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Clean Technologies Toward a Sustainable Future

Physicochemical, Biochemical and Biotechnological Approaches

Pradeep Verma and Maulin P Shah

Clean Technologies Toward the Development of a Sustainable Environment and Future

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Physicochemical, Biochemical, and Biotechnological approaches

Edited by

Pradeep Verma and Maulin P. Shah

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The Editors

Professor Pradeep Verma

Professor Verma completed his Ph.D. from Sardar Patel University Gujarat, India in 2002. In the same year, he was selected as a UNESCO fellow and joined the Czech Academy of Sciences Prague, Czech Republic. He later moved to Charles University, Prague to work as postdoctoral fellow. In 2004 he worked as a visiting scientist at UFZ Centre for Environmental Research, Halle, Germany. He was awarded a DFG fellowship that provided him with another opportunity to work as a postdoctoral fellow at Gottingen University, Germany. Later in 2007 he returned to India and joined Reliance Life Sciences, Mumbai, and worked extensively on biobutanol production, which attributed a few patents to his name. Later he was awarded JSPS Post-Doctoral Fellowship Programme and joined the laboratory of Biomass Conversion, Research Institute of Sustainable Humanosphere (RISH), Kyoto University, Japan. Professor Verma has also been the recipient of various prestigious awards such as the Ron Cockcroft award by Swedish society, and UNESCO Fellow ASCR Prague. Recently for his contribution to the area of fungal microbiology, industrial biotechnology, and environmental bioremediations, he has been awarded the prestigious Fellow Award from Mycological Society of India (2020), P.C. Jain Memorial Award (MSI), and Biotech Research Society of India (2021). In 2020 he was also awarded fellow of Biotechnology Research Society of India (BRSI) and Fellow of Academy of Sciences of AMI India 2021 (FAMSc). Furthermore, he has also been awarded the JSPS Bridge Fellow award in 2022 and a short-term visit to Kyoto University, Kyoto, Japan to strengthen ties between the two laboratories.

Professor Verma in 2009 began his independent academic career as a reader and founder head at the Department of Microbiology, Assam University. In 2011 he moved to the Department of Biotechnology at Guru Ghasidas Vishwavidyalaya (a Central University), Bilaspur, and served as an associate professor till 2013. He is currently working as a professor at the Department of Microbiology, CURAJ (Central University of Rajasthan) and was also the former head and dean, School of Life Sciences. He is a member of various National & International societies/academies and has also completed two collaborated projects worth 150 million INR in the area of microbial diversity and bioenergy.

Professor Verma is a group leader of the Bioprocess and Bioenergy laboratory at the Department of Microbiology, School of Life Sciences, CURAJ. His area of expertise involves microbial diversity, bioremediation, bioprocess development, lignocellulosic, and algal biomass-based biorefinery. He holds 12 international patents in the field of microwave-assisted biomass pretreatment and bio-butanol production. He has more than 65 research articles in peer-reviewed international journals and contributed to several book chapters (32 published; 11 in press) in different edited books. He has also edited 11 books for international publishers such as Springer and Elsevier. He is a guest editor to several journals such as *Biomass Conversion and Biorefinery* (Springer) *Agriculture* (mdpi), *Frontier in Nanotechnology* (Frontiers), and *International Journal of Environmental Research and Public Health* (mdpi). He is also an editorial board member for the *Journal Current Nanomedicine* (Bentham Sciences) and an acting reviewer for more than 60 journals of different publishers such as Springer, Elsevier, RSC, ACS, Nature, Frontiers, mdpi, etc.

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Preface

Water is an essential resource and crucial to sustaining life. Ever-increasing population, rapid urbanization, and industrialization have resulted in improper discharge of wastewater leading to hazardous impacts on human health and the environment. As a result, globally nations are adopting measures to retain water and improve the qualitative parameters of wastewater to make it harmless and reusable. These technologies are a fruitful approach for solving water shortages, removing contaminants, and recycling them back to the environment for various uses, for example, drinking, washing, etc.

Thus, considering the huge subject of wastewater treatment, this book focuses on various aspects such as bioremediation, soil management, water cleaning, and the role of biotechnology in environmental engineering. The book also emphasizes on the urgent need for developing new approaches for environmental cleaning, such as sensing and monitoring technologies for the detection of environmental pollution. The book is likely to serve as valuable reference material to understand the current challenges, research gaps, and advancements in wastewater treatment.

Additionally, the book looks at the use of nanotechnology in environmental cleaning, including waste management, and soil and water remediation. It discusses technologies available for carbon sequestration and their positive impact on creating a greener environment. Additionally, the book is focused on microbial-assisted remediation methodologies, for example, biodegradation of toxic dyes, and chemicals and also cellular sequestration and conversion of CO_2 into biomass and chemical products providing carbon neutral to negative alternatives to conventional fossil fuels.

The biodegradation of toxic dyes includes 1, 4-dioxane, which is an emerging organic pollutant and is produced as a by-product in several petrochemical-based industrial processes. It provides an overview and future perspective of microorganism-assisted reclamation of 1, 4-dioxane polluted sites and discusses the chemistry of dioxane, toxicity and the fate of dioxane in the environment, reclamation strategies, and different factors influencing reclamation, dioxane degrading monooxygenases, and microbial degradation pathways. The book also deals with the adverse impact of azo dyes and explains various mechanisms of dye degradation by microorganisms. Similarly, the impact of heavy metal on the environment, different microbes involved in the detoxification process and biotechnological solutions of heavy metal contamination have been discussed in detail.

Another aspect has included bacteria-assisted reclamation of petroleum hydrocarbon polluted sites. It also discusses the fate of petroleum hydrocarbons in the environment, and microbial hydrocarbon degradation pathways. Further, it focused on ecological contamination and gives deep insight into the status and advances of different computational techniques and omics approaches to enhance the process of bioremediation. The use of formulations or environmental enhancers is to biochemically strengthen selected bioremediation pathways.

The book also aims pedagogically to follow a problem-solving, data-based approach, starting by introducing the problem of Contaminants of Emerging Concern (CECs), and proposing a solution. For example, zooplankton and other microbes act to remove or reduce pollutants through the biological degradation of pollutants into nontoxic substances. Several parameters of bioremediation *in-situ* or *ex-situ* methods are discussed. It provides current information regarding the potential of biomass-based integrated biorefineries for wastewater treatment and resource recovery. Sustainable solutions can be applied to all types of industries including small and large, regardless of material, energy and water consumption levels. Thus, green technology can prove to be an asset in improving sustainability, enhancing green growth, and maintaining a balance between socioeconomic and environmental parameters, while preserving efficiency, productivity, and prosperity. The book aims to provide a comprehensive insight into the potential applications and also throws light on the recently developed advanced green technologies.

Acknowledgments

First of all, we would like to convey our gratitude to the publisher, IWA Publishing, for considering our thoughts to develop this book, which is the first of its kind covering various clean technologies. The current book became a reality because of the support from all researchers and academicians who have contributed to the book: the editor is thankful for their contributions. We would also like to thank all scholars for providing us with the necessary technical support and editorial assistance during the developmental stages of this book.

We are always thankful to God for his blessings and strength and motivation to always do well and contribute to the scientific world.

Professor Pradeep Verma
Dr. Maulin P. Shah

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Chapter 1

Microbes and wastewater treatment

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ABSTRACT

Wastewater is considered a source of water, power, and enriching nutrient for plants. Wastewater treatment is among the most significant biotechnological procedures for treating municipal and industrial sewage across the world. Conventional wastewater treatment methods, however, have constraints, primarily because they are cost-intensive to achieve the aim of wastewater remediation. Microorganisms, on the contrary, outperform humans when it comes to sewage water purification. Their ability to decompose a wide range of organic chemicals and cycle components such as nitrogen, phosphorus, and carbon is unparalleled in ecology. These characteristics have been successfully used in microbial wastewater treatment plants. This chapter discusses the necessity for wastewater treatment, and the function of diverse microbes in wastewater treatment, including new and developing technologies that use microbes for wastewater treatment and purification, such as microbial fuel cells, bioremediation, and activated sludge processes along with the challenges and prospects of using microorganisms in wastewater treatment.

Keywords: microbes, wastewater, microbial fuel cell, bioremediation, municipal waste

1.1 INTRODUCTION

The most pervasive material in the natural world is water. Liquid, solid, and invisible vapor are the three different states in which water may exist. The topmost portions of the Earth's crust and soil layer are where water forms the oceans, seas, lakes, rivers, and subterranean bodies. In the arctic and alpine areas, water occurs as ice and snow cover in a solid condition. Water is present in the air as water vapour, water droplets, and ice in addition to inhabiting the biosphere to some extent. The composition of the many minerals that make up the Earth's crust and core contains enormous amounts of water. Water is a necessary component of practically every living thing's everyday life. Most regions of the world have recently started to experience acute water scarcity, necessitating the use of wastewater reuse techniques. The amount of available freshwater is insufficient to meet the planet's consumption demands, and the majority of it is found in polar areas as snow and ice accumulate (Shiklomanov, 1993).

Concern is also raised by the poisoning of the aquatic environment with chemicals and pharmaceuticals. The growing population requires a steady supply of clean water for drinking, hygienic purposes, irrigation, and other applications. The daily water needs are further impacted by the pathogenic bacteria, micro irritants, and spores within water bodies that exist naturally. Currently, in addition to preserving the water cycle, wastewater reusing is required to satisfy water needs and safeguard environmental health by adhering to severe wastewater treatment regulations (Larsson *et al.*, 2007).

Every day, both our homes and businesses require water. The water we use is extracted from lakes, rivers, and the earth (groundwater), and the majority of it returns to these places after we consume it and pollute it. Wastewater is the term for this used water. Serious contamination results if it is not handled before being released into rivers. A mixture of ground water, surface water, and storm water, along with liquid or water-borne waste from homes, institutions, and commercial and industrial organizations can be referred to as wastewater. It typically has a high concentration of oxygen-demanding wastes, disease-causing pathogens, organic compounds, micronutrients that promote plant development, inorganic compounds, minerals, and sediments. Toxic substances could also be present (Metcalf & Eddy, 2004) (Figure 1.1).

1.2 NEED FOR WASTEWATER TREATMENT

Water purification continues to place a high emphasis on protecting our planet and the waterways that provide water to people worldwide (McCarty *et al.*, 2011). It is not just an issue for impoverished nations. And it is crucial for a number of reasons (Figure 1.2).

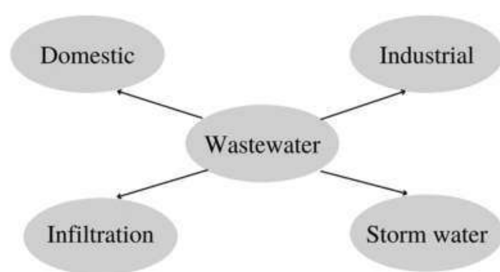

Figure 1.1 Classification of wastewater.

Figure 1.2 Need for wastewater treatment.

1.3 ROLE OF MICROBES IN WASTEWATER TREATMENT

Since microorganisms are arranged in species-rich structures in waste water treatment facilities, biodegradation of a larger spectrum of substrate is superior to that in pure culture (Cydzik-Kwiatkowska & Zielińska, 2016). The majority of the microorganisms used in biological wastewater treatment procedures are found in microbial aggregates, including biofilms, flocs, and granules. Extracellular polymeric substances (EPS), also known as complex high-molecular-weight mixtures of polymers, were found in activated sludge, biofilms, granulated sludge, and pure cultures after using a variety of electron microscopy techniques. By weak physicochemical interactions, EPS on the microbial surface is crucial in maintaining the microbial aggregates in a three-dimensional gel-like wet matrix. Hydrogen bonding, electrostatic interactions, hydrophobic contacts, and van der Waals interactions are examples of these weak forces. The metabolic waste products known as EPS build up on the bacterial cell surface (Liu & Fang, 2002). They are created by microbes in bioreactors after they consume organic substances found in wastewater. EPS builds up due to a variety of processes, including secretion, excretion, cell lysis, and sorption of waste water constituents. From an engineering perspective, EPS has certain crucial qualities including the ability to biosorb and the capacity to degrade. In addition to polysaccharides, proteins, nucleic acids, phospholipase, and other nonpolymeric molecules with smaller molecular weight, EPS is made up of a range of organic macromolecules. It has also been found that EPS contains organic compounds such humic chemicals and uronic acid (Pal & Paul, 2008).

1.4 COMMON MICROBES USED IN WASTEWATER TREATMENT

Microbes play a significant part in the process of recycling waste since they are the major actors and are primarily in charge of the degradability of inorganic organic compounds and the recycling of nutrients in the environment. Microbes are crucial throughout the fermentation process for recycling waste, treating wastewater, and producing alternative energy. The following group of bacteria is primarily present during the treatment of wastewater (Rani *et al.*, 2019).

1.4.1 Bacteria

They are largely in charge of extracting and converting these organic components in an effluent treatment, which is an essential part of the wastewater treatment process. Thus, these bacteria are crucial for the maintenance and proper operation of microbial treatment systems. Bacteria in wastewater treatment facilities are often present at concentrations of 10 ml. Typically, heterotrophic bacteria obtain energy from breaking down carbonaceous organic materials and utilize the same for cell formation and development. In the treatment of industrial wastewater, it was discovered that nearly 11 bacterial species were involved. They are *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus* sp., *Kosakonia oryzae*, and *Cronobacter sakazakii*.

