/10.1016/j.jenvman.2020.111370

Kumar V. and Dwivedi S. K. (2021b). Mycoremediation of heavy metals: processes, mechanisms, and affecting factors. *Environmental Science and Pollution Research*, **28**(9), 10375–10412, https://doi.org/10.1007/s11356-020-11491-8

Kumar R., Sharma P., Umar A., Kumar R., Singh N., Joshi P. K., AlAlharthi F. A., Alghamdi A. A. and Al-Zaqri N. (2020). In vitro bioadsorption of Cd^{2+} ions: adsorption isotherms, mechanism, and an insight to mycoremediation. *Processes*, **8**(9), 1085–1100, https://doi.org/10.3390/pr8091085

Kumar A., Sharma A., Chaudhary P. and Gangola S. (2021a). Chlorpyrifos degradation using binary fungal strains isolated from industrial waste soil. *Biologia*, **76**(10), 3071–3080, https://doi.org/10.1007/s11756-021-00816-8

Kumar A., Yadav A. N., Mondal R., Kour D., Subrahmanyam G., Shabnam A. A., Khan S. A., Yadav K. K., Sharma G. K., Cabral-Pinto M., Fagodiya R. K., Gupta D. K., Hota S. and Malyan S. K. (2021b). Myco-remediation: a mechanistic understanding of contaminants alleviation from natural environment and future prospect. *Chemosphere*, **284**, 131325, https://doi.org/10.1016/j.chemosphere.2021.131325

Lallawmsanga Leo V. V., Passari A. K., Muniraj I. K., Uthandi S., Hashem A., Abd_Allah E. F., Alqarawi A. A. and Singh B. P. (2019). Elevated levels of laccase synthesis by *Pleurotus pulmonarius* BPSM10 and its potential as a dye decolorizing agent. *Saudi Journal of Biological Sciences*, **26**(3), 464–468, https://doi.org/10.1016/j.sjbs.2018.10.006

Li P., Su R., Yin R., Lai D., Wang M., Liu Y. and Zhou L. (2020). Detoxification of mycotoxins through biotransformation. *Toxins*, **12**(2), 1–37, https://doi.org/10.3390/toxins12020121

Lin X., Li X., Sun T., Li P., Zhou Q., Sun L. and Hu, X. (2009). Changes in microbial populations and enzyme activities during the bioremediation of oil-contaminated soil. *Bulletin of Environmental Contamination and Toxicology*, **83**, 542–547, https://doi.org/10.1007/s00128-009-9838-x

Maadani Mallak A., Lakzian A., Khodaverdi E., Haghnia G. H. and Mahmoudi S. (2020). Effect of *Pleurotus ostreatus* and *Trametes versicolor* on triclosan biodegradation and activity of laccase and manganese peroxidase enzymes. *Microbial Pathogenesis*, **149**, 104473, https://doi.org/10.1016/j.micpath.2020.104473

Mathur M., Gola D., Panja R., Malik A. and Ahammad S. Z. (2018). Performance evaluation of two *Aspergillus* spp. for the decolourization of reactive dyes by bioaccumulation and biosorption. *Environmental Science and Pollution Research*, **25**(1), 345–352, https://doi.org/10.1007/s11356-017-0417-0

Mayans B., Camacho-Arévalo R., García-Delgado C., Alcántara C., Nägele N., Antón-Herrero R., Escolástico C. and Eymar E. (2021). Mycoremediation of soils polluted with trichloroethylene: first evidence of *Pleurotus* genus effectiveness. *Applied Sciences (Switzerland)*, **11**(4), 1–11, https://doi.org/10.3390/app11041354

Mishra A. and Malik A. (2013). Recent advances in microbial metal bioaccumulation. *Critical Reviews in Environmental Science and Technology*, **43**(11), 1162–1222, https://doi.org/10.1080/10934529.2011.627044

Molla A. H. and Khan H. I. (2018). Detoxification of textile effluent by fungal treatment and its performance in agronomic usages. *Environmental Science and Pollution Research*, **25**(11), 10820–10828, https://doi.org/10.1007/s11356-018-1361-3

Naeimi B., Foroutan R., Ahmadi B., Sadeghzadeh F. and Ramavandi B. (2018). Pb(II) and Cd(II) removal from aqueous solution, shipyard wastewater, and landfill leachate by modified *Rhizopus oryzae* biomass. *Materials Research Express*, **5**(4), 1–30, https://doi.org/10.1088/2053-1591/aab81b

Nam I. H., Murugesan K., Ryu J. and Kim J. H. (2019). Arsenic (As) removal using *Talaromyces* sp. KM-31 isolated from As-contaminated mine soil. *Minerals*, **9**(10), 1–11, https://doi.org/10.3390/min9100568

Negi B. B. and Das C. (2023). Mycoremediation of wastewater, challenges, and current status: a review. *Bioresource Technology Reports*, **22**, 101409, https://doi.org/10.1016/j.biteb.2023.101409

Noman E., Al-Gheethi A., Mohamed R. M. S. R. and Talip B. A. (2019). Myco-remediation of xenobiotic organic compounds for a sustainable environment: a critical review. *Topics in Current Chemistry*, **377**(17), 1–41, https://doi.org/10.1007/s41061-019-0241-8

Noman E., Al-Gheethi A. A., Talip B., Mohamed R. and Kassim A. H. (2020). Mycoremediation of Remazol Brilliant Blue R in greywater by a novel local strain of *Aspergillus iizukae* 605EAN: optimisation and mechanism study. *International Journal of Environmental Analytical Chemistry*, **100**(14), 1650–1668, https://doi.org/10.1080/03067319.2019.1657852

Pandey A. K., Gaur V. K., Udayan A., Varjani S., Kim S. H. and Wong J. W. C. (2021). Biocatalytic remediation of industrial pollutants for environmental sustainability: research needs and opportunities. *Chemosphere*, **272**, 129936, https://doi.org/10.1016/j.chemosphere.2021.129936

Park H. and Choi I. G. (2020). Genomic and transcriptomic perspectives on mycoremediation of polycyclic aromatic hydrocarbons. *Applied Microbiology and Biotechnology*, **104**(16), 6919–6928, https://doi.org/10.1007/s00253-020-10746-1

Patil P. D., Singh A. A. and Yadav D. G. (2021). Biodegradation of organophosphorus insecticide chlorpyrifos into a major fuel additive 2,4-bis(1,1 dimethylethyl) phenol using white-rot fungal strain *Trametes hirsuta* MTCC-1171. *Journal of the Indian Chemical Society*, **98**(9), 100120, https://doi.org/10.1016/j.jics.2021.100120

Planý M., Pinzari F., Šoltys K., Kraková L., Cornish L., Pangallo D., Jungblut A. D. and Little B. (2021). Fungal-induced atmospheric iron corrosion in an indoor environment. *International Biodeterioration and Biodegradation*, **159**, 105204, https://doi.org/10.1016/j.ibiod.2021.105204

Purnomo A. S., Andyani N. E. A., Nawfa R. and Putra S. R. (2020). Fenton reaction involvement on methyl orange biodegradation by brown-rot fungus *Gloeophyllum trabeum*. *AIP Conference Proceedings*, **2237**, 1–6, https://doi.org/10.1063/5.0005230

Rajta A., Bhatia R., Setia H. and Pathania P. (2020). Role of heterotrophic aerobic denitrifying bacteria in nitrate removal from wastewater. *Journal of Applied Microbiology*, **128**(5), 1261–1278, https://doi.org/10.1111/jam.14476

Roshandel F., Saadatmand S., Iranbakhsh A. and Ardebili Z. O. (2021). Mycoremediation of oil contaminant by *Pleurotus florida* (P. Kumm) in liquid culture. *Fungal Biology*, **125**(9), 667–678, https://doi.org/10.1016/j.funbio.2021.04.002

Rosu C. M., Avadanei M., Gherghel D., Mihasan M., Mihai C., Trifan A., Miron A. and Vochita G. (2018). Biodegradation and detoxification efficiency of azo-dye reactive orange 16 by *Pichia kudriavzevii* CR-Y103. *Water, Air, and Soil Pollution*, **229**(1), 1–18, https://doi.org/10.1007/s11270-017-3668-y

Sadañoski M. A., Benítez S. F., Fonseca M. I., Velázquez J. E., Zapata P. D., Levin L. N. and Villalba L. L. (2019). Mycoremediation of high concentrations of polychlorinated biphenyls with *Pleurotus sajor-caju* LBM 105 as an effective and cheap treatment. *Journal of Environmental Chemical Engineering*, **7**(6), 103453–103482, https://doi.org/10.1016/j.jece.2019.103453

Sadañoski M. A., Tatarin A. S., Barchuk M. L., Gonzalez M., Pegoraro C. N., Fonseca M. I., Levin L. N. and Villalba L. L. (2020). Evaluation of bioremediation strategies for treating recalcitrant halo-organic pollutants in soil environments. *Ecotoxicology and Environmental Safety*, **202**, 110929, https://doi.org/10.1016/j.ecoenv.2020.110929

Sellami K., Couvert A., Nasrallah N., Maachi R., Abouseoud M. and Amrane A. (2022). Peroxidase enzymes as green catalysts for bioremediation and biotechnological applications: a review. *Science of the Total Environment*, **806**, 150500, https://doi.org/10.1016/j.scitotenv.2021.150500

Senthivelan T., Kanagaraj J. and Panda R. C. (2016). Recent trends in fungal laccase for various industrial applications: an eco-friendly approach – a review. *Biotechnology and Bioprocess Engineering*, **21**(1), 19–38, https://doi.org/10.1007/s12257-015-0278-7

Sharma S., Tiwari S., Hasan A., Saxena V. and Pandey L. M. (2018). Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils. *3 Biotech*, **8**(4), 1–18, https://doi.org/10.1007/s13205-018-1237-8

Sheam M. M., Biswas S. K., Ahmed K. R., Syed S. B., Hossain M. S., Khan M. S. A., Hasan M. R., Zohra F. T. and Rahman M. M. (2021). Mycoremediation of reactive red HE7B dye by *Aspergillus salinarus* isolated from textile effluents. *Current Research in Microbial Sciences*, **2**, 100056, https://doi.org/10.1016/j.crmicr.2021.100056

Simanjorang S. W. and Subowo Y. B. (2018). The ability of soil-borne fungi to degrade polycyclic aromatic hydrocarbon (PAH). *AIP Conference Proceedings*, **2021**(2018), 030019, https://doi.org/10.1063/1.5062743

Singh A. K., Yadav P., Bharagava R. N., Saratale G. D. and Raj A. (2019). Biotransformation and cytotoxicity evaluation of kraft lignin degraded by ligninolytic *Serratia liquefaciens*. *Frontiers in Microbiology*, **10**, 1–11, https://doi.org/10.3389/fmicb.2019.02364

Skanda S., Bharadwaj P. S. J., Kar S. and Muthukumar V. S. (2023). Bioremoval capacity of recalcitrant azo dye Congo red by soil fungus *Aspergillus arcoverdensis* SSSIHL-01. *Bioremediation Journal*, **0**(0), 1–12, https://doi.org/10.1080/10889868.2021.1984198