1.4.2 Protozoa

They are larger than bacteria and are unicellular eukaryotic creatures. Protozoa are important in the process of treating wastewater because they feed on harmful bacteria. By digesting them, they eliminate floating bacteria as well as other suspended particles, giving wastewater an edge. Hence, the purity of wastewater effluent is improved. As with bacteria, certain protozoan species need very little oxygen to exist, while others can endure anaerobic environments. In aerobic activated sludge, the typical protozoan concentration ranges from 5×10 to 2×10. The many protozoa kinds found in wastewater treatment systems serve a variety of tasks. Flagellates (whose primary food source is the dissolved organic materials within effluent), ciliates (which eliminate floating bacteria and aid in pollutant clearing), crawling ciliates such as *Aspidisca* sp. and *Euplotes* sp. (which predominate activated sludge and indicate high treatment), *Chilodonella uncinata* are ciliated protozoan species that are frequently discovered in wastewater treatment plants (WWTPs).

1.4.3 Metazoa

Lagoon effluent in particular has a predominance of multicellular eukaryotics that are larger than most protozoa in size; however, their concentration in activated sludge is quite low (103/ml). Yet, they do highlight the problems of the therapeutic system. The rotifers and nematodes metazoan groups, which may be present in activated sludge, are known to serve distinct functions. They consume other microorganisms and clean the effluent. When hazardous amounts of effluents are present, they are the first to be impacted.

1.4.4 Filamentous bacteria

Long filaments of these bacteria are produced throughout their growth. The biomass of activated sludge often contains filamentous microorganisms. They are a regular part of the biomass of activated sludge and important for proper floc development. Their population is influenced by the nutritional circumstances (the wastewater system's dissolved oxygen (DO), pH, sludge age, temperature, the amount of nutrients that are readily accessible, and the amount of oil and grease). In activated sludge, filamentous bacteria of up to 25 distinct kinds have been identified. One of the major filamentous bacteria, *Nocardia* spp. causes foaming.

1.4.5 Algae

Algae are a class of photosynthesis-based organisms that are all around us and are important in the biological treatment of sewage. Algae play a variety of roles in the ecosystem because they may gather heavy metals, pesticides, poisonous organic and inorganic materials both within and outside of their cells. Algae improve the soil's nutrient content for plants and aid in their ecosystem mobilization. Excess nutrients may also be utilized in wastewater treatment systems to create biomass, which has a variety of uses, including the production of sustainable biofuel for the production of food (including proteins and carbohydrates), feed, and medicines (Rani *et al*., 2019).

1.4.6 Fungi

Fungi are multicellular creatures which hydrolyze intricate organic compounds while contending with other microorganisms in a mix culture. They are distinguished by the ability to oxidize ammonia to nitrite and nitrate, which impede bacterial growth, and even decompose organic material at low pH atmosphere. Fungi capture and adsorb suspended materials using its fungal hyphae to acquire the energy and nutrients they require. It has also been shown that some fungi release enzymes that aid in the substrate breakdown process during wastewater treatment. *Sphaerotilus natans*, *Aspergillus*, *Penicillium*, *Fusarium*, *Absidia*, and so on are among the most prevalent fungal species linked to waste decomposition (Rani *et al*., 2019).

1.5 MICROBIAL WASTEWATER TECHNIQUES

- Preliminary treatment involves removing bulky materials such as bottles, cans, and plastics that might obstruct downstream operations.
- Primary treatment: Elimination of grit, suspended particles, and oil.
- Secondary treatment: Usage of microorganisms in anaerobic or aerobic conditions to remove phenol, color, and oil while lowering biological oxygen demand (BOD).
- Tertiary treatment: Reverse osmosis, electrodialysis, and ion exchange are used for the effluent's ultimate purification and elimination steps.

Wastewater is treated biologically using techniques that have been around for a while to handle industrial effluents. These techniques, which use a membrane batch reactor, may be classified as aerobic and anaerobic reactor in batch mode. Moreover, there are anaerobic film reactors and filters (Rani *et al*., 2019) (Table 1.1 and Figure 1.3).

Table 1.1 Benefits and drawbacks of wastewater purification techniques.

Treatment Methods		Advantages	Disadvantages
Aerobic system	CASP	• High reduction of BOD and pathogen • Operated at a wide array of organic load as well as hydraulic rates	• Energy consumption is high Sludge require further treatment and appropriate discharge
Aerobic system	OD	• Operation management is easy • Less sludge	• Effluent suspended solids concentrations are relatively high • Require larger land area
Aerobic system	MBR	• Treated water is clarified and can be applied directly to recycling	• Aeration limitation and membrane pollution
Aerobic system	TF	• Low concentration and maintenance cost	• Poor transparency of treated water
Aerobic system	RBC	• Low operating cost and less sludge generation	• Poor transparency of treated water
Anaerobic system		• Biological waste produced is less in quantity Low maintenance in terms of nutrient requirements Production of methane as an end product	• The growth rate of microorganisms is slow • Production of odour
Facultative system		• Require little energy • Easy to operate	• Periodic removal of settled sludge is required

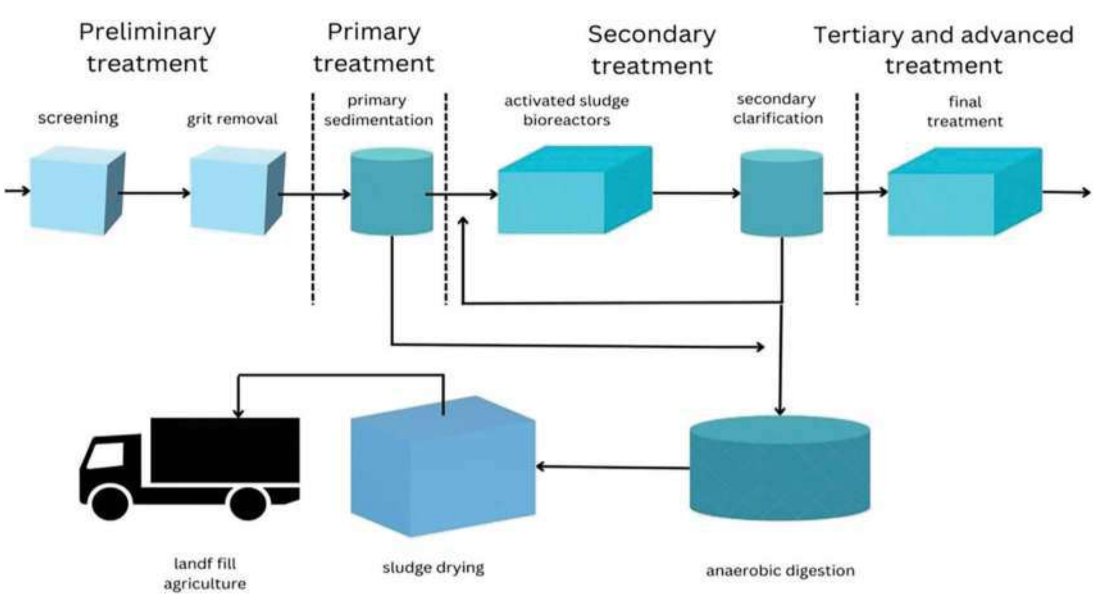

Figure 1.3 Typical wastewater purification system diagram.

1.5.1 Preliminary treatment

Large debris that might clog and hinder downstream processes, such as bottles and stones, are removed at the first stage of wastewater treatment. For screening and grit removal during initial treatment, tavern, ring, slashing, or bar screens that are slanted more on the side of inflowing water can be employed. These screens can catch particles as the sewage water passes past them. Following the removal of the mechanically or manually captured waste, fibrous materials may be additionally separated from the water. Grit removal, which entails getting rid of abrasive inorganic elements such as sand, gravel, and other heavy particulates, is essential to prevent obstruction and abrasive damage to the equipment or pipes that carry waste. There are additional varieties from grit channels, such as velocity channels, that reduce influent velocity and permit heavier abrasives to settle at ground level before removal (Chahal *et al*., 2016).

1.5.2 Primary treatment

Processes used in primary treatment are intended in eliminating suspended particles together with lowering particle BOD levels. The majority of the time, it is referred to as the first step of treatment and eliminates 25–50% of BOD, 65% of oil and grease, and 50–70% of total suspended solids (TSSs). Sedimentation, which includes materials settling under the effect of gravity, and flocculation are two important physical mechanisms for separating solids from wastewater. The most typical clarifiers or sedimentation tanks are round or rectangular in form. Contrary to rectangular tanks, which have water entering through one end and draining at the other, circular tanks have water entering along the centre and distributing outward axially. The tank's weir is a crucial component. The weirs are therefore meticulously crafted physical barriers that control flow rate to be the same. The inclusion of coagulants or flocculants allows for the separation of dissolved organic materials in addition to basic primary treatment techniques. The metal salt flocculent clumps the suspended colloidal particles, which facilitates their separation via settling or filtration. Mostly dissolved materials will be present in the outflow water, also referred to as primary effluent. The primary effluent moves into secondary treatment after it has been cleared.

1.5.3 Secondary treatment

The secondary treatment processes used on the primary effluent include a variety of biological treatment techniques to get rid of minerals and soluble inorganic as well as organic solids. Carbon oxidation and nutrient removal are two of the various functional processes that take place during secondary treatment. The carbon oxidation process, which transforms organic materials into carbon dioxide, water, and cellular detritus, is crucially dependent on microorganisms. In order to grow and reproduce, bacteria use the energy generated.

Prior to wastewater being released into the environment, two main nutrients—nitrogen and phosphorous—must be removed. Inorganic nitrogen is eliminated by the biological processes of nitrification and denitrification. As denitrification turns the nitrate into nitrogen gas, nitrification turns ammonia into nitrate. Nitrous oxide, a strong greenhouse gas, can be produced as a result of incomplete nitrification or denitrification. To get rid of phosphorus, physiochemical and biological processes might be combined. The growth of phosphate-accumulating organisms that can store orthophosphate is specifically supported by biological processes such as increased biological phosphorus removal plants. Secondary treatments come in a variety of forms which could be applied in biological nutrient elimination procedures (Chahal *et al*., 2016).

1.5.4 Activated sludge process

The biological removal of nutrients from wastewater is frequently accomplished by the activated sludge process (ASP). There are two main stages in an ASP. A heterogeneous and highly varied bacterial population breaks down pollutants at the initial stage while also converting the compounds into more streamlined and ecologically friendly by-products like nitrogen and carbon dioxide gas. The term 'activated sludge' refers to a heterogeneous microbial culture, as well as the biomass is frequently organized as microbial flocs, which are kept under suspension through mechanical mixing with aeration. An aeration tank along with aeration source make up the most essential ASP arrangement at this first stage. Incorporating anoxic and/or anaerobic regions that enhance overall nitrogen elimination through nitrification/denitrification and phosphorous assimilation has, however, resulted in numerous changes to this basic idea. The biomass is separated from the cleaned water in the second step of the ASP using secondary clarifier that employs gravity sedimentation. The cleared supernatant is transported for tertiary treatment, while the settled biomass is mainly recycled again to highest point of the ASP as return activated sludge (RAS). An ASP's main job is to biologically remove as well as stabilize nutrients, but it additionally possesses the ability to act as an effective therapeutic shield against diseases through predation along with connection, adsorption, and trapping to or inside the biological floc. Depending on how each WWTP operates and how the season affects pathogen density and treatment efficiency, there may be variations in accounts on the efficacy of ASP for pathogen elimination. It has been stated that between 1.5 and 2.5 log10 of *E. coli* have been eliminated, compared to between 1 and 3 log10 of *Cryptosporidium*. However, virus clearance differed between different WWTPs as well as appeared to vary for specific viral species. For instance, the reduction of rotavirus was between 7 and 8 log10, whereas the reduction of norovirus was between 1 and 6 log10.

1.5.5 Waste stabilization ponds

Large shallow reservoirs called waste stabilization ponds (WSPs) have been used to store wastewater for a longer duration to enable bacterial and algal communities to treat it biologically. WSPs provide a green treatment option with the advantages of low energy usage, low operating costs, and exceptionally efficient removal of organic debris and pathogens. In limited towns and villages, WSPs are commonly used as the only choice for sewage treatment, as an additional step of polishing after ASP, as one of several secondary treatments before discharge or reusing.

Pathogens are removed from WSPs by three main mechanisms: (1) unfavorable pond conditions (such as temperature, sunshine, and predator activity); (2) prolonged pond occupancy durations that result in natural mortality of microorganisms; and (3) particle binding and sedimentation. Pathogen eradication when the final discharge level of pathogens is both affected by pond depth, detention time, pond population, and pond geometry. By photooxidative DNA damage caused by sunlight and other physicochemical parameters such as temperature and pH, WSPs eliminate fecal coliforms, *E. coli*, and other dangerous microbes. *Cryptosporidium* suspended in a WSP has also been discovered to be inactivated by temperature and sunlight. Pathogens can be eliminated through predation by other microbes or zooplankton, particularly bacteria and protozoan parasites. WSPs demonstrated reductions about 2–4 log10, 3–6 log10, 1–2 log10, and up to 3 log10 for viruses, bacteria, protozoan cysts, and viruses, respectively. Protozoan removal for an Australian pond system was found to be highly seasonal, characterized by higher removal at the summer and autumn months (2.5–3 log10) while lower removal at the spring and fall months (0.5–1.2 log10). A number of facultative and maturation pools is the most common configuration, though there are many other pond design types. There is no pre-treatment employed, and the connection between the main facultative pond and the maturation ponds is fairly simple. An anaerobic pre-treatment stage is present before the facultative pond in more sophisticated plants. In the sections that follow, the various pond system types are discussed.

1.5.5.1 Anaerobic ponds

Anaerobic ponds eliminate organic mass while running without oxygen. They have brief retention durations and can reduce the organic load in wastewater by 40–70%. In these ponds, pathogens are primarily eliminated through sedimentation. Microorganisms often have low densities and hence slow settling rates. For sedimentation to occur, pathogens must be bounded onto heavier contaminants because the oocysts of *Cryptosporidium* settle at a rate of 30 mm/day. Large and rather dense helminth eggs easily settle by gravity and are eliminated in these ponds.