Šrédlová K., Škrob Z., Filipová A., Mašín P. and Holecová J. (2019). Biodegradation of PCBs in contaminated water using spent oyster mushroom substrate and a trickle-bed bioreactor. Water Research, 115274, 150, https://doi.org/10.1016/j.watres.2019.115274

Torres-Farradá G., Manzano-León A. M., Rineau F., Ramos Leal M., Thijs S., Jambon I., Put J., Czech J., Guerra Rivera G., Carleer R. and Vangronsveld J. (2019). Biodegradation of polycyclic aromatic hydrocarbons by native *Ganoderma* sp. strains: identification of metabolites and proposed degradation pathways. *Applied Microbiology and Biotechnology*, **103**(17), 7203–7215, https://doi.org/10.1007/s00253-019-09968-9

Uthayakumar H., Radhakrishnan P., Shanmugam K. and Kushwaha O. S. (2022). Growth of MWCNTs from *Azadirachta indica* oil for optimization of chromium(VI) removal efficiency using machine learning approach. *Environmental Science and Pollution Research*, **29**(23), 34841–34860, https://doi.org/10.1007/s11356-021-17873-w

Vara S. and Karnena M. K. (2020). Fungal enzymatic degradation of industrial effluents – a review. *Current Research in Environmental and Applied Mycology*, **10**(1), 417–442, https://doi.org/10.5943/CREAM/10/1/33

Vašinková M., Dlabaja M. and Kučová K. (2021). Bioaccumulation of toxic metals by fungi of the genus *Aspergillus* isolated from the contaminated area of Ostramo Lagoons. *IOP Conference Series: Earth and Environmental Science*, **900**(1), 1–8, https://doi.org/10.1088/1755-1315/900/1/012048

Vipotnik Z., Michelin M. and Tavares T. (2022). Biodegradation of chrysene and benzo[a]pyrene and removal of metals from naturally contaminated soil by isolated *Trametes versicolor* strain and laccase produced thereof. *Environmental Technology and Innovation*, **28**, 102737, https://doi.org/10.1016/j.eti.2022.102737

Viswanath B., Rajesh B., Janardhan A., Kumar A. P. and Narasimha G. (2014). Fungal laccases and their applications in bioremediation. *Enzyme Research*, **2014**, 1–21, https://doi.org/10.1155/2014/163242

Xiao P. and Kondo R. (2020). Biodegradation and biotransformation of pentachlorophenol by wood-decaying white rot fungus Phlebia acanthocystis TMIC34875. *Journal of Wood Science*, **66**(1), 1–11, https://doi.org/10.1186/s10086-020-1849-6

Xu P., Du H., Peng X., Tang Y., Zhou Y., Chen X., Fei J., Meng Y. and Yuan L. (2020a). Degradation of several polycyclic aromatic hydrocarbons by laccase in reverse micelle system. *Science of the Total Environment*, **708**, 134970, https://doi.org/10.1016/j.scitotenv.2019.134970

Xu X., Hao R., Xu H. and Lu A. (2020b). Removal mechanism of Pb(II) by *Penicillium polonicum*: immobilization, adsorption, and bioaccumulation. *Scientific Reports*, **10**(1), 1–12, https://doi.org/10.1038/s41598-020-66025-6

Yadav P., Rai S. N., Mishra V. and Singh M. P. (2021). Mycoremediation of environmental pollutants: a review with special emphasis on mushrooms. *Environmental Sustainability*, **4**(4), 605–618, https://doi.org/10.1007/s42398-021-00197-3

Zeng S., Qin X. and Xia L. (2017). Degradation of the herbicide isoproturon by laccase-mediator systems. *Biochemical Engineering Journal*, **119**, 92–100, https://doi.org/10.1016/j.bej.2016.12.016

Zhao W., Zhu G., Daugulis A. J., Chen Q., Ma H., Zheng P., Liang J. and Ma X. (2020). Removal and biomineralization of Pb 2 þ in water by fungus *Phanerochaete chrysoporium*. *Journal of Cleaner Production*, **260**, 120980, https://doi.org/10.1016/j.jclepro.2020.120980

Zhou X. W., Cong W. R., Su K. Q. and Zhang Y. M. (2013). Ligninolytic enzymes from *Ganoderma* spp: current status and potential applications. *Critical Reviews in Microbiology*, **39**(4), 416–426, https://doi.org/10.3109/1040841X.2012.722606

Zhuo R., Zhang J., Yu H., Ma F. and Zhang X. (2019). The roles of *Pleurotus ostreatus* HAUCC 162 laccase isoenzymes in decolorization of synthetic dyes and the transformation pathways. *Chemosphere*, **234**, 733–745, https://doi.org/10.1016/j.chemosphere.2019.06.113

doi: 10.2166/9781789063783_0301

Chapter 16

Bioremediation: a green tool to remediate refractory pollutants

*Shivangi Mudaliar[1], Komal Agrawal[2] and Pradeep Verma[1]**

[1]Bioprocess and Bioenergy Laboratory (BPEL), Department of Microbiology, Central University of Rajasthan, Bandarsindri, Kishangarh, Ajmer, Rajasthan 305817, India
[2]Department of Microbiology, School of Bio Engineering and Biosciences, Lovely Professional University, Phagwara, Punjab 144411, India
**Corresponding author: pradeepverma@curaj.ac.in; vermaprad@yahoo.com*

ABSTRACT

The rapid escalation of pollution in the environment by anthropogenic and household activities is responsible for the contamination of various ecological niches. The result of which is an increased demand for the development of sustainable and innovative wastewater treatment technologies. Bioremediation has been regarded as the most efficient technique to remove heavy metals from polluted water and soil. A wide range of microorganisms including bacteria, fungi, and yeasts can actively modify the toxic contaminants and has a decisive part in the degradation, and mineralization of harmful substances into less lethal forms. Primarily bioremediation focuses on *ex-situ* as well as *in-situ* treatment and the ever-evolving technology that can be used to manage diverse pollutants. Thus, the chapter emphasizes on the sources of pollutants along with several bioremediation technologies for the treatment of wastewater. Additionally, new techniques such as nanotechnology, electro bioremediation, and constructed wetlands, and so forth that are being implemented to minimize the industry expenses for the treatment of wastewater have also been discussed in the chapter.

Keywords: bio-based remediation, refractory pollutants, sustainable, bioremediation

16.1 INTRODUCTION

Water is vital for the maintenance of life on earth and the accelerated expansion of industries and agriculture has polluted the ecosystem with varied contaminants, including toxic heavy metals (Kumar & Dwivedi, 2021), polychlorinated biphenyls (Sadañoski *et al.*, 2019), and various agrochemicals (Bhadouria *et al.*, 2020). Their existence in the ecosystem is of great concern, as they are toxic and non-biodegradable by nature (Kour *et al.*, 2021). Industrial wastes are directly discharged into the aquatic environment, disrupting the photosynthetic process and altering the dissolved oxygen and pH level of water. In addition, treating and managing waste has been a challenging task (Amulya *et al.*, 2016) and a major problem associated with the waste is its mineralization. Several conventional approaches have been applied to transform this waste; however, it is a tedious process, and an expensive task (De Donno Novelli *et al.*, 2021) and the production of intermediate by-products can be more damaging than the original compound resulting in more deleterious impacts. Thus, these contaminants and their by-products require proper eradication through sustainable methods along with simultaneous restoration of the polluted site. Bioremediation is cost-effective and green means for the removal of refractory pollutants by treating wastewater. The front runners of this process are the microbial community and microalgae that result in sludge and treated water or biomass formation. Particularly microbes are capable of degrading, cleansing, and even gathering toxic organic and inorganic substances. Heavy metals can enter the environment from a variety of sources, including natural, industrial solid waste, agricultural, atmospheric sources, inland effluent, and more. Most parts of the world have been contaminated by activities such as electroplating, mining, metallurgical smelting, and the use of agricultural pesticides and fertilizers. In the past two decades, bioremediation technology has developed with the ultimate aim of efficiently restoring the damaged sites in a greener way with low cost. There is not even one bioremediation method that can be used as a sure approach to restore the affected habitats, despite the fact that researchers have established and modelled various bioremediation strategies. The majority of the problems relating to the bioremediation and biodegradation of contaminating compounds can be resolved by using native microorganisms found in damaged environments, because the ecological conditions are favorable for their survival and metabolic growth (Medfu Tarekegn *et al.*, 2020). All pollutants found in the environment must be eliminated, biodegraded, or inactivated. Any environmentally friendly treatment methods that can be described and shown to be efficient, with low costs and elevated capacity to remove pollutants, will make a significant contribution to resolving the environmental crisis we are currently facing. Thus, the current chapter will confer about various sources of pollutants and their effects on the environment. This chapter gives us a glimpse on the various bioremediation technologies that could be implemented for the remediation of wastewater.

16.2 SOURCES OF REFRACTORY POLLUTANTS AND THEIR CONSEQUENCES

Refractory pollutants from textile, paper, and agricultural sectors are the major source of contributors that cause harmful effects on aquatic bodies and the environment (Wen *et al.*, 2017). In the below sections, different pollutants discharged from various sectors have been elaborated (Figure 16.1).

16.2.1 Textile and apparel industries

The textile sector is a major water-consuming industry, nearly 15% of used dyes are released along with the discharged water directly (Rajhans *et al.*, 2021). Textile wastewater is characterized by high

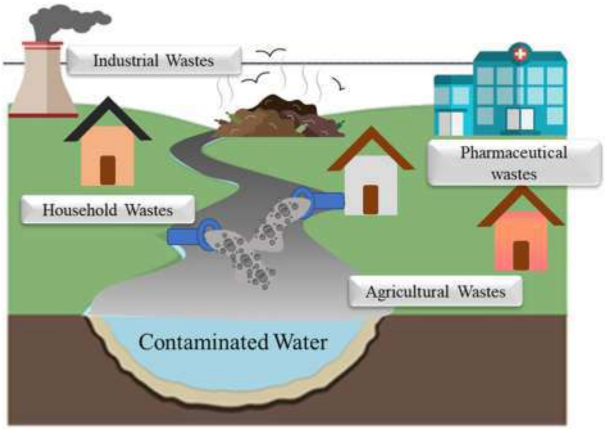


Figure 16.1 Major sources of refractory pollutants contaminating the soil and water.

pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), high dye content, total dissolved solids (TDS), total suspended particles (TSP), sulfates, and chlorides (Khan & Malik, 2018). Rajhans *et al.* (2021) showed for the first time the direct relationship between toxicity and COD, they have proved that the toxicity of wastewater was increasing with an increase in effluents COD in a very short period (Rajhans *et al.*, 2021). This contributes to the accumulation of toxic or xenobiotic pollutants in the environment that lasts for an extended duration and leads to severe health and environmental risks (Kishor *et al.*, 2021a). The most typical refractory pollutants from the textile industry are reactive azo dyes, aniline, and polyvinyl alcohol. Amidst pollutants aniline has been listed as a pollutant of concern by the United States Environmental Protection Agency (USEPA) as it is carcinogenic and mutagenic while polyvinyl alcohol being a water-soluble polymer causes the lack of dissolved oxygen. The removal of these pollutants through conventional methods is difficult due to their poor degradability (Yu *et al.*, 2022). The major contributor to textile wastewater is a reactive dye (Khan & Malik, 2018) coming out from the textile industries that contain different hazardous persistent coloring pollutants. Due to their several chromophore groups including azo (–N=N–), nitro (–N=O), carbonyl (–C=O), quinoid, and auxochrome groups like hydroxyl (–OH), carboxyl (–COOH), amine ($-NH_2$), and sulfonate ($-SO_3H$), textile dyes are aromatic, heterocyclic compounds that are persistent (Kishor *et al.*, 2021b). Compounds, such as Sudan I (azo dye) have toxic/carcinogenic character when transformed enzymatically to aromatic amines by intestinal bacteria (Piątkowska *et al.*, 2018). Synthetic dyes have been reported to cause cancer, skin diseases, and central nervous system disorders (Khan & Malik, 2018). The matrix of the textile industry's effluent is quite complex and contains a variety of aromatic compounds, color content and toxic metals, such as arsenic (As), lead (Pb), chromium (Cr), antimony (Sb), cadmium (Cd) and mercury (Hg) (Chandanshive *et al.*, 2020; Kishor *et al.*, 2021c). Khan and Malik (2018) showed that textile wastewater contains several heavy metals and organic compounds that inhibited the process of seed germination thus indicated the presence of toxic pollutants. Textile wastes contain a large number of contaminants that needs to be treated before their disposal into water. Many conventional and new treatments have been reported but their use is limited due to several drawbacks such as not being eco-friendly as well as causes harm to human health, so there is a need to opt for a biological approach. Biological tools have emerged as an effective way to degrade and detoxify textile industrial wastewater (Khandare & Govindwar, 2015; Kishor *et al.*, 2021a). Bioremediation technologies offer a sustainable and greener way for the removal of pollutants.

16.2.2 Paper and pulp industries

Paper and pulp industries release a huge amount of wastewater that remains toxic after secondary treatment and contain a large amount of lignin residues, and organic pollutants such as adsorbable organic halides (AOX) (Kumar *et al.*, 2020). AOX, are a group of a pollutant that includes chlorinated organic compounds, such as resin acids, phenols, chlorinated hydrocarbons, furans, and dioxin. Further, these organic compounds can be separated into two groups based on their molecular weight (MW), those having MW $>$ 1 kDa are high molecular weight (HMW) compounds, and those having MW $<$ 1 kDa are low molecular weight (LMW) compounds. Molecular weight is an important factor for evaluation of environmental behavior (Lindholm-Lehto *et al.*, 2015). These pollutants are discharged mainly by bleaching and pulping processes and affect both ecosystems as well as human health. Many severe respiratory disorders, cardiac problems, and eye and skin irritation have occurred because of the gaseous pollutants released by industries. Moreover, inorganic pollutants such as copper (Cu), zinc (Zn), nickel (Ni), and ferrous (Fe) have been found to cause neurotoxicity (Singh & Chandra, 2019). The physiochemical methods available are energy intensive, expensive, inefficient, and produce toxic sludge in bulk due to which the handling is difficult. To satisfy the discharge standards of environmental regulation, paper and pulp industries have started adopting biological methods for the remediation of wastewater (Kumar *et al.*, 2020; Dias *et al.*, 2020). Biological treatment using microorganisms, such as bacteria, fungi, algae, and yeast can help in reducing the amount of operation and easy handling. They are efficient in removing phenolics as well as color compounds unlike the conventional methods (Erkan & Engin, 2017; Qadir & Chhipa, 2015; Sharma *et al.*, 2020; Cen *et al.*, 2022). Howbeit, due to legislative pressures the water consumption has been reduced, also, by replacing the elemental chlorine with free chlorine the amount of AOX has been decreased. This has helped in reducing the number of pollutants generated by paper and pulp industries to a great extent. The technological advancements in the biodegradation approach could be an efficient method for the remediation of refractory pollutants. It could be an excellent, inexpensive tool for the removal of harmful organic wastes in a way (Singh *et al.*, 2018).

16.2.3 Agrochemical wastes

The rapidly growing population has increased the demand for food and food resources has made farmers utilize fertilizers and pesticides (Dhananjayan *et al.*, 2020; Nayak *et al.*, 2018) in an unregulated manner to meet product consumer needs globally. As a result, a large amount of contaminants gets washed off to nearby water bodies and affects the health of living bodies coming in contact (Akhtar & Mannan, 2020b). According to the American Heritage Science Dictionary, 'agrochemicals are substances such as hormones, fungicides, and insecticides that are frequently used to enhance the productivity of crops' (Bhadouria *et al.*, 2020). These chemical fertilizers consist of nitrogen, phosphorous, and potassium in their structure. Pesticides have subdivisions like insecticides (organochlorine, carbamate, pyrethrin, and rotenone), fungicides (sulfur metals, organomercurial, chlorophenols, phthalimides), herbicides (chlorates, arsenicals, chlorophenoxy), and so on (Gupta, 2019). It has been found that these chemicals affect the nervous system and also affect different parts of the animal and human body. Insecticides like organophosphate and carbamate are known acetylcholine inhibitors that primarily block the normal breakdown of acetylcholine. Besides, they show nicotinic and muscarinic effects such as manifested by salivation, lacrimation, urination, abdominal pain, vomiting, and diarrhea. While the nicotinic effect shows twitching of muscles, seizures, and tremors followed by convulsions (Gupta, 2019). Some chlorinated hydrocarbon compounds, heptachlor epoxide, lindane, and oxychlordane are also found in the fatty tissues after long exposure to these chemicals. Fungicides, such as chlorothalonil, captan, captafol, sulfur derivatives of dithiocarbamic acid, and dimethyldithiocarbamates are nontoxic and are being used widely by farmers. However, their hydrolyzed products such as ethylthiourea is carcinogenic (Gupta, 2019). Organic and inorganic pollutants of agriculture can cause more or less toxic effects to human as well as to the environment. Some compounds like anions (Cl^-, SO_4^{2-}) are recalcitrant in structure and requires further treatment before their final disposal into the environment. Microorganisms have the potential to degrade these agrochemicals even if they are present in low concentration.

16.2.4 Pharmaceutical and medical waste

Pharmaceutical compounds are biologically active, designed to prevent several different types of diseases in humans and animals. The usage of pharmaceutically active compounds (PhACs) has raised a lot of concern as they are vital for the maintenance of good health (Kumar & Bharadvaja, 2020). As a result, the discharge from pharmaceutical industries has also increased which ultimately pollutes the environment with incomplete transformation of the compounds. Luo *et al.* (2019) identified the composition of PhACs taken from different medical sites that showed the relative content of organic compounds. Wastewater from antibiotic pharmaceutical industries was reported to have 15.86% of 5-(phenylmethoxy)-1H-indole-3-carbaldehyde, 22.28% amyl acetate, and 14.27% N, N-dimethylformamide, including dodecyl mercaptan, decamethylcyclopentasiloxane with total of 53 organic compounds in the influent while only 30 in the effluent. By contrast, the effluent from traditional Chinese medicine pharmaceutical plant had 25 more organic compounds than the influent and this depicted that the incomplete degradation of organic matters in the treatment plants and increased the number of refractory pollutants in the effluent. Amongst all pollutants, the highest relative content in organic compound class was acids (63.65%), esters (44.96%), and heterocyclic compounds (30.24%) while in effluents it was esters (52.66%) and organosilicon compounds (42.46%) (Luo *et al.*, 2019).

Various techniques, such as membrane separation, advanced oxidation process, flocculation, sand filter process, and so on have been efficiently used for the elimination of contaminants. Likewise, bioremediation is also found to be effectively removing a small amount of recalcitrant compounds (Kumar & Bharadvaja, 2020) (Table 16.1).

16.3 APPROACHES FOR BIOREMEDIATION OF REFRACTORY POLLUTANTS

For the treatment of refractory pollutants, bioremediation is a promising technique and is a better alternative to conventional techniques. This process is extensively used as it is economical and eco-friendly technology. There are various approaches for bioremediation of pollutants. Some of the approaches have been discussed below.

16.3.1 Bioremediation

Removal of environmental pollutants by a biological process where microorganisms and their metabolic competence are being used for the breakdown of a broad range of chemicals is known as bioremediation. Microorganisms convert hazardous compounds into non-hazardous compounds. This approach is cost-effective and eco-friendly and it mineralizes the contaminants completely. Even the smallest number of toxic contaminants can be removed by bioremediation. Also, bioremediation helps in restoring the polluted site to its original condition without harming the environment (Jobby *et al.*, 2018). Microorganisms that live in groundwater can naturally degrade the harmful contaminants. The microbes convert these chemicals into water and harmless gases, for example, CO_2. The varying structures of the different components present in industrial wastes demand separate treatment methodologies. A natural system using microbes could be established to degrade these contaminants. The bioremediation could be classified into *ex situ* and *in situ* according to the treatment site, pollution range, pollutant category, and treatment cost (Haripriyan *et al.*, 2022) as depicted in the below-given picture. Fungi, bacteria, plants, actinomycetes, and algae are being used prominently for the elimination of contaminated soil and water (Zhang *et al.*, 2020). Microbes are capable of transforming or detoxifying the contaminants in the water in a sustainable way (Figure 16.2).

16.3.2 Fungi

In recent years, presence and persistence of organic and inorganic contaminants have augmented rapidly. It is necessary to devise a strategy that is capable of overcoming these challenges and offering *in-situ* treatment of the pollutants. The use of fungi or their derivatives to redress environmental contaminants can be a cost-effective, environment-friendly, and successful method in addressing the growing issue of soil and water pollution. Mycoremediation methods mostly rely on microorganisms (fungi) native to the contaminated sites (Akhtar & Mannan, 2020a). Mycoremediation is a significant procedure that uses fungus to eliminate petroleum hydrocarbons from the environment. Due to their complex enzymatic systems, quick ability to adapt to harmful organic contaminants, and harsh environmental circumstances, fungi have benefits. Bilen Ozyurek *et al.* (2021) investigated the potential of spore suspension, live biomass, and cell-free culture supernatant of *Aspergillus ochraceus* in the degradation of petroleum. Fungal discs showed 94% success rate than spore suspension which was 87% in petroleum degradation. The strain *A. ochraceus* has a high degradation capacity. Fungi have phenomenal competence to grow in the presence of heavy metals and pesticides. Dey *et al.* (2020) in their work proved the accumulation of metals by *Aspergillus fumigatus* in the order Zn (98%) > Pb (95%) > Cd (63%) > Cr (62%) > Ni (46%) > Cu (37%) from a cocktail of metals and lindane (30 mg/L). Furthermore, the results of transmission electron microscopy coupled with energy-dispersive x-ray analysis (TEM-EDAX) were used to determine the uptake of the metals Cd, Cu, and Pb in the membrane of the cytoplasm and the accumulation of the metals Cr, Ni, and Zn in the cytoplasm of the fungus. FTIR revealed the involvement of fungal cell walls in metal chelation (Dey *et al.*, 2020). In addition, the production of edible mushrooms from industrial and agricultural wastes may be a technique with

Table 16.1 Ecotoxicological effects of various pollutants from different industries.