1.5.5.2 Facultative ponds

In facultative ponds, both aerobic and anaerobic activities occur. Pathogens are removed from these wetlands through a complex process that includes sedimentation, inactivation using sunlight, a pH level that is elevated, low carbon dioxide levels, and elevated oxygen concentrations. The photic, heterotrophic, and anaerobic zones are just a few of the many operational layers or areas that make up these wetlands. The most shallow zone is anaerobic and produces gases such as methane and carbon dioxide while removing organic material that has collected in sediment without using oxygen. The growth of algae is encouraged by carbon dioxide inside the heterotrophic region, providing air for heterotrophic aerobes to decompose organic materials. The uppermost layer, also known to be photic zone, where algae photosynthesis occurs at high rates, becomes extremely oxygenated. Heterotrophic bacteria use this oxygen in the aerobic breakdown of organic materials. Facultative ponds typically have retention periods of 5–30 days and are shallow, measuring between 1 and 2.5 m in depth.

1.5.5.3 Maturation ponds

Maturation ponds have a retention period of roughly 20 days and are 1–2 m deep. Although they also serve to extract nutrients, their primary use is to remove pathogens. It is more practical to avoid short circuits by using a succession of tiny maturation ponds as opposed to a single one. Because of the efficient pathogen elimination provided by solar radiation (UV penetration), a pH level that is elevated, high oxygen concentration, as well as low levels of nutrients, maturation ponds are typically shallower than other ponds. Maturation ponds can eliminate all protozoa and helminth eggs as well as 99% of coliforms. Both industrialized nations like Australia and developing nations such as India frequently employ them, but the mechanisms by which enteric viruses are eliminated are poorly understood and need further research.

1.5.5.4 High-rate algal ponds

A less common pond shape for treating primary wastewater is a rapidly growing algal pond (HRAP). To promote the development of green microalgae, which reduces the organic load and pathogen counts, these ponds are usually small and well-mixed. An additional advantage of HRAP is that it has the potential to inactivate pathogens; one study found that the infectivity of *Cryptosporidium* was reduced by 97% as a result. Using algae to remove toxins like heavy metals, alongside secondary treatment, HRAP also provides some tertiary therapy. Even though HRAP uses more energy compared to various systems, it still outperforms them in terms of expense and energy efficiency, especially when energy-efficient propellers are employed. Before HRAP is applied to main effluent, anaerobic ponds or clarifiers can be used as a pre-treatment process to remove particles from wastewater. If carbon is a limiting component in the effluent, CO_2 aeration can increase HRAP's effectiveness. Compared to WSPs, HRAP systems are a desirable alternative for urban or semiurban areas that are rapidly growing and require a decentralized treatment of waste solution that is affordable with minimal disturbance to land (Chahal *et al.*, 2016).

1.5.5.5 Tertiary treatment and disinfection

Tertiary treatment is the ultimate polishing step required to obtain recycled water of a suitable quality; it entails a number of biological and chemical processes. The treatment methods selected are influenced by the final use that is decided. When human interaction with the recycled water is infrequent, secondary treated effluent may be appropriate for use in applications like underground irrigation or woodlot irrigation. However, the need for tertiary cleaning rises as the danger of human exposure to the recycled water does. To reduce the quantity of pathogens to levels that comply with healthcare standards, the effluent must generally be correctly treated and/or disinfected. Risk analyses that take into account exposure pathways, exposure levels, infectious dosages, and disease outcomes produce these target numbers. Lime or alum can be used to precipitate out nutrients such as phosphorus, and, less frequently, microalgae (using HRAP) have also been shown to be successful at removing nitrogen and phosphorous. Pathogens can be physically eliminated using filtration methods such as flotation of dissolved air filtration, microfiltration for the removal of microorganisms and protozoans, as well as ultrafiltration to remove viruses. Larger pathogens like bacteria and protozoa can be effectively removed using membrane filtration techniques. The extra benefit of filtration is that it removes particles, which helps the subsequent disinfection procedures that are necessary to destroy any lingering bacteria.

The final and possibly most important step in tertiary treatment is the decontamination of the effluent prior to utilization (at least with respect to microbiological safety). UV rays and chlorine treatment are both widely used disinfection techniques. Chlorine is introduced to treated effluent for set periods of time to increase microbe exposure and inactivation. Then, any chlorine that is left over is neutralized before being discharged into the atmosphere or kept in aquifers. Traditionally, chlorine disinfection goals are determined using contact time, or CT, that is calculated as the combination of the chlorine dose (in mg/L) and the time (in minutes). As a result, the same CT can be acquired using either an increased dose/small period of time or a low dose/high period of time. Amount of free chlorine that is readily available, which is influenced by pH and temperature, has an impact on the CT. This is important because, compared to warm water, cold water necessitates significantly higher CTs for pathogen inactivation (e.g., a CT of 8 mg min/L for viruses at 5°C versus a CT of 3 mg min/L for viruses at 20°C). CTs of the main enteric pathogens have been determined for disinfection with chlorine of drinking water or wastewater, and these have been set in numerous guidelines. Chloramine is a significantly weaker oxidant than chlorine, and it requires high level of CTs to disinfect water to the same degree. Common gut bacterial pathogens with chlorine CTs of 1 mg min/L or less include *Salmonella*, *Campylobacter*, and *E. coli*. Viruses are inactivated by chlorine, despite having a little bit more resistance than intestinal bacteria. Certain protozoan parasites, most notably *Toxoplasma* are resistant to chorine.

Working with UV light is safer, involves fewer stages, and does not produce any disinfection by-products in comparison to working with chlorine and different ways of producing chlorine. Fair enough, building the required infrastructure for UV disinfection can be more costly than chlorination. When wastewater is treated with UV light, it is exposed to a UV-C illumination source, usually a UV lamp enclosed in a quartz sleeve inside of a steel conduit and attached to reinforced concrete. Although some viruses, most notably the adenovirus, can withstand UV radiation well, UV is very efficient toward bacteria alongside intestinal protozoans. Combining UV with chlorination can be particularly effective since it allows for the most economical use of each treatment method. For the elimination of viruses and *Cryptosporidium*, appropriate concentrations of chlorine are used. Other cleaning techniques have been created and researched as well. Peracetic acid has also been regarded as a powerful disinfectant due to its potent bactericidal, fungicidal, sporicidal, and virucidal properties. Ozone has been shown to be effective with viruses, protozoan cysts, and helminth eggs. Wastewater can be disinfected using conductive-diamond electrochemical oxidation (CDEO), and by preventing *E. coli* cells from congregating, CDEO and ultrasonic technology improved disinfection effectiveness. Yet it does not seem like this technique has been adopted for widespread commercial application (Chahal *et al.*, 2016).

1.6 MICROBIAL FUEL CELLS

A particular kind of bio-electrochemical fuel cell technology is the microbial fuel cell (MFC). It produces electricity by transferring electrons from reduced chemicals produced by microbial oxidation on the anode to oxidized compounds like oxygen upon this cathode into an auxiliary electrical circuit (Jatoi *et al.*, 2020).

1.6.1 MFC configuration

Two electrodes and a membrane separating these two compartments make up the majority of a microbial cell. As a result of microorganisms being oxidized at the anode location, electrons and protons are produced there and begin to transfer to the cathode. Unlike protons, which move through the membrane, electrons move across the circuit. When electrons and protons reach the cathode, they reduce the oxygen to create water. Most microbial cells are aided by mediators because they lack electrochemical activity (Jatoi *et al.*, 2020).

1.6.2 Mechanism of MFC

The electron transport process served as the foundation for the working of MFCs. Similar to how energy production is impacted, it also depends on a number of variables, such as the propensity of microorganisms to transfer electrons, the size of the electrodes' surfaces, the strength of the electrolyte, and the rate of the kinetic oxygen reaction. The three categories of these components are kinetic limits, ohmic restrictions, and transport restrictions. The MFC shows the worth of identifying limiting parameters and later changing to improve overall performance by treating wastewater through batch or continuous inputs. The transmission of electrons was impacted by the microbial cell wall and electron mediator. pH 7 and an impedance of more than 500 V are the ideal values (Jatoi *et al*., 2020).

1.6.3 Wastewater from MFC

The substrate is crucial for any microorganism's growth and evolution since it provides nutrients. Similar to this, it is believed that the substrate is the main organic element of MFC responsible for energy generation. There are many different kinds of wastewater that have been employed, including home wastewater, swine wastewater, food processing wastewater, wastewater from the chocolate business, and wastewater from farm lands, municipal, and industrial sources. Microbial fuel are popular for how they operate in terms of converting substrate into energy and lowering wastewater-related environmental issues (Jatoi *et al*., 2020).

1.7 MFCs WITH SYNTHETIC WASTEWATER AS SUBSTRATES

Carbon removal from wastewaters using MFCs has demonstrated high rates (>90%). Acetate, glucose, sucrose, and xylose are just a few of the many organic substrates employed in the MFCs as synthetic wastewaters for microbial oxidation in the anode chamber. For exo-electrogenic bacteria, acetate is the most straightforward and typical substrate employed in MFCs. Since acetate offers a straightforward metabolism, these bacteria can biodegrade it easily. Acetate is also a by-product of various metabolic processes for higher-order carbon sources. For instance, when wastewater sludge is digested anaerobically, carbonaceous matter is transformed into shorter-chain organic acids like acetic acid. In comparison to household wastewater and glucose substrates, MFCs with acetate substrates exhibited higher power densities. The power generated with acetate (506 mW/m^2, 800 mg/L) was up to 66% higher than that produced with butyrate (305 mW/m^2, 1000 mg/L) using a single-chamber MFC. A recent study examined the coulombic efficiency (CE) and power output of four different substrates. After butyrate (43.0%), propionate (36.0%), and glucose (15.0%), MFC fed with acetate displayed the highest CE (72.3%). The MFC based on acetate-induced consortia achieved more than two times the maximum electric power and half of the ideal external load resistance compared to the MFC based on consortia induced by a protein-rich wastewater when acetate was compared with it as a substrate in MFC. Another often researched substrate in MFDs is glucose. A glucose fed-batch MFC with 100 mM ferric cyanide as the cathode oxidant produced a maximum power density of 216 W/m^3. This study compared anaerobic sludge to glucose to assess its suitability as a fuel for MFC electricity production. Anaerobic sludge provided very little substrate to a baffle-chamber membrane-less MFC, and only a little amount of electricity (0.3 mW/m^2) could be produced. Yet, a maximum power of 161 mW/m^2 was produced when glucose was present in the same system. Acetate and glucose were used as MFC substrates in a different study, and the energy conversion efficiency (ECE) of each was examined. With acetate, the ECE was 42%; however, with glucose, it was only 3%, resulting in a low current and 14 power density. Glucose-fed due to electron loss by competing bacteria, MFC produced the lowest CE, but its relatively diversified bacterial structure allowed for the largest power density and significantly greater substrate use. The findings of numerous independent investigations demonstrate that complex wastewaters have power outputs that are five times lower or less than those of discrete substrates. The potential to handle high strength substrates is indicated by the substrate removal rates for fake wastewater, which can reach up to 8.9 kg COD(chemical oxygen demand)/m^3 reactor/day, compared to actual wastewater substrate removal rates, which vary from 0.5 to 2.99 kg COD/m^3 reactor/day (Gude, 2016).

1.8 MFCs WITH ACTUAL WASTEWATER AS SUBSTRATES

Municipal wastewaters are classified as low energy density carriers or feedstocks for MFCs due to their lower BOD concentrations, which are typically less than 300 mg/L. Due to the anaerobic conditions in the anode chamber, MFCs may also treat wastewaters with high energy density and BOD concentrations surpassing 2000 mg/L. These industrial waste streams include those from the food processing industry, breweries, dairy farms, animal feeding facilities, and other waste streams. With relatively low quantities of organic nitrogen, the effluent from the food processing industry is rich in organic acids and quickly biodegradable carbohydrates. Depending on the BOD and amount of water used in the processing, MFCs can generate electricity from the wastewater used to process food products in the range of 2–260 kWh/ton of product. The US milk dairy farms' low BOD wastewaters have the capacity to generate 46 MW of electricity, whereas the high BOD wastewater from the dairy industry has the ability to generate up to 1960 MW of electricity. Animal wastewaters from the livestock-related industry are frequently particularly high in organic material content (∼100 000 mg/L COD for animal wastes) and may contain high levels of nitrogen-containing components, like proteins, and harder to degrade organic materials, like cellulose. In addition, 15 lipids may also be present in slaughterhouse wastewaters from the livestock-related business in addition to carbs, organic acids, and proteins. Although brewery wastewater concentrations vary, they are typically between 3000 and 5000 mg/L COD, making them around 10 times more concentrated than domestic wastewater. The fact that it has a high carbohydrate content (high energy density) and a low ammonium nitrogen concentration may make it a good substrate for MFCs. A maximum power density of 528 mW/m^2 was attained when 50 mM phosphate buffer was introduced to the wastewater during the treatment of beer brewery wastewater using air cathode MFC. In this instance, when both wastewaters were evaluated at equal intensities, the maximum power produced by brewery wastewater was less than that obtained by household wastewater (Gude, 2016).

1.9 BIOREMEDIATION

To decontaminate contaminated environments, a method known as bioremediation involves dissolving complicated contaminants into intermediates that are either safe for humans or other living organisms to consume. A crucial role of bioremediation is played by microorganisms, including fungi, bacteria, algae, and so on. Not all microorganisms have the ability to convert contaminants into simple, innocuous molecules that can be consumed (Ojhaa *et al.*, 2021).

1.9.1 Principle

Biodegradation is the foundation of the concept of biological restoration. Using the bioremediation method, environmental waste can be biologically decomposed to a safe form or at proportions beneath the corresponding limits of concentration. This is done under closely supervised conditions set by the relevant regulatory authorities. Utilizing organisms that are living, most commonly bacteria, bioremediation breaks down hazardous materials or chemicals to less harmful form that can be found in wastewater. It is an ecological treatment choice as well as feasible technology, but the environment can exhibit how successful it is. The essential microorganisms such as bacteria, mold, and flora have the biological ability of breaking down harmful pollutants in water and also clean it up. A moderately priced technology is available on-site. This technique requires the growth of microbial consortiums or promoted microflora. They can carry out required duties and are native to contaminated areas. The aforementioned colonies of bacteria can be produced in a variety of methods, such as by promoting growth, adding nutrients, including a terminal electron acceptor, or regulating the relative humidity and temperature. Throughout the course of bioremediation procedures, microorganisms consume these contaminants as food or energy sources. Under certain conditions, specific native microbes may already be at the location, while others may be collected and added in the purified medium via a bioreactor. In spite of the fact that bioremediation relies on the growth and activity of microorganisms, it is important to emphasize

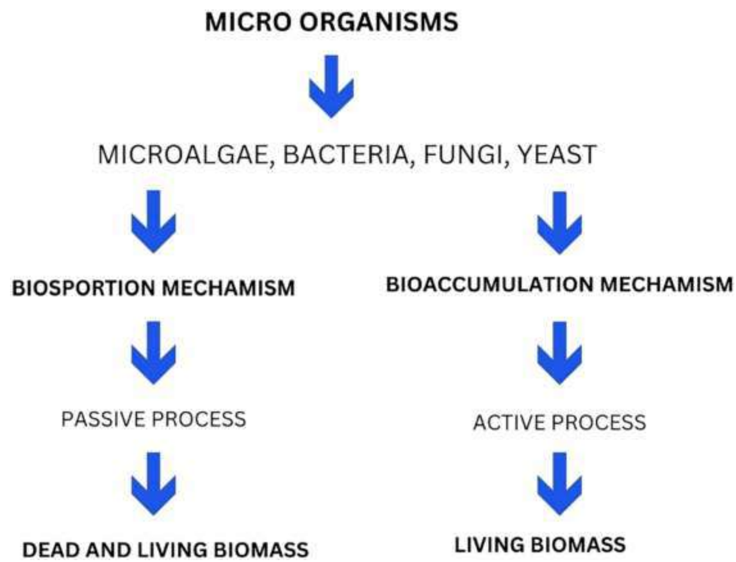

Figure 1.4 Methods of bioremediation for wastewater.

that the environmental factors that influence the growth and degradation rates of microbes have a significant impact on how effective bioremediation is. To locate the appropriate microorganisms in the right place for an effective degradation process under the required environmental conditions, the bioremediation method generally depends on doing so. It is doable to improve the biological mechanisms employed in bioremediation techniques and focus on removing harmful chemicals from water. This procedure results in waste mineralization and reduces the need for further treatment by converting waste into substances such as water, carbon dioxide, biomass, or other non-hazardous materials. The word 'bioremediation' refers to the management of different substances. Chemicals used in agriculture, petroleum-based components, and potential substances that are not biodegradable such as solvents that are chlorinated, chlorofluorocarbons, and various other industrial organic substances can all be broken down by microorganisms in addition to municipal waste and process water. The microorganisms may be transported to the contaminated location from elsewhere or they may be isolated and endemic to the polluted area. Living organisms convert contaminants through reactions that occur as part of their metabolic activities. The behavior of numerous species can also contribute to the biodegradation of a chemical (Ojhaa *et al.*, 2021) (Figure 1.4).

1.9.2 Methods of bioremediation of wastewater

1.9.2.1 Bacteria

There is a wide spectrum of bioremediation potential for bacteria from a financial and environmental standpoint. Heavy metal emissions from industrial uses become a big environmental problem such as Cd, Cr, Cu, and Hg. The hazardous heavy metal chromium is commonly used in the metalworking, tanning process, textile coloring, and electroplating manufacturing sectors. Several microorganisms, including *Desulfovibrio vulgaris*, *Arthrobacter*, *Pseudomonas* sp., *Serratia marcescens* and *Ochrobactrum* sp. have been seen to convert highly soluble and poisonous Cr (VI) into lower soluble and fewer toxic Cr. (III). *Arthrobacter psychrolactophilus* sp. 313 lowered the protein content of wastewater. They are utilized for the sewage from industrial facilities. In the course of the treatment, a variety of different bacteria, including *Pseudomonas* and *Bacillus subtilis*, were employed (Mishel *et al.*, 2023).

1.9.2.2 Applications of oxygenic photosynthetic bacteria (cyanobacteria in bioremediation)

Cyanobacteria are frequently used in wastewater purification for the removal of metal contaminants, petroleum-based chemicals, and colorants. Since cyanobacteria need nitrogen for their metabolic processes, they are usually quite effective at removing nitrate. To get rid of the nitrate-producing *Synechococcus* sp. strains and *Synechocystis minima* CCAP148014, distinct cyanobacterial species were used. Phosphorus and nitrogen were successfully extracted by the *Phormodium tenue* strain and the *Phormodium bohneri* strain. The cyanobacteria strains *Anabaena variabilis*, *Anabaena oryzae*, and *Tolypothrix ceytonica* were additionally treated using a mixture of domestic and commercial effluents. Organic detritus has been eliminated, respectively, by *A. variabilis* and *A. oryzae* were used to remove particles. Heavy metals such as copper, cobalt, manganese, zinc, and lead have been recovered from sewage wastewater by the plants *Anabaena subcylindrica* and *Nostoc muscorum*. Copper was obtained using *Nostoc* PCC 7936 and *Cyanospira* capsule. (II). Zn and Cu were both removed by *T. certonic*. Crude oil was effectively degraded by *Oscillatoria salina*, *Aphanocapsu* sp. *terenbans*, and *Plectonema*. *Nostoc ellipsosporum* strain B1453–7 and *Anabaena* sp. PCC 7120 both broke down lindane contaminants. It was discovered that *Oscillatoria formosa* NTDM 02 was particularly efficient at removing dye from the textile sector. Bioremediation using anoxygenic photosynthetic bacteria *Rhodobacter sphaeroides* Z08 was introduced to the PPB during the treatment of pharmaceutical wastewater. *R. sphaeroides* IL106 also eliminated phosphorus. The *Rubrivivax gelatinosus* strain SS51 and SY40 was able to break down the organic pollutants in the wastewater used to make latex. *Rhodobium marium* NW16 and *R. sphaeroids* KMS24 have been discovered to be effective heavy metal extractors. *R. palustris* WS17 was utilized to degrade pesticides. Used in the fading of colors is Purple non-sulfur Bacteria (PNSB), *R. palustris* ASI.2353 (Mishel *et al.*, 2023).

1.9.2.3 Algae

Algae are crucial to the process of naturally purifying water. They can be utilized for recovering precious metal ions such as gold and silver as well as for the sorption of toxic and radioactive metal ions. They contribute to the clean-up of nutrients by growing quickly and assimilating C, N, and P from wastewater. It is an alternate method of treating sewage effluent that is also cost-effective and environmentally friendly. Some researchers have acknowledged the practical application of microalgae for obtaining nutrients from various wastes to stop potential deterioration of the water quality of wastewater. Textile wastewater (TWW) contains organic dyes as well as the minerals (phosphate, nitrate, micronutrients, etc.) required for the growth of algae (a potential source of carbon). Clothing wastewater can be effectively bioremediated using microalgae that can utilize nutrients and dyes (TWW). Microalgae use the colors and nutrients in wastewater for their growth. Wastewater can be bioremediated using a culture of *Chlorella vulgaris* and *S. quadricauda*. Citric acid and diluted ethanol from the wastewater industry are produced using *C. vulgaris*. It accelerates the decline in effluent BOD and COD concentrations. *C. vulgaris* and *S. quadricauda* were used to remove nitrate. *S. quadricauda* successfully extracted the phosphate. During growing, *C. vulgaris* uses phosphorus to eliminate phosphate. Clothing wastewater is bioremediated using *C. vulgaris* strain UMACC 001 (TW). Both viable and non-viable algae have been used to remove color from dyes and wastewater. Two techniques, namely bioconversion and bioaccumulation, or biosorption operation, are used to bioremediate TWW utilizing microalgae. During the bioconversion process, these colors are ingested by microalgae as a source of carbon and converted to metabolites. Microalgae can nonetheless function as a biosorbent and attract the pigments to their surface. In simultaneous TWW bioremediation, both outcomes are conceivable. The aggregation of microalgae may be caused by adsorption, enzyme breakdown, or both. Microalgae have a high sorption potential due to traits like their enormous surface area along with powerful attraction to binding to azo dyes. The cells biosorb metal ions on the top of dead algae's cell membrane. It may be more effective, less costly, and environmentally safe to remove heavy metal ions from wastewater using an algae-based biosorption process. The removal of reactive dye from TWW has been proven to be possible using the nonviable biomass of *Spirogyra*. Basic dyes can be removed by living material from macroalgae such as *Caulerpa scalpelliformis*. Certain algae, like *Phormidium*, have the ability to bioconvert colors into simpler chemicals. Immobilized algae are used to draw color from the surface of textile pigments. Both *Spirulina* and *Chlorella* are effective at cleaning up sewage. Some of the microalgae that can convert azo dyes into simple amines include *Scenedesmus bijugatus*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, and *Oscillatoria tenuisin*. *Chlorella* sp. and *Scenedesmus* sp. microalgae G23 and *Cosmarium* sp. were commonly used to treat various types of wastewater because they are more efficient (Mishel *et al*., 2023).

1.9.2.4 Fungi

Heavy metals are generally consumed by filamentous fungi. Utilizing the ability of fungal biomass, biosorbents may be used to remove radionuclides and heavy metals from contaminated waterways. White-rot fungi like *Pycnoporus sanguineus laccase* and *Phanaerochaete chrysosporium* can all devastate straw, sawdust, or corn cobs. Metal ions are taken up by *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Saccharomyces*, and *Fusarium*. Utilizing *Penicillium*, heavy metals can be bioabsorbed (Cr, Pb and As). Radionuclides can be bioabsorbed by the microbes *Penicillium*, *Rhizopus*, and *Saccharomyces* (U, Th, and Sr). For the bioremediation of distillery wastewater, *Trametes pubescens* MB 89, *Pycnoporus cinnabarinus*, and UD4 were used. White-rot fungus can degrade high-intensity phenolic wastes. Fungi known as white-rot fungi break down industrial waste. To decolorize and reduce the COD of molasses wastewater, it may be feasible to use the organisms *Funalia trogii* and *Pleurotus pulmonarius*. White-rot fungi, which include edible mushrooms like *Lentinula* and *Pleurotus*, as well as several yeast species can all be utilized to treat olive oil mill wastewater (OMWW). They reduce OMWW color, phenolics, and COD. White-rot fungi, such as *Coriolus versicolor* and *Funalia trogii*, *Geotrichum candidum*, *Lentinula* (*Lentinus*) *edodes*, and *Phanerochaete* sp., were employed for the remediation of OMWW. Most viable possibilities are fungi that play a key role in bioremediation. White-rot fungi are one of them and provide a number of benefits for environmental pollutant degradation. Endosulfan-degrading fungi such *Aspergillus terreus*, *Cladosporium oxysporum*, *Mucor thermohyalospora*, *Fusarium ventricosum*, *Phanerochaete chrysosporium*, and *Trichoderma harzianum* are utilized. Fungi of the Zygomycetes class and *Aspergillus*, *Mucor*, and *Penicillium* were employed to breakdown and detoxify textile effluent and crude oil. For the breakdown of polychlorinated biphenyls, *Penicillium chrysogenum* and *Fusarium solani* are utilized (PCB). Pinus is combined with *Rhizopogon roseolus*, *Suillus bovines*, and cadium extraction. A few plant-related fungi are used to decolorize textile industry waste (Mishel *et al*., 2023).

1.9.2.5 Yeast

Yeast helps with absorption of heavy metals. In OMWW bioremediation, yeasts including *Trichosporon cutaneum* and *Saccharomyces* sp. are employed. Yeasts are successful at removing mono- and polyphenols from the body as well as lowering COD levels. Because harmful chromophores can be absorbed, collected, and degraded into simpler compounds, these are used to clean TWW. They can be used as biosorbents for dye biosorption and contain enzymes for pigment breakdown. Yeasts like *Candida krusei* and *Saccharomyces cerevisiae*, and so on, were used to break down colors and other compounds (Mishel *et al*., 2023).