Industries	Pollutants	Types of Pollutants	Ecotoxicological and Human Effects	References
Pharmaceuticals and medical	Endocrine disruptive compounds (EDC)	Chlororganics Imidazoles Triazoles Paraquat Bisphenol A Estrone Phthalates	• Thyroid dysfunction • Change of sex in aquatic animals • Antiandrogenic effects by mimicking estrogenic actions • Modifies the hormone levels in the body • Affects fertility by acting as a reproductive toxicant • Gene suppression, activation with alteration in the hormonal feedback system	Pironti *et al.* (2021)
		Polychlorinated biphenyls Dioxins Heavy metals Diclofenac Naproxen	• Causes acute renal failure • Inhibits the growth of cyanobacteria and algae • Neurotoxicity and behavioral changes are been observed for some drugs	Khan *et al.* (2020)
Paper and pulp	Adsorbable organic halogens (AOX)	Organochlorines Organophosphates	• Causes phytotoxicity and chromosomal aberrations • Various androgenic and carcinogenic components are being found in the effluent that results in the deteriorating health of organisms • AOX inhibits cytochrome oxidase and damages the electron transport system. • The bleaching process is responsible for respiratory, reproductive, genetic, chronic, and skin damage	Mandeep Gupta *et al.* (2019)
Textiles and apparel	Heavy metals	Chromium (Cr) Cadmium (Cd) Lead (Pb) Mercury (Hg) Nickel (Ni) Copper (Cu)	• Causes mutagenic, carcinogenic, or teratogenic effects on different species depending on the concentration • Exposure to Cd affects osmoregulation and Ca metabolism by affecting carbonic anhydrase (enzyme) in the tissues • Cd is highly toxic and thus decreases the number of freshwater mussels • Smoking and tobacco are the sources of Cd exposure in humans. • Exposure to Hg vapors in the process of amalgamation of gold • Welders exposed to welding fumes had increased levels of Cr Cd, Ni, and Pb in their blood which results in oxidative stress • Cd, Pb, and Hg are nephrotoxic, particularly in the renal cortex. The accumulation of metals in the body leads to several histopathological problems.	Ali *et al.* (2019)

(*Continued*)

Table 16.1 Ecotoxicological effects of various pollutants from different industries. (*Continued*)

Industries	Pollutants	Types of Pollutants	Ecotoxicological and Human Effects	References
	Dyes	Congo red Azure B Disperse orange Crystal violet	• Toxic to aquatic organisms • It stops the light from penetrating the photic zone and depletes oxygen levels thus hindering the biological function of aquatic flora and fauna • Potentially carcinogenic for humans • Can cause dermatitis and central nervous system problems • Long-term exposure can cause bladder cancer, colorectal cancer, and colon cancer	Al-Tohamy *et al.* (2022)
Agricultural	Pesticides	Insecticide	• Few are carcinogenic, they cause liver damage, muscle tremors, carcinogenicity, and convulsions • Endosulfan are persistent organic pollutants (POP) that cause endocrine disruption, sex hormone hindrance, and even cancer • Some enter the bloodstream and cause more severe effects on health such as vomiting, cramps, diarrhea, headache, and even death • Pesticides such as parathion disrupt the synthesis of macromolecules like DNA, RNA and proteins and can be neurotoxic	Pandey *et al.* (2018)
		Herbicide	• Affects the liver and kidney in animals when exposed to high levels of herbicides • Nonspecific alterations such as depression of the central nervous system, myocardium problems, gastrointestinal problems • Also, weakness, ataxia, fatigue, tachycardia, vomiting, sweating, abdominal pain • Application of herbicide affects the ecology, number and microorganism distribution in soil	Magnoli *et al.* (2020)
		Fungicide	• Affects liver cells • High reactive oxygen species production due to oxidative stress in humans • Can be a potential risk factor for the pathogenesis of non-alcoholic fatty liver disease in humans • Few are partly responsible for the development of Parkinson's disease	Kara *et al.* (2020); Kwon *et al.* (2021)

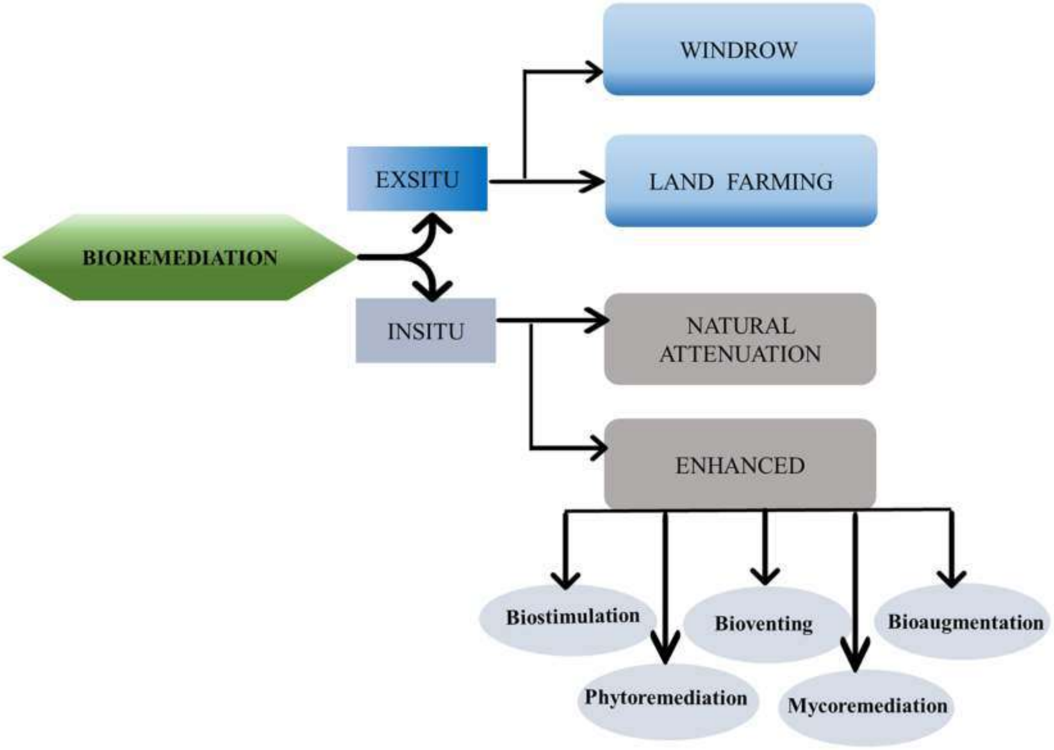


Figure 16.2 Pictorial illustration of the classification of bioremediation on the basis of the treatment site and their subclassifications.

added value that can turn what would otherwise be considered trash into diets and feedstuffs. In addition to producing healthy mushrooms, it reduces the genotoxicity and toxicity of mushroom species (Kulshreshtha *et al.*, 2014). Thus, more study is required to fully understand the capabilities of fungi as a remediating tool and their safety characteristics for consumption and the treatment of wastewater.

16.3.3 Bacteria

The emerging condition provided the stimulus to look for new sources for the treatment of textile dye. A better and more sustainable option is bacteria, which could be used for the remediation of pollutants from various sources contaminating soil and water (Gangola *et al.*, 2019). The textile industry without proper treatment contains a good amount of synthetic dye and their degradation in an eco-friendly manner is a global challenge. Afrin *et al.* (2021) showed the potential of a bacterial consortium of *Enterococcus faecium*, and *Pseudomonas aeruginosa* for the hydrolysis of Novacron dyes. The treated dyes were then used for agricultural purposes and found that it was non-toxic to agriculturally valuable bacteria (Afrin *et al.*, 2021). Other than synthetic dyes several hydrocarbons are also found in the wastewater and their degradation can be done biologically. Polycyclic aromatic hydrocarbon (PAH) adversely affects the health of humans. This study used nine natural bacterial strains isolated naturally for the degradation of four kinds of PAH. The study was conducted on several cultural factors and their effects on PAHs degradation. In the study, it was found that a bacterial mixture can increase the degradation rates of phenanthrene, and benzo pyrene and the rate was over 83%. They concluded that the efficient degradation was due to the cooperation of dioxygenase and dehydrogenase enzymes (Xu *et al.*, 2022). Industries are the dominant factors for the economic development of society and the need to have efficient biological remediation is persistent. To enhance the efficiency of degradation of bacteria, the study of genetic engineering has been introduced. Genetically engineered microorganisms (GEMs) facilitated methods for the elimination of metals/metalloids, which are thought to be both environmentally safe and economically practical strategies. At low concentrations, metals and metalloids are extremely poisonous and primarily contribute to the survival equilibrium of environmental activity. However, because they are not biodegradable, metals and metalloids remain in nature and bio-accumulate in the food chain (Sharma *et al.*, 2021). GEMs are projected to be an extremely effective process when compared to other procedures because they are highly capable of detoxifying (Table 16.2).

16.3.4 Plants

Plants can be found everywhere in different habitats and be a promising bioremediation tool for various waste effluents and meets all the requirements of the green purification process. This plant-based technology also helps in the restoration of habitats with reduced remedial costs. Zhang *et al.* (2021) while performing remediation with plants on slightly Cd-contaminated soil via three cropping systems found the highest Cd abstraction amount (148 h/ha) in monoculture. In addition, the net value of 1.88×10^4 US$ was achieved using the Monte-Carlo simulation. The metabolic and extractive potential of plants makes them capable of biodegradation. Recent developments in omics and nanotechnology are creating new avenues for the competent treatment of emerging contaminants effectively (Rai *et al.*, 2020). The uptake of organic/xenobiotic compounds in plants occurs through passive uptake. Several transporters like the phosphate transporter1 family (PHT1), a phosphate-H^+ symporter containing 520–550 amino acids with ~molecular mass of 58 kDa help transport arsenic (Rai *et al.*, 2020). The phytoremediation process takes place in wetlands also called natural marsh or root wetlands. Under reducing conditions like wetlands, one of the most poisonous and common inorganic forms of As is AsIII. It can attach to sulfhydryl groups in proteins and restrict general protein functioning (Abedin *et al.*, 2002). In comparison to other treatments, phytoremediation is easy to operate, can tolerate the lack of wastewater, as well as its maintenance cost is also low. Studies show that phytoremediation has almost 100% efficiency and is environmentally friendly in eliminating organic compounds from polluted water (Gatidou *et al.*, 2017). Singh *et al.* (2021) investigated the use of water macrophytes, that

Table 16.2 Microorganisms involved in the degradation of refractory pollutants.

Microorganism	Name	Pollutants	References
Bacteria	*Bacillus subtilis*	Endosulfan	Ahmad (2020)
	Bacillus velezensis	Slaughter wastewater	Li *et al.* (2018)
	Bacillus aryabhattai	Lignin and color	Zainith *et al.* (2019)
	Bacillus cohnni (RKS9)	Congo red	Kishor *et al.* (2021a)
	Bacillus albus MW407057	Methylene blue	Kishor *et al.* (2021b)
Fungi	*Aspergillus flavus* *Aspergillus niger* *Penicillium chrysogenum*	Endosulfan	Ahmad (2020)
	Aspergillus sp.	Platinum and palladium	Godlewska-Żyłkiewicz *et al.* (2019)
	Aspergillus sydowii *Fusarium lichenicola*	Polyaromatic hydrocarbon compounds (PAH)	Obire *et al.* (2020)
	Aspergillus sp.	Heavy metals	Vašinková *et al.* (2021)
	Ganoderma luicdum	Phenanthrene Chrysene	Agrawal *et al.* (2018)
	Ganoderma applanatum *Laetiporus sulphureus*	NSAIDs	Bankole *et al.* (2020)
Microalgae	*Desmodesmus* sp. *WR1*	17-α Estradiol	Wang *et al.* (2020)
	Chlorella vulgaris	Sulfamethoxazole	Xie *et al.* (2020)

is *Pistia stratiotes* aided in removing the pollutants of plant mill effluent (PME). They found that using 75% of PME treatment gave the best result in terms of removal with the highest biomass and chlorophyll enrichment. Their work was innovative in terms of phytoremediation of significant physicochemical and microbiological pollutants of paper mill effluent (PME) by *P. stratiotes*. Textile wastes are also prone to be toxic to the environment. Mahajan *et al.* (2019) showed the phytoremediation potential of *Chara vulgaris* for the treatment of textile wastes. *C. vulgaris* competently decreased BOD, COD, pH, EC, and TDS of the 10–5% of concentrated textile effluent within 120 h of treatment. It also can be used for the tertiary-level treatment of textile effluents. While performing the process one needs to consider higher efficiency such as the type of plant to be used, process time, number, also the type of soil and wetland. Also, the plant used should have a high rate of biomass growth, high content of salts, phosphate, and nitrogen, and be less sensitive to varying compositions of soil and wastewater (Justin *et al.*, 2010). The installation of this system occurs in two types: first is abiotic, based on hydrolysis, adsorption, and photodegradation and second is biological, through bioaccumulation and metabolism in tissue (Gatidou *et al.*, 2017). The different biological processes of phytoremediation are phytovolatilization, phytodegradation, phytoaccumulation, phytostabilization, and rhizofiltration (Polińska *et al.*, 2021). The drawback of this method is that the accessibility of plants all-round the year is difficult and consequently, this is considered a secondary or tertiary form of treatment. It is a slow process and that's why it is suggested that it should be combined with other agents to augment the process (Khandare & Govindwar, 2015). This approach has emerged as an eco-friendly, passive, cost-effective, and solar energy-driven method for cleaning the environment in comparison to other physio-chemical techniques. Amidst remediation approaches, phytoremediation is a good option for the future but still, the studies remain on the laboratory scale. There is a need to take this to a large-scale study at the contaminated site to find out more avenues of research in the removal of refractory pollutants (Figure 16.3).

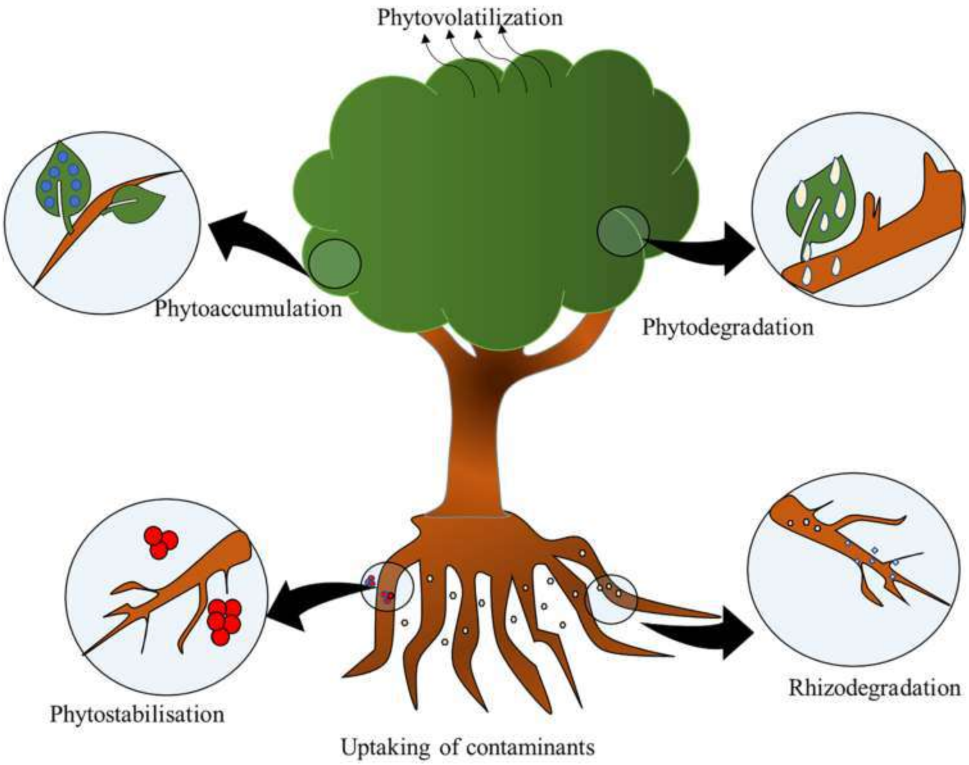


Figure 16.3 Contaminant uptake and degradation through different biological processes of phytoremediation.

16.4 OTHER TECHNOLOGICAL ADVANCEMENTS IN BIOREMEDIATION

Bioremediation is considered a feasible technology for the degradation of pollutants in soil and water. However, the rate and degree of deterioration of contaminants may often be constrained due to their limited solubility, and high hydrophobicity. In recent years, to overcome such problems many new promising hybrid technologies such as electro-bioremediation, nano remediation, and constructed wetlands (CWs) have been implemented in the cleanup of water/soil polluted with refractory contaminants. In the below section, the new techniques have been discussed.

16.4.1 Electro-bioremediation

A key microbial electrochemical technology (MET) that is emerging as a viable alternative for treating groundwater is electro-bioremediation (Ceballos-Escalera *et al.*, 2021). MET is based on bio-electrochemical systems that use solid electron conductors (e.g., electrodes) to execute oxidation and reduction processes by electroactive microorganisms. Thus, the anode and cathode can be used by these electroactive microorganisms as an endless source of electron acceptor or donor, respectively. Direct current (DC) is supplied across electrodes introduced into the polluted soil during the electro-bioremediation procedure for the generation of an electric field for boosting bioremediation efficiency (Li *et al.*, 2020). The structure of microbial population and activity is disturbed by electrochemical reactions and electrokinetic processes. Li *et al.* (2020) showed the outcome of an electric field on the microbes of soil through the examination of changes in the extent of PAH degradation and microorganisms. The outcomes indicated that the microbial activity and population structure was influenced by the intensity of voltage. In this study, the optimal electric strength for enhancing the activity of microbes and further degradation of PAHs was found to be 2 V/cm (voltage gradient). The microbial population structure was influenced by the voltage gradient as well as the electrode reaction. For efficient use of electro-bioremediation, choosing a suitable electric field that has no adverse effects on microbes and will encourage microbes to use their maximum degrading activity is crucial. Hence, to choose a suitable electric field, it is necessary to understand the changes in the microbe community and activity under different electric fields. Understanding the changes in microorganism communities and activity under different electric fields is a requirement for choosing a suitable electric field. Recently, the effects of electric fields on microorganisms have been studied thoroughly, such as polarity reversal, electrode configuration and the electric field strength (or current) (Barba *et al.*, 2017; Li *et al.*, 2015). Soil or water contaminated with refractory pollutants electro-bioremediation is a promising technology for remediation (Figure 16.4).

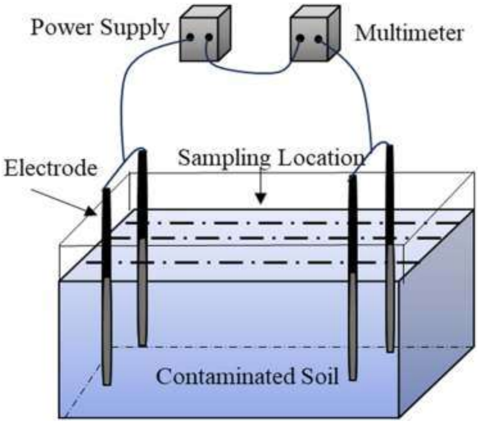


Figure 16.4 Schematic representation of experimental set-up of electro-bioremediation.

16.4.2 Nano bioremediation

The term 'nano remediation' refers to the use of nanoscale materials for the cleanup of the environment, and it provides quick, low-cost, and effective solutions for the remediation of affected sites (Kahraman *et al.*, 2022). Nanoparticles such as zero-valent iron (Karthick *et al.*, 2019) iron oxide (Karthick *et al.*, 2019) and silica (Chattopadhyay & Karthick, 2017) have been reported to be efficient for soil remediation. The advancement of nanotechnology and the incorporation of nanomaterials – defined as particles with sizes of 100 nm or less in at least one dimension and particular nanoparticles – signify a ground-breaking strategy to advance bioremediation beyond its limitations. This integrated method comprises a broad range of potential applications with less costs and the least negative impacts on the environment for treating pollutants in groundwater and wastewater (Cecchin *et al.*, 2017), residues contaminated with heavy metals and hydrocarbons (Darwesh *et al.*, 2021) and either organic or inorganic amalgams in soil (Cecchin *et al.*, 2017). Several studies showed that nanoparticles possess distinct properties including enhanced catalysis and adsorption as well as increased reactivity. Previously, only physical and chemical processes were used to create nanoparticles. Howbeit, in search of sustainable methods for the production microorganisms and plant extracts are being utilized. In a study, iron nanoparticles were mycosynthesized using *Fusarium oxysporum*, size ranging from 1 to 3 nm. This nanoparticle was significantly successful as an antimicrobial and adsorbing agent and was used for the redressal of municipal wastewater (Darwesh *et al.*, 2021). It is evident that this technique is more intelligent, safe, ecologically friendly, inexpensive, and green than either technology alone, the field of environmental remediation may be greatly altered in the long run by this amalgamation of the two technologies, known as nano-bioremediation.