1.10 ACTIVATED SLUDGE PROCESS

The activated sludge system must also disperse the microbes using an efficient mixing system in order for them to come into direct contact with all of these substrates. The microbes must also mature into floes, three-dimensional aggregated microbial populations. This is perhaps most crucial. These flocs must possess excellent settling properties in order to successfully separate from the liquid supernatant in the clarifiers. It is necessary to give enough O_2, which is expensive, in order to mix the biomass in the reactor and supply what is needed by the aerobically respiring organisms that are in charge of breaking down these substrates. The respective advantages of the various widely used aeration techniques and the associated health risks related to aerosol production. The strength or concentration and composition of the wastes change significantly hourly, daily, and seasonally, and the ASP must be able to manage significant variations in flow rates that have a knock-on impact. The metabolic activity and rate of microbial development will be greatly impacted by similar variations in the temperatures of mixed liquor. The process's by-product, microbial detritus, also referred to as sludge, is still a resource that is vastly underutilized in most countries. Given all of these erratic factors, it can be considered astonishing that activated sludge facilities operate as efficiently as they do. Conventional plants' basic design has not changed much, but there is a general consensus that they will likely remain the most popular systems for aerobic waste treatment well into the 21st century due to their dependability, adaptability, and versatility. A rectangular basin with motorized surface agitators or submerged diffusers providing the mixing and aeration still makes up the bulk of aerobic reactors. The clarifier is still used to inoculate the inbound raw wastes with the majority of the separated biomass or sludge, which is separated from the liquid supernatant after the effluent mixed liquor is passed through it, the main factors influencing creativity and change in activated sludge plant design. When these systems were first developed, their primary objectives were to remove carbonaceous material from residential sources that contained naturally existing organic compounds and to produce a treated liquid effluent with low enough BOD and suspended particle levels to be securely discharged into another body of water. The so-called 30:20 standard for BODs and 55 as the allowable limit for treated effluent in conventional plants are adopted by many countries. The effluent had to meet standards for reducing NH_3 level later because it was believed that NH_3 was more hazardous to fish than NO^{3-} and as a consequence, plant design was altered to encourage nitrification. In response to the public's growing concern over eutrophication's long-term effects on the environment over the past 20 years, plants that can microbiologically extract both nitrogen (N) and phosphorus (P) are presently being created. It is expected that as governmental bodies impose stricter requirements for effluent quality in response to public pressure, these occasionally expensive devices will become more common. The addition of more reactor tanks alters the environment in a number of ways, especially in terms of the biomass's availability to oxygen, which encourages the development of particular physiological groups. For example, denitrification is enabled by the incorporation of anoxic zones with low DO levels but abundant NO^{3-} and the existence of anaerobic zones with low levels of both NO^{3-} and O_2 gives the bacteria required to break down phosphate (P). Activated sediment testing was done in batches in the laboratory. Two-liter laboratory dividers made of Plexiglas were used. To inoculate the wastewater, activated sludge from a plant that treats domestic sewage was used. Every day, the aeration was turned off to enable the sludge to settle. Then, the supernatant was taken out and fresh wastewater was poured into the column. Until a significant amount of modified sludge was produced, this procedure was repeated. Numerous studies were conducted to find out how the aeration time affected the activated sludge. One such example is a different column that already contained a predetermined quantity of sludge (3–4 g/L) was added to the pre-treated wastewater. One hour to twenty-four hours of confinement were examined. Adjustments were made to maintain the DO content at a minimum of 2 mgO_2/l. The characterization of the treated wastewater and sludge analysis were conducted after 60 min of settling (Seviour *et al.*, 1998).

1.11 CONCLUSION

Understanding the role performed by the microbial community structure of the organisms participating in the treatment processes is essential and crucial for having a better understanding and control measures of wastewater treatment operations. Enzymes produced by microorganisms that participate in the breakdown of environmental pollutants are typically quite selective in the substrates they catalyze (Jatoi *et al.*, 2020). Microorganisms can, however, develop new enzymes to manufacture energy and nutrients from other substrates or under new growth conditions following an acclimation period when they are exposed to new growth conditions or substrates (Gude, 2016). To use the potential of the microorganisms for bioremediation, it is crucial to control their activities

(Ojhaa *et al*., 2021). To ensure affordability while maintaining public health protection, wastewater must be appropriately treated. This is a crucial factor to take into account because recycled water would not be accepted without it. Customers will utilize the least-expensive water available, though, if it is too pricey. Treatment to remove or inactivate pathogens is one of the biggest expenses involved in producing reused water. Although chemical contaminants are also significant, health authorities frequently concentrate on those that cause acute disease, particularly when wastewater is reused and prolonged human exposure is unlikely (Krantz and Kifferstein 1996). To provide the best possible treatment for reducing the danger from pathogens in wastewater, it is necessary to understand how pathogens behave during the wastewater treatment and disinfection processes as well as the variables that affect these processes. Association with particles is one of the main elements that affects how pathogens behave in water. Understanding the nature of the pathogen is crucial. This presents a chance to improve wastewater treatment while lowering treatment costs: reduced amounts of association can improve disinfection, whereas higher levels of association can improve removal via sedimentation processes. This strategy seems to combine the benefits of each of the technologies that were previously discussed while attempting to get around the problems of nutrient scarcity and a lack of petroleum-degrading bacteria. It turned out to be an interesting detour because it offers the possibility of an economically viable and scientifically advantageous method through the use of locally accessible support material and bacteria that have been isolated from prior contamination. Moreover, the local biota, nutrient availability, and other environmental parameters crucial for getting the greatest results have to be considered in an efficient restoration method. It was thought that bacteria might have an enhancing effect on the biodegradation of persistent organics in the bioremediation of persistent organics-contaminated soil and particularly groundwater environments by increasing the bioavailability of these pollutants to microorganisms. Last but not least, a combination of technologies that are strictly regulated and given enough time will prove to be quite significant. Biological treatment techniques have frequently been regarded as the most comprehensive, environmentally friendly, and economically advantageous treatment solutions.

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Chapter 2

Elucidation of omics approaches and computational techniques for wastewater treatment: A deep insight

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ABSTRACT

Ecological contamination has been growing rapidly over the past few decades. Bioremediation, a microbial degradation process, is considered to be one of the most ecological and environment safe and financially friendly remediation methods for ecological parts polluted with chemical-based contaminants. Toward this pursuit, the traditional approaches are skimpy, and individuals must enthusiastically think about the current developments in high-throughput 'omics' technologies to remove hazardous and highly polluted contaminants from the environment. The sequencing of various microbial genomes and their functional studies along with metabolic pathway reconstruction and systems biology provide strategies to enhance the degradation and decomposition of pollutants. The computational techniques and omics approaches provide genome-based analysis of the microbial genomes including genomics, proteomics, and system biology along with various tools to determine the structural and functional aspects of microbes and their biodegradation pathways. The current chapter gives a deep insight into the status and advances on different computational techniques and omics approaches to enhance the process of bioremediation. It is a great step against the degradation of the environment and restoring natural resources.

Keywords: bioremediation, bioinformatics, omics, genomics, proteomics, systems biology, data mining

2.1 INTRODUCTION

Cleaner production (CP) is the process of successfully utilizing resources and energy, as well as removing hazardous raw materials and reducing the toxicity of all emissions and solid waste. It is the most proactive technique for reducing waste, negative environmental effects, and health concerns by using energy and natural resources in a more organized and efficient manner (Giannetti *et al.*, 2020). On the contrary, the obligation to conserve natural resources and maintain global ecosystems in order to support health and well-being today and in the future is known as environmental sustainability. In 2015, the United Nations highlighted the Agenda 2030 for sustainable development goals (SDG), which emphasized overcoming any deficiency linked to the policies, strategies, and applications that may otherwise put a roadblock in the path of achieving CP through SDG (UN, 2015). Therefore, there is a pressing need to establish more sophisticated research tools and techniques that may play an inter/multidisciplinary role to address the issue related to CP leading to achieving the future concept of sustainable development goals.

Currently, activities carried out by humans can create and generate a lot of toxic chemical waste because of the increase in industries and factories which release toxic waste into the environment. These activities have led to an increase in pollution in the land soil and water because of the toxic fumes released by this chemical waste that affects the air as well. Even after implementing various rules for managing, maintaining, and disposal of this waste, hundreds of such cases are being reported daily for mismanagement of toxic chemical waste (Umadevi *et al.*, 2015). This pollutant contaminating the environment has drawn attention across the world, taking into account their unwanted slow degrading compounds. Several xenobiotics and aromatic hydrocarbons along with choro- and nitro-aromatic compounds have raised concerns about the environment changing them into highly toxic mutagenic and cancer-causing agents among living organisms. A variety of microorganisms are to be considered as the best medium among other organisms for the procedure to bioremediate the environmental contaminants into the geological biochemistry due to their diverse, versatile character, and adaptability in adverse conditions (Fulekar & Sharma, 2008). These microorganisms have been continuously able to display a great range of contamination which happens to be the ability to degrade, restore natural and raw environmental conditions. It has been shown, however, that many toxins are abnormal, that is, microorganisms do not modify or modify some other metabolic compounds that can accumulate them into the environment. So that makes it important to study other forward pathways that are leading towards complete mineralizing of the pollutants (Mohanta *et al.*, 2015). Bioremediation is considered to have a great potential to recover and regenerate the environment from contamination and pollutants, but there has been a great lack of information regarding the factors which control the growth and metabolism among the considered microorganism in the pollution-ridden environment more often have been able to act as an obstacle to limit the implementation of the bioremediation process Garg (2020). The schematic representation of biodegradation process is presented in Figure 2.1.

2.2 BIOREMEDIATION

Bioremediation can be defined as the process in which microorganisms are made to rapidly remove the unwanted dangerous organic pollutants which affect the ecological levels in soil, sediment, chemical substances, and groundwater (Singh *et al.*, 2021). Biodegradation is a natural process to recycle waste to nutrients that can be used by other organisms. Degradation is caused by microbes that consume nutrients such as carbon, nitrogen, and phosphorus from pollutants that are subject to long-term adaptation turn toxins into a friendly environment. By utilizing the energy of biodegradation, people can reduce waste and purify environmental pollution. Digestion accelerates energy degradation and converts organic matter into energy-efficient products (Vishnoi & Dixit, 2019). The mechanism of bioremediation is presented in Figure 2.2.

Water pollution treatment continues to break down old energy for degradation and harm organic things so that the release of water into the environment does not cause pollution problems. Bioremediation uses microorganisms to clean up oil spills and other contaminants. In the case of bioremediation, therefore, it provides the technology to eliminate pollution by promoting the same destructive process that occurs in nature (safe, cheap, and appropriate treatment) (Chakraborty *et al.*, 2012; Rawat & Rangarajan, 2019). Bioremediation on infected sites usually works in two ways (a) support the growth of contaminants consumed by microorganisms in the contaminated area, (b) special microorganisms have been added that break down contaminants.

2.3 BIOREMEDIATION AND OMICS

Bioremediation can be considered as an umbrella concept that can take in and covers multiple stage binders belonging to different groups transport toxic waste from contaminated sites. There is a lot of

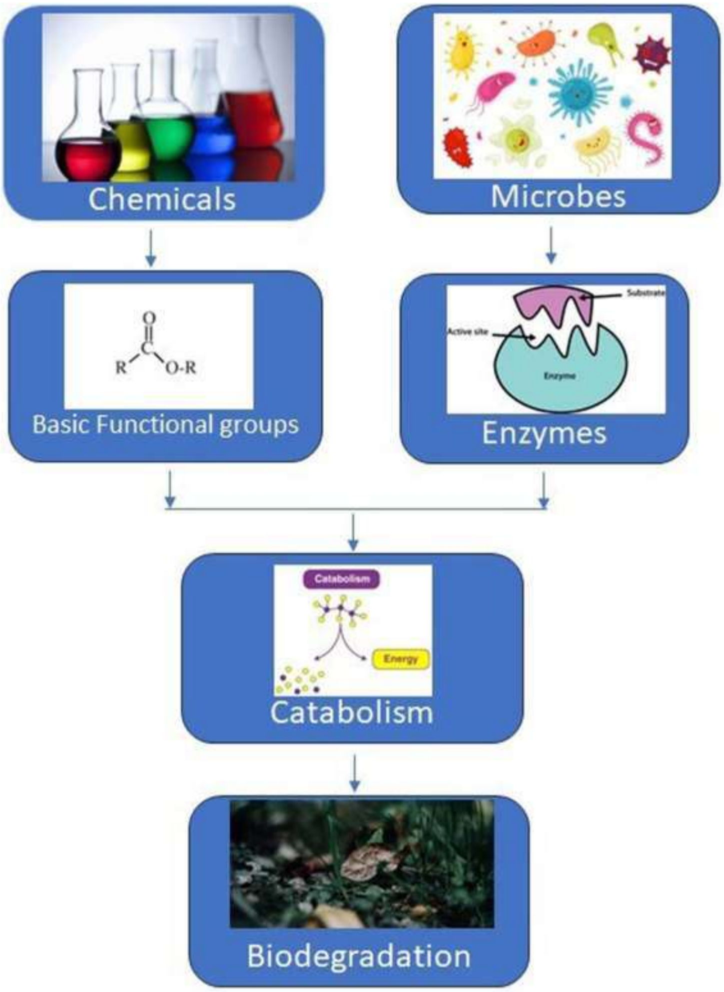

Figure 2.1 Schematic representation of biodegradation process.

information out there for tech support tools, and it is part of the process across all places. This includes distribution/management of chemical toxins, chemical composition, and environmental properties of these chemicals, microbial enzymes, metabolic agents, and degree of degrading action level (Rawat & Rangarajan, 2019).

Bioremediation, in contrast to these approaches, is a long-term and cost-effective way for removing hazardous contaminants from our environment using a diverse range of microorganisms. However, there are certain drawbacks to bioremediation, such as the fact that it takes a long time and has a narrow action range. As a result, significant work will be required to make the process quicker, more effective, and capable of acting on a wide spectrum of organic contaminants and heavy metals (Zhang *et al.*, 2010). *In-silico* bioremediation process is presented in Figure 2.3.