16.4.3 Constructed wetlands

CWs are artificial, human-made wetlands that are created and designed to imitate natural wetland systems for the treatment of water. Intricate physical, chemical, and biological processes are used by these systems, which are mostly composed of vegetation, substrates, soils, microorganisms, and water, to remove various contaminants or improve the quality of the water. CW can be classified into two types according to wetland hydrology: free water surface (FWS) and subsurface flow (SSF). FWA is similar to a natural wetland, while in the SSF system, water flows vertically or horizontally through the substrates. SSF can further be categorized into vertical and horizontal flow. Early in the 1950s, Käthe Seidel made the initial attempt to explore the potential of CWs for wastewater treatment in Germany. Subsequent CW studies were conducted and used for wastewater treatments in the 1960s and 1970s. The application of CWs was initially applied to remediate conventional domestic and municipal wastewater. Currently, the use of CWs has been significantly increased to clear out industrial and agricultural effluents, mine drainage, landfill leachates, contaminated river and lake waters, and urban and highway runoff. It has also been developed in a variety of climatic conditions, including tropical, arid, and warm climates (Wu *et al.*, 2014). FWS CWs are more effective at removing organics and suspended particles than SSF CWs are at removing nitrogen and phosphorus. However, their therapeutic effectiveness and sustainable use are typically constrained in cold weather or after the degradation of the plant (Vymazal, 2011). In comparison to FWS CWs, SSF CWs are very effective in the removal of organics, microbial pollution, suspended solids, and heavy metals, and they are less cold-sensitive and easier to insulate for winter operation. However, these CWs are permanently underwater, and the ability to remove nitrogen depends on the availability of oxygen and carbon sources. In addition, without the use of specialized media with high sorption capacities, poor phosphorus removal is frequently obtained (Babatunde *et al.*, 2010). The remediating effect of macrophytes in CWs depends on how well the species fits the particular climatic and operational circumstances as well as on the species' morphological features and has been the subject of intensive research over the last three decades (Qadiri *et al.*, 2021). Qadiri *et al.* (2021) experimentally tested and compared the involvement of three macrophytes in eliminating toxic compounds from combined wastewater (graywater and septic effluent) using horizontal sub-surface flow type constructed wetlands

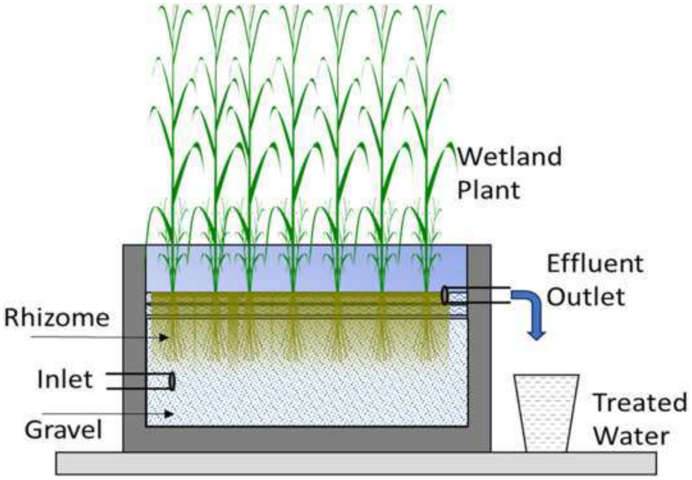


Figure 16.5 Schematic representation of constructed wetland system for wastewater treatment.

(HSSF-CW) in sub-tropical conditions. They monitored the standard wastewater parameters (COD, nitrogen, phosphorus, and solids) in the inlet and outlet of the CW microcosms, the fate of nutrients through the planted microcosms and the growth rate of the selected species. *Phragmites karka* exhibited better removal efficiencies with organic loading removal rates of 10 ± 1.3/g^2/d, nitrogen removal rates of 2.6 ± 0.4/g^2/d, phosphorus removal rates of 1.3 ± 0.2/g^2/d, and solids removal rates of 3.6 ± 1.2/g^2/d. This showed that in CWs, macrophytes with well-developed root systems have a greater ability to remove pollutants. Considering the successful and sustainable implementation of CWs, future research must concentrate on inclusive assessment of plants and substrates in field trials under real-world situations, optimization of environmental and operational parameters (Figure 16.5).

16.5 CHALLENGES AND PROSPECT

Bioremediation has emerged as a cost-effective alternative to remediation technologies. With advantages, they have several disadvantages such as harming the environment by producing secondary pollutants and having a detrimental impact on the ecosystem. From the aforementioned, it is obvious that bioremediation strategies are varied and have successfully restored sites that have been contaminated with various pollutants. Microorganisms are essential to the process of bioremediation. The variety, abundance, and population structure of microbes in contaminated environments provide a better understanding of the outcome of these strategies, as long as the other environmental factors that could hinder microbial activities that are kept at optimum levels (Azubuike *et al.*, 2016). Because some substances cannot be broken down by biological processes, bioremediation is severely constrained. If a material is biodegradable at all, it may in some situations undergo further processing and degradation that results in the production of harmful material. Due to the occurrence of some site-specific limiting variables, a specific bacterial strain that is effective at one site may occasionally fail to function well at other sites. The nature of toxic compounds, the presence of an optimum amount of nutrients, and the metabolic activities of microorganisms are thought to contribute to the complication of biological processes. The method is more time-taking and labor-intensive because it calls for soil excavation, construction, and modification of a specialized site plan (Vishwakarma *et al.*, 2020). Most of the time, the process takes place in remote, underground locations far from populated areas. In the case of soil bioremediation, aging is one of the greatest obstacles faced during remediation. Hence, a proper solution to exploit the bioavailability of pollutants in soil arises. Likewise in groundwater remediation, the geological settings need to be understood (Vishwakarma *et al.*, 2020).

The scientific community's persistent hard work in this field has been successful in pushing the envelope. It is anticipated that the rapidly evolving bioaugmentation technologies will improve our ability to control a cell's genetic makeup and help us overcome limits brought on by microbiological variables. New techniques for bioremediation can be developed with a deeper understanding of biotransformation at the ecological and genetic levels. Newer strategies are being developed to enhance the bioavailability of microbes to add value to the efficiency of bioremediation. The main goal is to assess the applicability of a theoretically suggested solution to guarantee that the cleanup objectives are achieved. Based on their understanding of molecular biology, researchers are also exhibiting a propensity to create improved techniques that are predicted to be accurate, efficient, and affordable. The *in-situ* characterization of the physiochemical parameters also appears promising and has the potential to transform field evaluation procedures in the future

16.6 CONCLUSION

The main problem of the era is pollution, and scientific societies are dedicating a major deal of attention to this problem. A microbial remediation is a powerful tool for enhancing the natural biodegradation processes, as microorganisms acclimatize speedily with lethal environments. For the formation of an ecologically stable, new, and feasible bioremediation approach, a thorough understanding of the microbial populations and their response to the environment and to contaminants is crucial. It is now generally known that many microorganisms are capable of degrading toxins found in the environment. The use of bioremediation to clean up contaminated soils and aquifers areas has become increasingly common. Furthermore, the applications of new emerging techniques could be a game changer along with the study of genetically modified microbes. A commonly recognized substitute for environmental cleanup is bioremediation, particularly because it promises to do so economically and with fewer risks of transmitting pollutants to other media.

REFERENCES

Abedin M. J., Feldmann J. and Meharg A. A. (2002). Uptake kinetics of arsenic species in rice plants. *Plant Physiology*, **128**(3), 1120–1128, https://doi.org/10.1104/pp.010733

Afrin S., Shuvo H. R., Sultana B., Islam F., Rus'd A. A., Begum S. and Hossain M. N. (2021). The degradation of textile industry dyes using the effective bacterial consortium. *Heliyon*, **7**(10), e08102, https://doi.org/10.1016/j.heliyon.2021.e08102

Agrawal N., Verma P. and Shahi S. K. (2018). Degradation of polycyclic aromatic hydrocarbons (phenanthrene and pyrene) by the ligninolytic fungi *Ganoderma lucidum* isolated from the hardwood stump. *Bioresources and Bioprocessing*, **5**(1), 1–9, https://doi.org/10.1186/s40643-018-0197-5

Ahmad K. S. (2020). Remedial potential of bacterial and fungal strains (*Bacillus subtilis*, *Aspergillus niger*, *Aspergillus flavus and Penicillium chrysogenum*) against organochlorine insecticide Endosulfan. *Folia Microbiologica*, **65**(5), 801–810, https://doi.org/10.1007/s12223-020-00792-7

Akhtar N. and Mannan M. A. (2020a). Mycoremediation: expunging environmental pollutants. *Biotechnology Reports*, **26**, e00452, https://doi.org/10.1016/j.btre.2020.e00452

Akhtar N. and Mannan M. A. U. (2020b). Mycoremediation: an unexplored gold mine. In: New and Future Developments in Microbial Biotechnology and Bioengineering: Recent Advances in Application of Fungi and Fungal Metabolites: Environmental and Industrial Aspects, J. Singh and P. Gehlot (eds). Elsevier BV, Amsterdam, Netherlands, https://doi.org/10.1016/B978-0-12-821007-9.00002-4

Al-Tohamy R., Ali S. S., Li F., Okasha K. M., Mahmoud Y. A. G., Elsamahy T., Jiao H., Fu Y. and Sun J. (2022). A critical review on the treatment of dye-containing wastewater: ecotoxicological and health concerns of textile dyes and possible remediation approaches for environmental safety. *Ecotoxicology and Environmental Safety*, **231**, 113160, https://doi.org/10.1016/j.ecoenv.2021.113160

Ali H., Khan E. and Ilahi I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, **2019**, 1–14, https://doi.org/10.1155/2019/6730305

Amulya K., Reddy M. V., Rohit M. V. and Mohan S. V. (2016). Wastewater as renewable feedstock for bioplastics production: understanding the role of reactor microenvironment and system pH. *Journal of Cleaner Production*, **112**, 4618–4627, https://doi.org/10.1016/j.jclepro.2015.08.009

Azubuike C. C., Chikere C. B. and Okpokwasili G. C. (2016). Bioremediation techniques – classification based on site of application: principles, advantages, limitations and prospects. *World Journal of Microbiology and Biotechnology*, **32**(11), 1–18, https://doi.org/10.1007/s11274-016-2137-x

Babatunde A. O., Zhao Y. Q. and Zhao X. H. (2010). Alum sludge-based constructed wetland system for enhanced removal of P and OM from wastewater: concept, design and performance analysis. *Bioresource Technology*, **101**(16), 6576–6579, https://doi.org/10.1016/j.biortech.2010.03.066

Bankole P. O., Adekunle A. A., Jeon B. H. and Govindwar S. P. (2020). Novel cobiomass degradation of NSAIDs by two wood rot fungi, *Ganoderma applanatum* and *Laetiporus sulphureus*: ligninolytic enzymes induction, isotherm and kinetic studies. *Ecotoxicology and Environmental Safety*, **203**, 110997, https://doi.org/10.1016/j.ecoenv.2020.110997

Barba S., Villaseñor J., Rodrigo M. A. and Cañizares P. (2017). Effect of the polarity reversal frequency in the electrokinetic-biological remediation of oxyfluorfen polluted soil. *Chemosphere*, **177**, 120–127, https://doi.org/10.1016/j.chemosphere.2017.03.002

Bhadouria R., Das S., Kumar A., Singh R. and Singh V. K. (2020). Mycoremediation of agrochemicals. In: Agrochemicals Detection, Treatment and Remediation: Pesticides and Chemical Fertilizers, Majeti N. and V. Prasad (eds). Butterworth-Heinemann, UK, LTD, https://doi.org/10.1016/B978-0-08-103017-2.00022-2

Bilen Ozyurek S., Avcioglu N. H. and Seyis Bilkay I. (2021). Mycoremediation potential of *Aspergillus ochraceus* NRRL 3174. *Archives of Microbiology*, **203**(10), 5937–5950, https://doi.org/10.1007/s00203-021-02490-5

Ceballos-Escalera A., Pous N., Chiluiza-Ramos P., Korth B., Harnisch F., Bañeras L., Balaguer M. D. and Puig S. (2021). Electro-bioremediation of nitrate and arsenite polluted groundwater. *Water Research*, **190**, 116748, https://doi.org/10.1016/j.watres.2020.116748