On-site examination of uncultivable microorganisms is now possible because of the development of culture-independent genomics tools. Other 'omics' platforms, like transcriptomics, proteomics, metabolomics, interactomics, fluxomics, and so on, can observe dynamic variations (in terms of mRNA, proteins, and metabolites) occurring in a cell over time. However, a single 'omics' study is insufficient; for example, transcriptomics can measure the total mRNA present in a cell at a particular moment but does not provide information on the amount of expressed proteins, their cellular location, biological activity, or other factors (Chen *et al.*, 2005). Bioremediation is a complicated biological process that

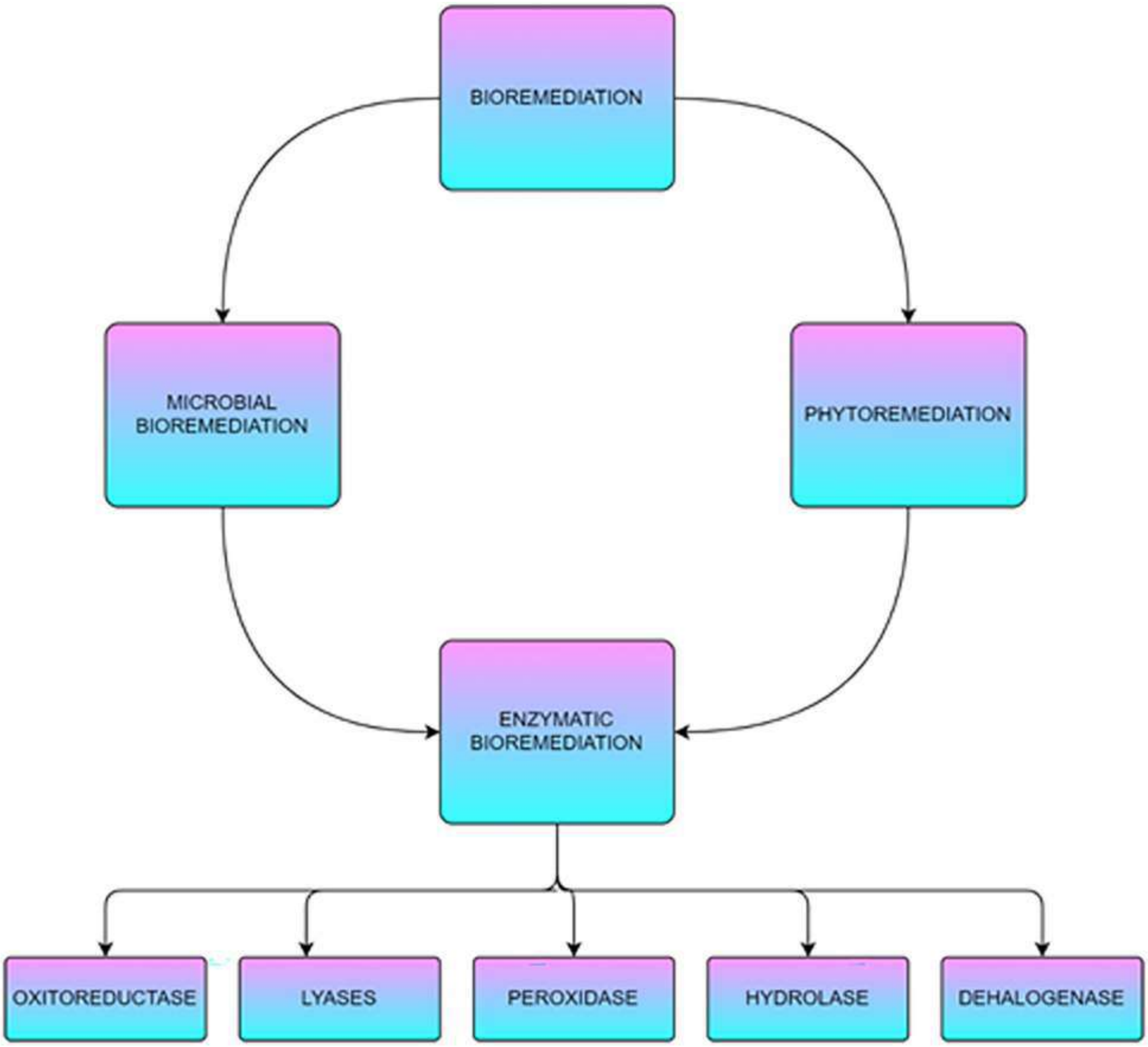

Figure 2.2 Overview of bioremediation highlighting enzymes.

involves several metabolic pathways and interactions between the entire microbial population present at a polluted location, rather than just individual bacteria. Multiple-omics research, rather than single 'omics' approaches, can give a better knowledge of the metabolic and regulatory mechanisms of microbial bioremediation, allowing such complex biological systems to be fully characterized.

2.4 BIOREMEDIATION AND GENOMICS

Non-molecular technology: Currently, most microbiology of bioremediation processes uses 'readability studies'. In this study, samples from contaminated environments and other contaminants are taken to the laboratory and the degree of deterioration or degradation is documented resulting in the immobilization of pollutants (Singh & Nagaraj, 2006). These studies assess the potential metabolic activity of microbe populations, but biomedical causative microorganisms or specific stimulants may or may not be considered when performing bioremediation.

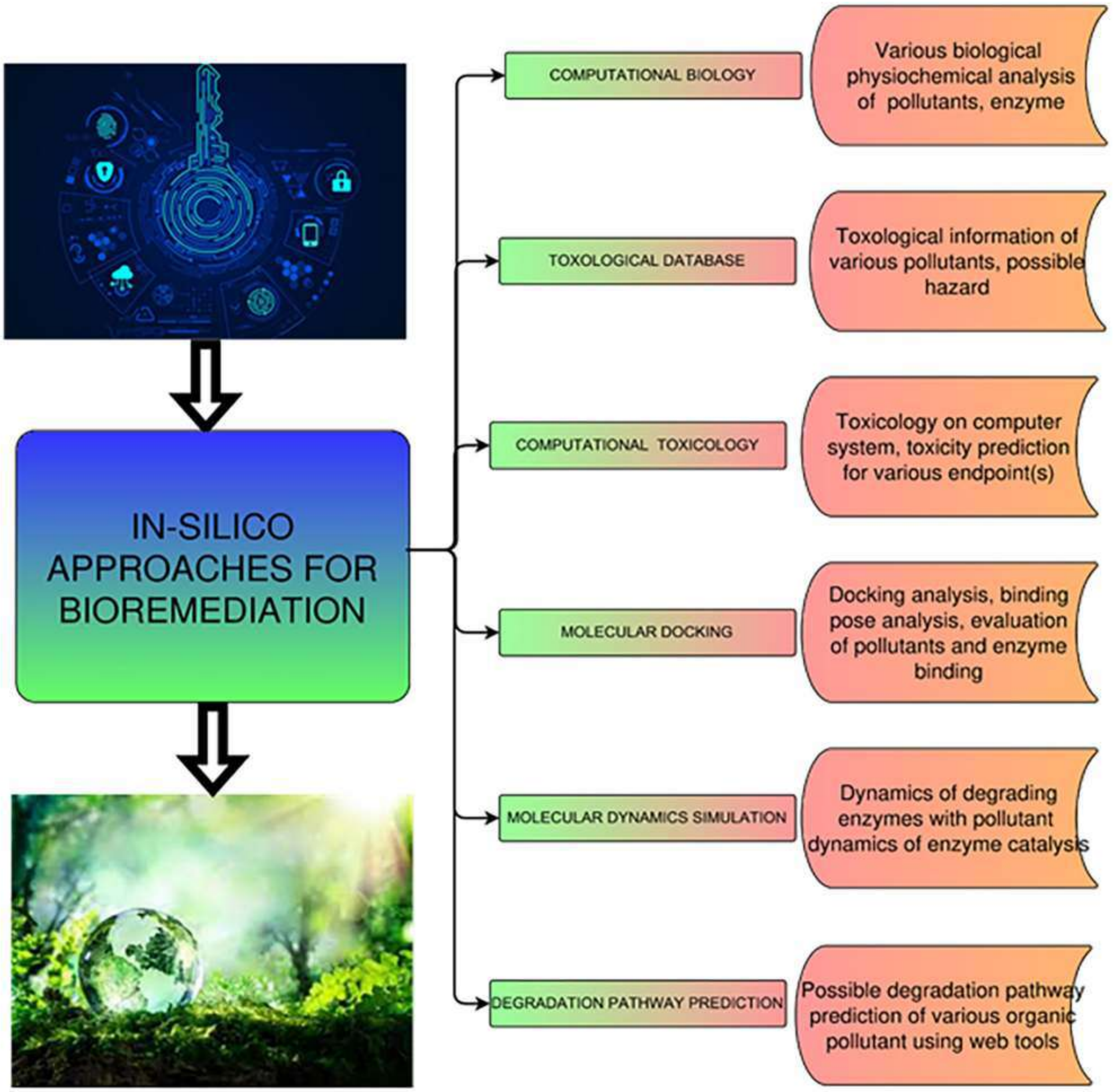

Figure 2.3 Brief outline of *in-silico* strategies for bioremediation process.

The developmental process of the body was thoroughly studied and bioremediation is usually treated because of the efforts of our ancestors. The isolation of pure culture and the voluntary work of such men remain important for the development of molecular analysis and interpretation. Recovery of criminal isolates of microorganisms. Bioremediation processes can be unique because, as described below, the monitoring of these isolates provides an opportunity to study not only the biodegradation process but also the physiological side that can control their biodegradation processes (Eyers *et al.*, 2004). Evolutionary methods are much more useful for dual-fuel adaptation of the biodegradation process than the step-by-step variation introduced by rational conception. Similarly, recent advances in genome switching between species allowing the exchange and reproduction of different traits of the same species have accelerated the search for new microorganisms that can compound-complex contaminants (Lovley, 2003).

2.4.1 *In-silico* toxicity of the compounds

Toxicity of a compound in the environment is important to be contained to a level where it is tolerable in the environment, however, knowledge about the levels of toxicity in the given compounds, are very important to be considered to further develop technologies and research to find out the level of toxicity of the chemicals before focusing on the bioremediation process (Parthasarathi & Dhawan, 2018). Thus, there has been development in the fields of omics and bioinformatics to make a system of database and tools developed for determining the bioremediation technology of any chemical. The pharmaceutical industry has developed and ingrained several *in-silico* procedures to understand the pharmacodynamic, pharmacokinetic, and toxicological profiles of compounds. Figure 2.4 presents the *in-silico* approaches to predict toxicology.

The following list gives a summary of different homology models, pharmacophores, molecular modeling approaches, databases, toolbox, methodology, and so on, which is useful in calculating and knowing the toxicity of the chemical compounds (Khan *et al.*, 2013).

Tools for toxicity prediction:

(1) HazardExpert (CompuDrug) (http://www.compudrug.com/)
(2) OncoLogic (USEPA) (http://www.epa.gov/oppt/st/pubs/oncologic.htm)

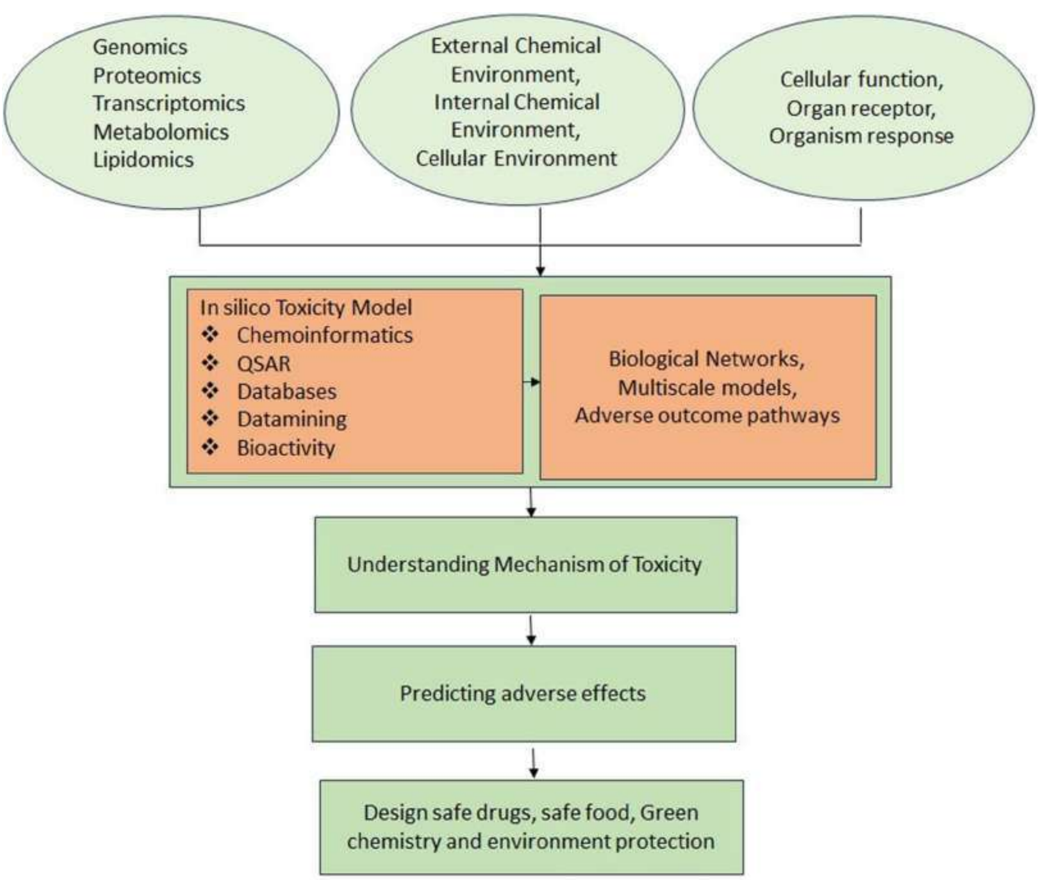

Figure 2.4 *In-silico* approaches to predict toxicology.

(3) MolCode Toolbox http://www.molcode.com
(4) ACD/Tox Suite (ToxBoxes) (http://www.acdlabs.com/products/pcadmet/tox/tox/)
(5) ADMET Predictor http://www.simulations-plus.com/Products.aspx?pID=13
(6) Derek (Lhasa Ltd) (http://www.thasalimited.org/)

Database for toxicity prediction

(1) Acutorbase https://acubase.amwaw.edu.pl
(2) SuperToxic http://bioinformatics.chante.de/supertoxic/index.php?site-home
(3) Terra-Base http://www.terrabaseinc.com/
(4) Carcinogenic Potency Database http://toxnet.nlm.nih.govicg-tinsis/htmlgenCPDB.htm

2.5 SYSTEM BIOLOGY APPROACH IN BIOREMEDIATION

In a contaminated environment, bioremediation entails applying a variety of chemical structures to a complex multispecies metabolic network. The complexity of such processes is becoming increasingly accessible to systems biology's conceptual framework and techniques. The accessibility of biodegradative microorganism genes, genomes, and metagenomes allow scientists to analyze and even forecast chemical fates using the comprehensive metabolic network that comes from linking all known biochemical transactions. The freely diffusible metabolic pool shapes the landscape of pan enzymes that microbial communities embody (Chakarborty *et al.*, 2012). Figure 2.5 is describing the three dimensions to define the activity of each bioremediation process (Roy & Kar, 2016). New computational resources are increasingly helping to develop higher biocatalysts due to the biodegradation and biotransformation of desired chemicals, which are beneficial to the developing field of synthetic biology. Scenarios play a role in which the body consists of several factors. Various physical conditions like flow voltage, electron acceptor, and other properties, diverge in catalysis. These abiotic surroundings over the configuration of the ancestral and bacterial population and the availability of a particular species. However, it is necessary to consider the different levels of combination that differentiate the occurrence of different types of catalytic activity in individual genomes in order to characterize the extensive application of these catalysts to attack humans (Lovley, 2003, Pinto *et al.*, 2021).

2.6 METAGENOMICS IN BIOREMEDIATION

It includes the investigation of the general genes of specific habitats delivering straighten trees to all basins of ecological genes without restriction linked to laboratory cultivation of microbial species.

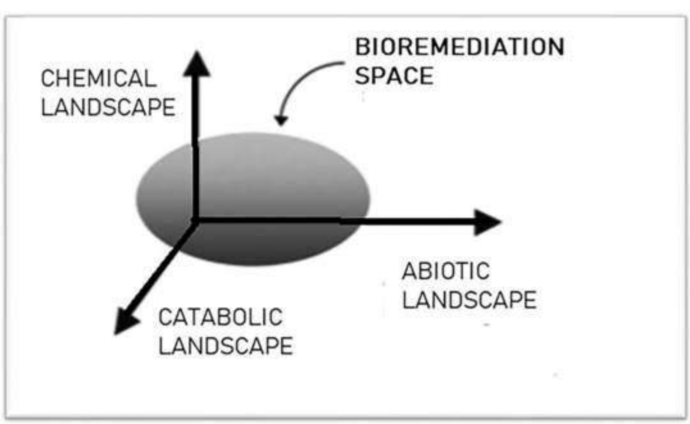

Figure 2.5 Bioremediation space showing chemical, catabolic, and abiotic landscapes.

Metagenomics offers an excellent opportunity to restore unknown processes or sequences in the environment. It is very hard to describe differences among DNA genetics from complex environmental problems, such as soil, fertilizer, soil, or soil, in terms of quality, litter, and full access to microbial species and other species (Thomas *et al*., 2012). Research guidelines for metamorphic libraries can be adapted to study functions and/or different types. By applying interest, clones are examined or selected, and heterologous markers of the desired character are obtained (Cowan *et al*., 2005). Library research is facilitated by autonomous collections system of the microbial colony, robotic pipelines, microtiter plates, database-assisted data management, and complex analysis focusing on multiple biomolecules based on the sensor array. Due to several constraints, such as entry size, heterologous expression of cloned cells, as well as post-transcriptional (relative to post-translational material) and the secretion of foreign proteins, 'hit' you are very limited.

However, the filtration and screening rate can be increased by the following ways:

- Using the host with different expressions.
- Develop strategies to use variety/social genomes to first choose the desired brand building and then the metagenomic libraries.
- Experience and humidity allow simultaneous testing practices of the cultures to filter out the different clones.
- Develop new areas of research for high-throughput screening.

Sequence-based analyses are mostly applied to identify members of known gene families or new versions of known protein functional classes. Extensive DNA input libraries offer more possibilities to recover a complete set of functional genes and combined them with potential metabolic functions among a specific microorganism (Daniel, 2005). Although shotgun sequencing may be useful for comparing different media, it is not known whether this sequence will provide information about certain genes and functional interactions. The 'omics' approaches involved in bioremediation is presented in Figure 2.6.

2.7 MICROARRAY ANALYSIS IN BIOREMEDIATION

The ability of a single-stranded DNA or RNA molecule to hybridize with a probe connected to a solid support is the basis for microarrays. The presence of single mismatch probes on the array ensures probe–target specificity, allowing sequence-specific signals to be distinguished from nonspecific signals (Gentry *et al*., 2006). In environmental genetics, the following three chief classes of microchips have been established: Phylogenetic oligonucleotide arrays (POAs),

(1) Functional gene arrays (FGAs),
(2) Community genome arrays (CGAs).

The search form for POAs and FGAs types is dependent on the sequence program loaded from databases. These subclasses do not have access to functions related to unidentified functional activities and phylogenetic correlations. Given the large reservoir of molecular sequences, which is unknown to the environment, the main weakness lies in the 'traditional' microarray analysis (Gentry *et al*., 2006). Conditions for using the infringement adjustment service, which allows you to find the clone that has genes that have been expressed (or repressed) because of the therapy. The next stage will be to integrate metagenomics with microarray using probes derived directly from the DNA environment without any or any culture step. As the competition progresses, use the information that the first one does not require much to hope for, but the metagenomics array (MGA) technology has enormous promise for high-throughput screening of natural settings, of the microbial communities being examined (Zhou, 2003). The purpose of this microarray element is to provide a direct link between biogeochemical processes of microbial communities and their functional activities in different environments.

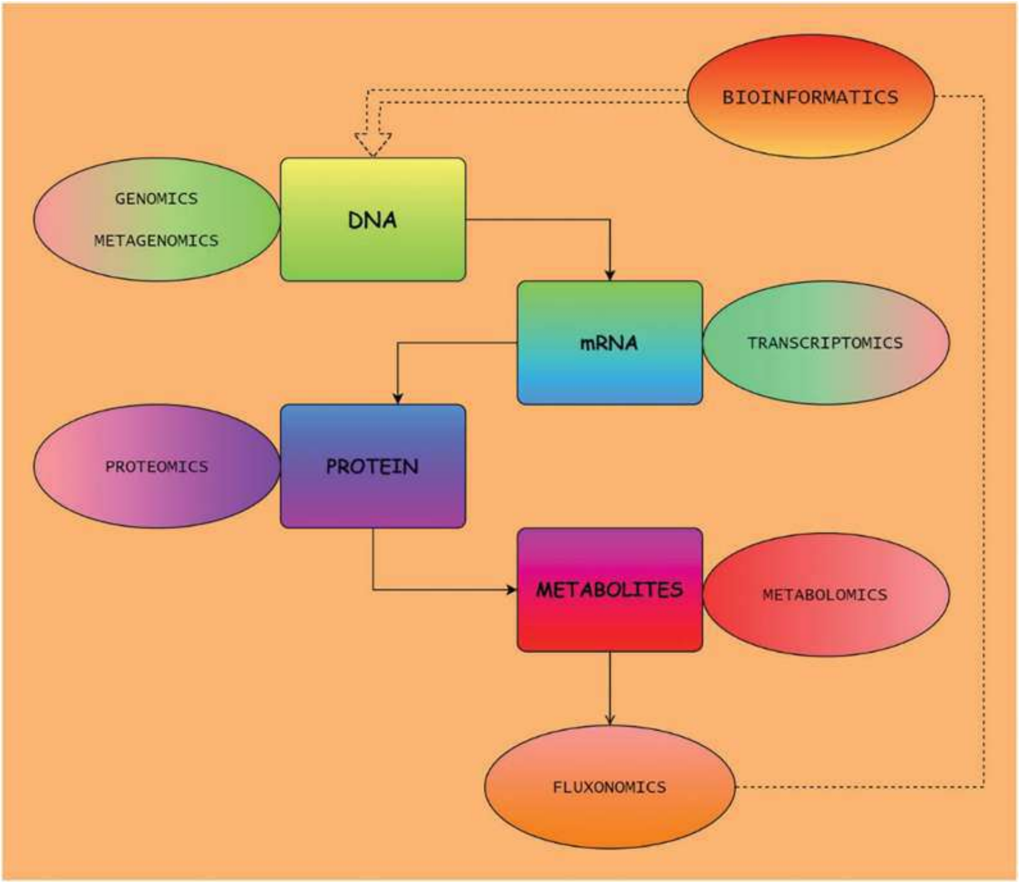

Figure 2.6 Schematic view of 'omics' approaches involved in bioremediation.

2.8 SINGLE CELL SEQUENCING APPROACH IN BIOREMEDIATION

DNA sequencing from single cells has provided new insights into the world of bacteria. Without creating a culture, it is becoming more common to identify bacterial species directly from clinical samples. Recent technological developments often lead to the almost complete completion of genomic data for this sometimes-inaccessible species. New bioinformatic technologies also facilitate the assembly of single-cell genomes. The usage of single-cell sequencing along with metagenomic analysis is proving to be an effective new strategy for the analysis of bacterial communities (Lasken, 2012).

2.9 NEXT-GENERATION SEQUENCING IN BIOREMEDIATION

The next-generation sequencing (NGS) platform can create enormous amounts of monoclonal and digital DNA data in parallel. An increasingly convenient solution at a lower sequence price tools will be provided to the environmental science community. For example, NGS has revolutionized environmental science and contributed to metagenomics and meta-transcriptomics (Bihari, 2013). In various molecular microbiology laboratories, state-of-the-art technologies are playing the same role in bioremediation and biodegradation as they enter the omics age. Gigabytes of monoclonal and digital DNA data can be generated simultaneously on modern NGS machines. As the cost of sequencing in the environmental science community drops, less-expensive tools and solutions are offered (Singh, 2006).

2.10 METAPROTEOMIC IN BIOREMEDIATION

The term 'metaproteomic' refers to the study of proteins. When compared to the process of genomics for the functional examination of a colony of microorganisms, studying the total protein content of a specific environment has more promise. Protein prediction and further identification are highly in facilitation with the relevant metagenomic sequence data available. As expected, there was a discrepancy in the proportion of proteome proteins associated with the same function as the genes in the function-related genomes of each protein function category (Singh, 2006).

MS advancements have enhanced peptide analysis for protein identification and aided in the development of the area of environmental proteomics. MS determines the composition of molecules by measuring the peptide mass that comes from certain amino acid pairings. Proteins are frequently digested using proteases to yield peptides short enough for MS analysis, referred to as PMF, in order to examine these amino acid combinations. Controlled peptide fragmentation gives a succession of overlapping fragment ions that differ by the mass of specific amino acid, allowing the entire or partial amino acid sequence of the peptide to be determined. Because one MS analyzer is often used to pick samples, this phenomenon is also known as MS/MS or tandem MS (Wilmes & Bond, 2006).

MALDI–TOF–MS is commonly used to detect and identify viruses, bacteria, fungal spores, and low-mass chemicals in environmental samples, as well as to identify proteins of interest from 2-D gels. Many site-specific bacteria may be detected using the complicated mass spectra of environmental samples, which can be utilized to develop characteristic fingerprinting databases. MALDI–TOFMS can identify certain bacterial signature proteins and biomarkers (primary and secondary metabolites) from site-specific samples for taxonomic identification of possible microorganisms in the context of bioremediation. Another interesting analytical technology for site-specific samples is MALDI–TOF–MS–SELDI–TOF–MS, which is a type of direct sample analysis on a microchip. The researchers looked at several differentially expressed signature proteins in blue mussels (*Mytilus edulis*) exposed to PAHs and heavy metals (Lay, 2001)

SELDI–TOF–MS. Although SELDI analysis has been effective in clinical research for discovering possible biomarkers, its repeatability and specificity have been questioned. Another new technique is ESI MS/MS used to detect different epoxide hydrolases, peroxisomal antioxidant enzymes, and sarcosine oxidase from a peroxisomal protein pattern associated with marine pollution exposure. Thus, the identification of proteins/enzymes from any site-specific bacterium using 2-DE and MS might open the door for cell-free bioremediation in the future (Mishra *et al.*, 2021).

2.11 META-TRANSCRIPTOMICS IN BIOREMEDIATION

Meta-transcriptomics refers to the analysis of the transcriptome collectively amongst all the microorganisms of a certain specific habitat. Researchers have led to the development of a transcriptomics approach based on the collection and analysis of microbial transcriptomes among marine and freshwater microbial organisms. Environmental transcriptomics procedures provide promising tools for the study while expressing natural genes for the organic microbial communities without biasing known sequences. This easily applicable high-throughput approach has not been tested for the process to analyze microbial communities in contaminated areas (Futschik *et al.*, 2018; Malik *et al.*, 2021).

2.12 METABOLOMICS IN BIOREMEDIATION

Metabolomics is the study of every primary and secondary metabolite with a low molecular weight (b1000 Da) found in and around cells developing under certain physiological circumstances. Metabolomics is the study of the end products, or metabolites, generated by a living organism under certain circumstances. Unlike previous studies that looked at a limited number of metabolites, metabolomics looks at all the metabolites in a biological system, therefore there is no bias in the metabolites analyzed. In a site-specific organism, however, metabolites are part of an in vivo metabolite flow that controls whole metabolic pathways. Furthermore, metabolism-based broad fluxes enable us to locate physiological regulatory situations in an organism. The major difficulty in metabolomics is figuring out how to get the most out of the hidden information in varied metabolite compositions. Hundreds of primary and secondary metabolites are released by a microbial cell during the course of its existence in response to environmental or cellular changes (Futschik *et al.*, 2018).

Metabolomics is a step beyond metabolite fingerprinting in that it tries to measure every single molecule in its functional role rather than merely building an inventory of the metabolites in a cell. Any metabolomics investigation must start with the measurement of all metabolites in a biological system. This may be accomplished via metabolite isolation and characterization methods that combine automation and miniaturization, such as sampling, extraction of particular chemical classes, storage temperature, sample preparation, and analysis. As a result, changes in the reporter organisms' metabolism during the encounter with any pollution are easily identified, and the end products, that is, metabolites, clearly showed the amount of contamination and feasible bioremediation procedures (Mashego *et al.*, 2007).