Cecchin I., Reddy K. R., Thomé A., Tessaro E. F. and Schnaid F. (2017). Nanobioremediation: integration of nanoparticles and bioremediation for sustainable remediation of chlorinated organic contaminants in soils. *International Biodeterioration and Biodegradation*, **119**, 419–428, https://doi.org/10.1016/j.ibiod.2016.09.027

Cen Q., Wu X., Cao L., Lu Y., Lu X., Chen J., Fu G., Liu Y. and Ruan R. (2022). Green production of a yellow laccase by *Coriolopsis gallica* for phenolic pollutants removal. AMB Express, Vol. **12**, pp. 96–111, https://doi.org/10.1186/s13568-022-01434-6

Chandanshive V., Kadam S., Rane N., Jeon B. H., Jadhav J. and Govindwar S. (2020). In situ textile wastewater treatment in high rate transpiration system furrows planted with aquatic macrophytes and floating phytobeds. *Chemosphere*, **252**, 126513, https://doi.org/10.1016/j.chemosphere.2020.126513

Chattopadhyay P. and Karthick R. A. (2017). Characterization and application of surfactant foams produced from ethanol-sodium lauryl sulfate-silica nanoparticle mixture for soil remediation. *Macromolecular Symposia*, **376**(1), 1–4, https://doi.org/10.1002/masy.201600182

Darwesh O. M., Shalaby M. G., Abo-Zeid A. M. and Mahmoud Y. A. G. (2021). Nano-bioremediation of municipal wastewater using myco-synthesized iron nanoparticles. *Egyptian Journal of Chemistry*, **64**(5), 2499–2507, https://doi.org/10.21608/EJCHEM.2021.60921.3307

De Donno Novelli L., Moreno Sayavedra S. and Rene E. R. (2021). Polyhydroxyalkanoate (PHA) production via resource recovery from industrial waste streams: a review of techniques and perspectives. *Bioresource Technology*, **331**, 1–13, https://doi.org/10.1016/j.biortech.2021.124985

Dey P., Malik A., Mishra A., Kumar D., Von Bergen M. and Jehmlich N. (2020). Mechanistic insight to mycoremediation potential of a metal resistant fungal strain for removal of hazardous metals from multimetal pesticide matrix. *Environmental Pollution*, **262**, 114255, https://doi.org/10.1016/j.envpol.2020.114255

Dhananjayan V., Jayanthi P., Jayakumar S. and Ravichandran B. (2020). Agrochemicals impact on ecosystem and bio-monitoring. In: Resources use Efficiency in Agriculture, S. Kumar, R. S. Meena, and M. K. Jhariya (eds), Springer, Singapore, https://doi.org/10.1007/978-981-15-6953-1_11

Dias C., Reis A., Santos J. A. L. and Lopes da Silva T. (2020). Concomitant wastewater treatment with lipid and carotenoid production by the oleaginous yeast *Rhodosporidium toruloides* grown on brewery effluent enriched with sugarcane molasses and urea. *Process Biochemistry*, **94**, 1–14, https://doi.org/10.1016/j.procbio.2020.03.015

Erkan H. S. and Engin G. O. (2017). The investigation of paper mill industry wastewater treatment and activated sludge properties in a submerged membrane bioreactor. *Water Science and Technology*, **76**(7), 1715–1725, https://doi.org/10.2166/wst.2017.351

Gangola S., Joshi S., Kumar S. and Pandey S. C. (2019). Comparative analysis of fungal and bacterial enzymes in biodegradation of xenobiotic compounds. Smart Bioremediation Technologies: Microbial Enzymes, 169–189, https://doi.org/10.1016/B978-0-12-818307-6.00010-X

Gatidou G., Oursouzidou M., Stefanatou A. and Stasinakis A. S. (2017). Removal mechanisms of benzotriazoles in duckweed *Lemna minor* wastewater treatment systems. *Science of the Total Environment*, **596–597**, 12–17, https://doi.org/10.1016/j.scitotenv.2017.04.051

Godlewska-Zyłkiewicz B., Sawicka S. and Karpińska J. (2019). Removal of platinum and palladium from wastewater by means of biosorption on fungi *Aspergillus* sp. and yeast *Saccharomyces* sp. *Water (Switzerland)*, **11**(7), 1–17, https://doi.org/10.3390/w11071522

Gupta P. K. (2019). Concepts and Applications in Veterinary Toxicology – an Interactive Guide | Pawan Kumar Gupta. Springer, Switzerland, https://doi.org/10.1007/978-3-030-22250-5%0A

Haripriyan U., Gopinath K. P., Arun J. and Govarthanan M. (2022). Bioremediation of organic pollutants: a mini review on current and critical strategies for wastewater treatment. *Archives of Microbiology*, **204**(5), 1–9, https://doi.org/10.1007/s00203-022-02907-9

Jobby R., Jha P., Yadav A. K. and Desai N. (2018). Biosorption and biotransformation of hexavalent chromium [Cr(VI)]: a comprehensive review. *Chemosphere*, **207**, 255–266, https://doi.org/10.1016/j.chemosphere.2018.05.050

Justin M. Z., Pajk N., Zupanc V. and Zupančič M. (2010). Phytoremediation of landfill leachate and compost wastewater by irrigation of *Populus* and *Salix*: biomass and growth response. *Waste Management*, **30**(6), 1032–1042, https://doi.org/10.1016/j.wasman.2010.02.013

Kahraman B. F., Altin A. and Ozdogan N. (2022). Remediation of Pb-diesel fuel co-contaminated soil using nano/bio process: subsequent use of nanoscale zero-valent iron and bioremediation approaches. *Environmental Science and Pollution Research*, **29**(27), 41110–41124, https://doi.org/10.1007/s11356-022-18857-0

Kara M., Oztas E., Ramazanoğulları R., Kouretas D., Nepka C., Tsatsakis A. M. and Veskoukis A. S. (2020). Benomyl, a benzimidazole fungicide, induces oxidative stress and apoptosis in neural cells. *Toxicology Reports*, **7**, 501–509, https://doi.org/10.1016/j.toxrep.2020.04.001

Karthick A., Roy B. and Chattopadhyay P. (2019). Comparison of zero-valent iron and iron oxide nanoparticle stabilized alkyl polyglucoside phosphate foams for remediation of diesel-contaminated soils. *Journal of Environmental Management*, **240**, 93–107, https://doi.org/10.1016/j.jenvman.2019.03.088

Khan S. and Malik A. (2018). Toxicity evaluation of textile effluents and role of native soil bacterium in biodegradation of a textile dye. *Environmental Science and Pollution Research*, **25**(5), 4446–4458, https://doi.org/10.1007/s11356-017-0783-7

Khan H. K., Rehman M. Y. A. and Malik R. N. (2020). Fate and toxicity of pharmaceuticals in water environment: an insight on their occurrence in South Asia. *Journal of Environmental Management*, **271**, 111030, https://doi.org/10.1016/j.jenvman.2020.111030

Khandare R. V. and Govindwar S. P. (2015). Phytoremediation of textile dyes and effluents: current scenario and future prospects. *Biotechnology Advances*, **33**(8), 1697–1714, https://doi.org/10.1016/j.biotechadv.2015.09.003

Kishor R., Purchase D., Saratale G. D., Ferreira L. F. R., Bilal M., Iqbal H. M. N. and Bharagava R. N. (2021a). Environment friendly degradation and detoxification of Congo red dye and textile industry wastewater by a newly isolated *Bacillus cohnni* (RKS9). *Environmental Technology and Innovation*, **22**, 101425, https://doi.org/10.1016/j.eti.2021.101425

Kishor R., Purchase D., Saratale G. D., Saratale R. G., Ferreira L. F. R., Bilal M., Chandra R. and Bharagava R. N. (2021b). Ecotoxicological and health concerns of persistent coloring pollutants of textile industry wastewater and treatment approaches for environmental safety. *Journal of Environmental Chemical Engineering*, ***9***(2), 105012, https://doi.org/10.1016/j.jece.2020.105012

Kishor R., Saratale G. D., Saratale R. G., Romanholo Ferreira L. F., Bilal M., Iqbal H. M. N. and Bharagava R. N. (2021c). Efficient degradation and detoxification of methylene blue dye by a newly isolated ligninolytic enzyme producing bacterium *Bacillus albus* MW407057. *Colloids and Surfaces B: Biointerfaces*, **206**, 111947, https://doi.org/10.1016/j.colsurfb.2021.111947

Kour D., Kaur T., Devi R., Yadav A., Singh M., Joshi D., Singh J., Suyal D. C., Kumar A., Rajput V. D., Yadav A. N., Singh K., Singh J., Sayyed R. Z., Arora N. K. and Saxena A. K. (2021). Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: present status and future challenges. *Environmental Science and Pollution Research*, **28**(20), 24917–24939, https://doi.org/10.1007/s11356-021-13252-7

Kulshreshtha S., Mathur N. and Bhatnagar P. (2014). Mushroom as a product and their role in mycoremediation. *AMB Express*, **4**(1), 1–7, https://doi.org/10.1186/s13568-014-0029-8

Kumar L. and Bharadvaja N. (2020). Microbial remediation of heavy metals. In: Microbial Bioremediation & Biodegradation, M. P. Shah (ed.), Springer, Singapore, https://doi.org/10.1007/978-981-15-1812-6_2

Kumar V. and Dwivedi S. K. (2021). Mycoremediation of heavy metals: processes, mechanisms, and affecting factors. *Environmental Science and Pollution Research*, **28**(9), 10375–10412, https://doi.org/10.1007/s11356-020-11491-8

Kumar V., Thakur I. S. and Shah M. P. (2020). Bioremediation approaches for treatment of pulp and paper industry wastewater: recent advances and challenges. In: Microbial Bioremediation & Biodegradation, M. P. Shah (ed.), pp. 1–48, https://doi.org/10.1007/978-981-15-1812-6_1

Kwon H. C., Sohn H., Kim D. H., Jeong C. H., Kim D. W. and Han S. G. (2021). Effects of flutriafol fungicide on the lipid accumulation in human liver cells and rat liver. *Foods*, **10**(6), 1–19, https://doi.org/10.3390/foods10061346

Li T., Guo S., Wu B., Zhang L. and Gao Y. (2015). Effect of polarity-reversal and electrical intensity on the oil removal from soil. *Journal of Chemical Technology and Biotechnology*, **90**(3), 441–448, https://doi.org/10.1002/jctb.4312

Li W., Jia M. X., Deng J., Wang J. H., Lin Q. L., Liu C., Wang S. S., Tang J. X., Zeng X. X., Ma L., Su W., Liu X. Y., Cai F. and Zhou L. Y. (2018). Isolation, genetic identification and degradation characteristics of COD-degrading bacterial strain in slaughter wastewater. *Saudi Journal of Biological Sciences*, **25**(8), 1800–1805, https://doi.org/10.1016/j.sjbs.2018.08.022

Li F., Guo S., Wang S. and Zhao M. (2020). Changes of microbial community and activity under different electric fields during electro-bioremediation of PAH-contaminated soil. *Chemosphere*, **254**, 126880, https://doi.org/10.1016/j.chemosphere.2020.126880

Lindholm-Lehto P. C., Knuutinen J. S., Ahkola H. S. J. and Herve S. H. (2015). Refractory organic pollutants and toxicity in pulp and paper mill wastewaters. *Environmental Science and Pollution Research*, **22**(9), 6473–6499, https://doi.org/10.1007/s11356-015-4163-x

Luo Q., Wang J., Wang J. H., Shen Y., Yan P., Chen Y. P. and Zhang C. C. (2019). Fate and occurrence of pharmaceutically active organic compounds during typical pharmaceutical wastewater treatment. *Journal of Chemistry*, **2019**, pp. 1–12, https://doi.org/10.1155/2019/2674852

Magnoli K., Carranza C. S., Aluffi M. E., Magnoli C. E. and Barberis C. L. (2020). Herbicides based on 2,4-D: its behavior in agricultural environments and microbial biodegradation aspects. A review. *Environmental Science and Pollution Research*, **27**(31), 38501–38512, https://doi.org/10.1007/s11356-020-10370-6

Mahajan P., Kaushal J., Upmanyu A. and Bhatti J. (2019). Assessment of phytoremediation potential of *Chara vulgaris* to treat toxic pollutants of textile effluent. *Journal of Toxicology*, **2019**, pp. 1–11, https://doi.org/10.1155/2019/8351272

Mandeep Gupta G. K., Liu H. and Shukla P. (2019). Pulp and paper industry-based pollutants, their health hazards and environmental risks. *Current Opinion in Environmental Science and Health*, **12**, 48–56, https://doi.org/10.1016/j.coesh.2019.09.010

Medfu Tarekegn M., Zewdu Salilih F. and Ishetu A. I. (2020). Microbes used as a tool for bioremediation of heavy metal from the environment. *Cogent Food and Agriculture*, **6**(1), pp. 1–19, https://doi.org/10.1080/23311932.2020.1783174

Nayak S. K., Dash B. and Baliyarsingh B. (2018). Microbial remediation of persistent agro-chemicals by soil bacteria: an overview. *Microbial Biotechnology*, **2**, 275–301, https://doi.org/10.1007/978-981-10-7140-9_13

Obire O., Aleruchi O. and Wemedo S.A. (2020). Fungi in biodegradation of polycyclic aromatic hydrocarbons in oilfield wastewater. *Acta Scientific Microbiology*, **3**(4), 220–224, https://doi.org/10.31080/asmi.2020.03.0572

Pandey C., Prabha D. and Negi Y. K. (2018). Mycoremediation of common agricultural pesticides. 155–179, https://doi.org/10.1007/978-3-319-77386-5_6

Piątkowska M., Jedziniak P., Olejnik M., Żmudzki J. and Posyniak A. (2018). Absence of evidence or evidence of absence? A transfer and depletion study of Sudan I in eggs. *Food Chemistry*, **239**, 598–602, https://doi.org/10.1016/j.foodchem.2017.06.156

Pironti C., Ricciardi M., Proto A., Bianco P. M., Montano L. and Motta O. (2021). Endocrine-disrupting compounds: an overview on their occurrence in the aquatic environment and human exposure. *Water (Switzerland)*, **13**(10), 1–32, https://doi.org/10.3390/w13101347

Polińska W., Kotowska U., Kiejza D. and Karpińska J. (2021). Insights into the use of phytoremediation processes for the removal of organic micropollutants from water and wastewater; a review. *Water (Switzerland)*, **13**(15), pp. 1–19, https://doi.org/10.3390/w13152065

Qadir I. and Chhipa R. (2015). Critical evaluation of some available treatment techniques for textile & paper industry effluents: a review. *American Chemical Science Journal*, **6**(2), 77–90, https://doi.org/10.9734/acsj/2015/15484

Qadiri R. Z. Z., Gani K. M., Zaid A., Aalam T., Kazmi A. A. and Khalil N. (2021). Comparative evaluation of the macrophytes in the constructed wetlands for the treatment of combined wastewater (greywater and septic tank effluent) in a sub-tropical region. *Environmental Challenges*, **5**, 100265, https://doi.org/10.1016/j.envc.2021.100265

Rai P. K., Kim K. H., Lee S. S. and Lee J. H. (2020). Molecular mechanisms in phytoremediation of environmental contaminants and prospects of engineered transgenic plants/microbes. *Science of the Total Environment*, **705**, 135858, https://doi.org/10.1016/j.scitotenv.2019.135858

Rajhans G., Barik A., Sen S. K., Masanta A., Sahoo N. K. and Raut S. (2021). Mycoremediation and toxicity assessment of textile effluent pertaining to its possible correlation with COD. *Scientific Reports*, **11**(1), 1–11, https://doi.org/10.1038/s41598-021-94666-8

Sadañoski M. A., Benítez S. F., Fonseca M. I., Velázquez J. E., Zapata P. D., Levin L. N. and Villalba L. L. (2019). Mycoremediation of high concentrations of polychlorinated biphenyls with *Pleurotus sajor-caju* LBM 105 as an effective and cheap treatment. *Journal of Environmental Chemical Engineering*, **7**(6), 1–28, https://doi.org/10.1016/j.jece.2019.103453

Sharma J., Kumar V., Kumar S. S., Malyan S. K., Mathimani T., Bishnoi N. R. and Pugazhendhi A. (2020). Microalgal consortia for municipal wastewater treatment – lipid augmentation and fatty acid profiling for biodiesel production. *Journal of Photochemistry and Photobiology B: Biology*, **202**, 111638, https://doi.org/10.1016/j.jphotobiol.2019.111638

Sharma P., Sirohi R., Tong Y. W., Kim S. H. and Pandey A. (2021). Metal and metal(loids) removal efficiency using genetically engineered microbes: applications and challenges. *Journal of Hazardous Materials*, **416**, 125855, https://doi.org/10.1016/j.jhazmat.2021.125855

Singh A. K. and Chandra R. (2019). Pollutants released from the pulp paper industry: aquatic toxicity and their health hazards. *Aquatic Toxicology*, **211**, 202–216, https://doi.org/10.1016/j.aquatox.2019.04.007

Singh J., Sharma D., Kumar G. and Sharma N. R. (2018). Microbial bioprospecting for sustainable development. Microbial bioprospecting for sustainable development. 1–397, https://doi.org/10.1007/978-981-13-0053-0

Singh J., Kumar V., Kumar P., Kumar P., Yadav K. K., Cabral-Pinto M. M. S., Kamyab H. and Chelliapan S. (2021). An experimental investigation on phytoremediation performance of water lettuce (*Pistia stratiotes* L.) for pollutants removal from paper mill effluent. *Water Environment Research*, **93**(9), 1543–1553, https://doi.org/10.1002/wer.1536

Vašinková M., Dlabaja M. and Kučová K. (2021). Bioaccumulation of toxic metals by fungi of the genus *Aspergillus* isolated from the contaminated area of Ostramo Lagoons. *IOP Conference Series: Earth and Environmental Science*, **900**(1), 1–9, https://doi.org/10.1088/1755-1315/900/1/012048

Vishwakarma G. S., Bhattacharjee G., Gohil N. and Singh V. (2020). Current status, challenges and future of bioremediation. In: Bioremediation of Pollutants: from Genetic Engineering to Genome Engineering, V. C. Pandey and V. Singh (eds), Elsevier, UK. INC, https://doi.org/10.1016/B978-0-12-819025-8.00020-X

Vymazal J. (2011). Plants used in constructed wetlands with horizontal subsurface flow: a review. *Hydrobiologia*, **674**(1), 133–156, https://doi.org/10.1007/s10750-011-0738-9

Wang R., Li F., Ruan W., Tai Y., Cai H. and Yang Y. (2020). Removal and degradation pathway analysis of 17-estradiol from raw domestic wastewater using immobilised functional microalgae under repeated loading. *Biochemical Engineering Journal*, **107700**, 1–31, https://doi.org/10.1016/j.bej.2020.107700

Wen X. J., Niu C. G., Zhang L., Liang C. and Zeng G. M. (2017). An in depth mechanism insight of the degradation of multiple refractory pollutants via a novel SrTiO3/BiOI heterojunction photocatalysts. *Journal of Catalysis*, **356**, 283–299, https://doi.org/10.1016/j.jcat.2017.10.022

Wu S., Kuschk P., Brix H., Vymazal J. and Dong R. (2014). Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. *Water Research*, **57**, 40–55, https://doi.org/10.1016/j.watres.2014.03.020

Xie B., Tang X., Yong H., Deng S. and Shi X. (2020). Biological sulfamethoxazole degradation along with anaerobically digested centrate treatment by immobilized microalgal-bacterial consortium: performance, mechanism and shifts in bacterial and microalgal communities. *Chemical Engineering Journal*, **388**, 124217, https://doi.org/10.1016/j.cej.2020.124217

Xu M., Wu M., Zhang Y., Zhang H., Liu W., Chen G., Xiong G. and Guo L. (2022). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by bacterial mixture. *International Journal of Environmental Science and Technology*, **19**(5), 3833–3844, https://doi.org/10.1007/s13762-021-03284-4

Yu J., Shu S., Wang Q., Gao N. and Zhu Y. (2022). Evaluation of Fe^{2+}/peracetic acid to degrade three typical refractory pollutants of textile wastewater. *Catalysts*, **12**(7), 684, https://doi.org/10.3390/catal12070684

Zainith S., Purchase D., Saratale G. D., Ferreira L. F. R., Bilal M. and Bharagava R. N. (2019). Isolation and characterization of lignin-degrading bacterium *Bacillus aryabhattai* from pulp and paper mill wastewater and evaluation of its lignin-degrading potential. *3 Biotech*, **9**(3), 1–11, https://doi.org/10.1007/s13205-019-1631-x

Zhang H., Yuan X., Xiong T., Wang H. and Jiang L. (2020). Bioremediation of co-contaminated soil with heavy metals and pesticides: influence factors, mechanisms and evaluation methods. *Chemical Engineering Journal*, **398**, 125657, https://doi.org/10.1016/j.cej.2020.125657

Zhang J., Cao X., Yao Z., Lin Q., Yan B., Cui X., He Z., Yang X., Wang C. H. and Chen G. (2021). Phytoremediation of Cd-contaminated farmland soil via various *Sedum alfredii*-oilseed rape cropping systems: efficiency comparison and cost-benefit analysis. *Journal of Hazardous Materials*, **419**, 126489, https://doi.org/10.1016/j.jhazmat.2021.126489

Increasing global population growth and the continued exploitation of land, water and other natural resources is causing the release of harmful gases and toxic compounds into the environment. To combat this pollution and mitigate its impact, a number of physicochemical, biochemical, and biotechnological approaches have been developed. Each chapter of this book focuses on one specific technology, discussing its principle, methodology, recent advances, limitations, technological and economic feasibility, and its prospects for the future.

Cover images: zooplankton, biogas plant, water hyacinth

iwapublishing.com
@IWAPublishing

ISBN 9781789063776 (paperback)
ISBN 9781789063783 (eBook)
ISBN 9781789063790 (ePub)

ISBN 9781789063776
9 781789 063776