2.13 MOLECULAR DOCKING APPROACHES IN BIOREMEDIATION

Molecular docking can be defined as a technique that can be used to calculate the most appropriate binding position for a molecule to another molecule with a minimal amount of energy conformation while there is the formation of a molecular complex. Docking can be done between protein and a small molecule, protein–protein, protein–DNA, protein–RNA, protein–lipid, protein–sugar, polymer–ligand, and so on where a substrate is the bigger metabolite molecule and a smaller one is the ligand (Shockcor & Holmes, 2002). Molecular docking speeds up the selection process and improves the enzymatic properties of certain pollutants through rapid computational scrutiny. This technique offers the anomalous potential to predict the function of enzyme binding and degradation or adsorption of pollutants and provides clarity while depicting the relationship and communication between pollutants and enzyme molecules (Basharat *et al.*, 2020). This allows one to infer whether the active site of a particular enzyme contains a dye molecule, based on damage to the stearic acid residue and the nature of the active site pocket. The association between proteins and contaminants is verified by evaluating thousands of potential conformational docking poses. Scanning the entire surface of the protein attempts to blindly bind proteins to unknown active sites. The link also provides information about the regulation of brokers in the active pocket of the site and speculates on the productive or unfavorable link. The resulting coloration of the active site residues and the binding properties of the test compound is likely to be used for site-specific mutagenicity testing (Bhatt *et al.*, 2020).

2.14 CONCLUSION AND FUTURE PERSPECTIVE

CP is the process of successfully utilizing resources and energy, as well as removing hazardous raw materials and reducing the toxicity of all emissions and solid waste. It is the most proactive technique for reducing waste, negative environmental effects, and health concerns by using energy and natural resources in a more organized and efficient manner. Today, there is a pressing need to establish more sophisticated research tools and techniques that may play an inter/multidisciplinary role to address the issue related to CP leading to achieving the future concept of sustainable development goals. Bioinformatics and omics data technology have contributed a lot to the bioremediation process. A very large chunk of data has been extracted and is used for the *in-silico* analysis advancement. Omics approaches have been able to provide a medium for the analysis of different components of cells such as functional genes, proteins, metabolic interactions, and regulatory pathways. Bioinformatics has facilitated and fastened the cellular analysis process to understand the microbial working and their impact on treating the environment and bioremediation process. Thus, these multiple omics tools and databases are providing valuable information for the purpose to recognize the mechanism involved in the bioremediation process to use microbial technology to work against pollutants. In conclusion we can state that the future holds more understanding about the molecular mechanism and using it to manipulate cellular working by incorporating bioinformatics to demonstrate and the ultimate goal is to translate these computational data and processes to the nature against the different pollutants and the heavy metals.

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Chapter 3

Bioremediation: role of zooplankton in urban waters

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ABSTRACT

The various everyday developments of our urban society have led to the improvement of industries and agricultural growth causing the mismanagement of wastewaters coming out from these industries. This wastewater exerts many faceted pollution issues leading to the biomagnification and thus affecting human population. Bioremediation is one of the most effective methods of removing hazardous impurities from wastewater. Due to their role as bio indicators in showing the status of any environment, zooplankton have been used to remove the effects of hazardous pollutants from wastewater and thus aid in bioremediation for a while. Zooplankton and other microbes act to remove or reduce pollutants through biological degradation of pollutants into nontoxic substances. Several parameters decide the correct method of bioremediation among *in situ* or *ex situ* methods. Processes like bio stimulation, bioaugmentation, bioventing, bio piles and bio attenuation are required to develop a complete bioremediation process.

Keywords: bioremediation, wastewater, zooplankton, biomagnification, pollutants

3.1 INTRODUCTION

The growing urban population has led to the large-scale production of urban waste—be it untreated or partially treated. Since the unplanned urban society requires more and more amount of water, hence the requisition of water has increased and will increase in future also. Pollutants from several sources such as nutrients, pathogens, plastics, chemicals and so on are found in the urban wastes. The sewage produced every day is not treated and only one-third is treated resulting in waste accumulation and leading to the contamination of the local water bodies where there is constant inflow of untreated or partially treated sewage wastes. Due to the huge amounts of untreated sewage wastes, the search for clean water becomes really challenging and the entire habitat service provided by the lentic and lotic ecosystems are hindered (Prakash *et al.*, 2022).

The urban wastewater is usually disposed in several ways but in all cases the cost of treatment is preferred to be reduced (Ramachandra & Solanki, 2007). Due to the population explosion, most of the waterbodies are getting depleted to meet the various needs (agricultural–irrigational, industrial, and domestic) of the population leading to the deterioration of the water quality. Domestic sewage, agricultural run-offs, and industrial effluents are the sources from where the urban surface water bodies get polluted and cause several enteric diseases in humans.

Among the major pollutants polluting the soil, environment and water are heavy metals, hydrocarbons, polychlorinated biphenyls, industrial effluents, pharmaceutical chemicals, xenobiotics, polyaromatic hydrocarbons (PAHs), and radionuclides are the industrial contaminants that are threats to human health as well as various ecosystems. To minimize and eradicate these pollutants and their harmful effects, several techniques were used but they all seem to exert long-term dangers to the environment. Therefore, bioremediation was the only method that can be long-term and eco-friendly to eradicate contaminants with the help of microorganisms like zooplankton. In addressing water contamination issues in urban areas, bioremediation emerges as a sustainable and environmentally friendly approach that utilizes microorganisms such as zooplankton to effectively eliminate contaminants over a long period of time (Singh *et al*., 2022). This method stands out as the sole viable alternative that combines long-term efficacy and ecological considerations (Singh *et al*., 2022).

The crucial role of zooplankton as an important community in maintaining the ecological balance for other biotic components of the urban waters are discussed in the following portions. The role of zooplankton as bioindicators, how they assist in bioremediation process in wastewaters along with the parameters assisting in the process and how the zooplankton cumulatively help in wastewater treatments with other urban water organisms are discussed.

3.2 URBAN WATERS AND ZOOPLANKTON AS A PART OF ITS DYNAMIC POPULATION

Various works take place in an urban area like transportation of goods by various vehicles and various other anthropogenic events that lead to change in soil, air, and water composition polluting the corresponding areas. There were different methods of land use in the urban areas, which resulted in the production of land wastes both solid and liquid produced as a result of various anthropogenic reasons resulting from industries, vehicles, construction along with domestic, all created pollution of the urban waters, which is more deteriorated in quality and not fit for usage. Untreated industrial wastes get released in the urban waters and increases the percentage in wastes like heavy metals, inorganic compounds, and untreated industrial effluents resulting in highly polluted urban waters.

Zooplankton being an integral part of the urban freshwater ecosystem and its population is affected by the changes in water quality. They have an integral role in the material cycle of any waterbody where they reside by being a functional entity of the urban water ecosystem. They act as an important link in the aquatic ecosystem and the members belong to Protozoa, Cladocera, Copepoda, Rotifera, and so on. Studies reveal that the number of zooplankton reduces in waterbodies when sewage water gets mixed (Menezes-Sousa *et al*., 2018). The plankton's role in pollutants dynamics as a tool for ecotoxicological studies (see *Orbital: The Electronic Journal of Chemistry*, pp. 346–354).

Several types of urban waters are present which show complex internal systems. Unlike urban lakes, urban and semi-urban ponds are very important in acting as natural tertiary waste disposal areas where natural sewage treatment is done. The zooplankton are found to be present in both polluted industrial and municipal waste waters where they maintain their own freshwater ecosystem (Vidali, 2001).

Zooplankton have a growth dynamic like that of algal bloom of high-rate algal ponds (HRAPs) and can furnish all the information regarding the habitat they live in. The zooplankton have individuals as small as (<20 μm) unicellular protozoa and as large as multicellular rotifers and micro-crustaceans (>200 μm) along with copepods and cladocerans (5 mm) (Alprol *et al*., 2021).

3.3 ROLE OF ZOOPLANKTON IN PROVIDING SIGNIFICANT AND VALUABLE ROLE IN URBAN WATERS

Zooplankton remain a very important part of the aquatic ecosystems of any type of water. On the one hand they are an important biotic component in the aquatic food chain where they feed on the producers and in turn, they themselves are eaten by fishes. In comparison to the phytoplankton, zooplankton are one of the required biological components of environmental impact assessment (EIA) are more diverse and their variability in any ecosystem is dependent on seasons, patchiness, and diurnal-vertical migration (Figure 3.1).

In case of urban waters their function gets more pronounced as the urban waters need more attention since they get polluted due to the various anthropogenic hazards that not only pollutes the water, but also shift the homeostatic balance to a quality that becomes a threat to the aquatic flora and fauna residing in the same waterbody. Given that the growth and distribution of zooplankton are heavily influenced by various abiotic factors such as temperature, salinity, pH, pollution, stratification, as well as biotic factors such as resource competition, any alteration or disruption in these factors caused by environmental hazards can be detected through observable fluctuations in the zooplankton cycle and population sizes. Therefore, a slight change or malfunctioning of any of this due to other environmental hazards may be known by the changes in the zooplankton cycle and their population numbers.

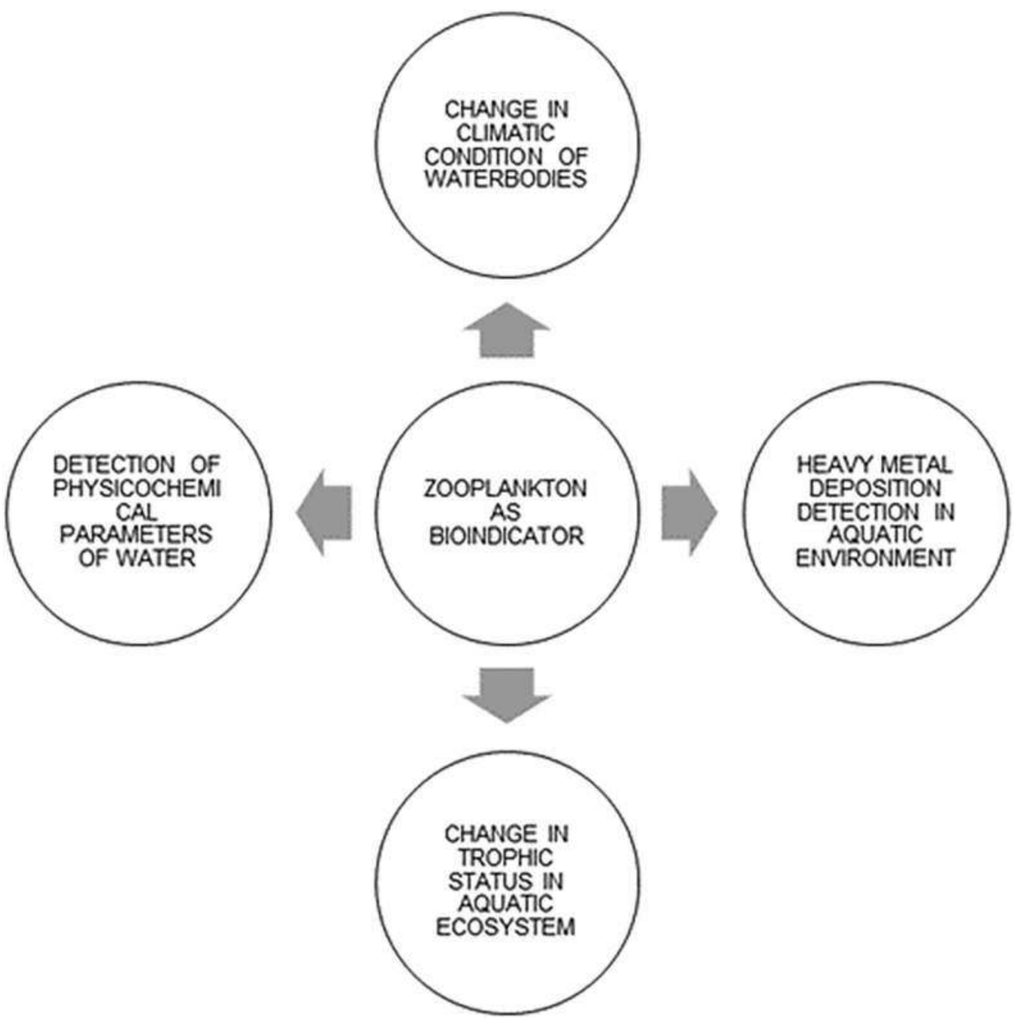

Figure 3.1 Role of zooplankton as ecosystem service provider in any aquatic biome.

The three basic stages of any traditional wastewater treatment are:

(1) Primary treatment in which the solids are removed.
(2) Secondary treatment in which aerobic degradation of soluble organic compounds are used to remove the effluents.
(3) Tertiary treatment in which the dissolved nutrients like nitrogen and phosphorus are removed to stop eutrophication of the incoming waters (Pous *et al*., 2021).

Zooplankton usually act on the treatment stages to bioremediate.

3.4 ZOOPLANKTON AS BIOINDICATOR SPECIES

Any kind of major or minor changes in the environment and conditions which make the biogeography and health of the environment can be assessed and identified by bioindicators. Zooplankton are the best examples of bioindicators who can screen water quality and indicate the severity of water pollution (Hays *et al*., 2005). Biological monitoring or biomonitoring is a method where the changes in any environment are measured by any kind of biological response. So, while enquiring about the water quality in any area aquatic biomonitoring can be of great help. Ecosystem components, especially the biotic ones are used to enquire about the abrupt environmental changes in the ecosystem from time to time. The biotic ecosystem components involved in biomonitoring process is known as bioindicators (Campos *et al*., 2022) (Figure 3.2).

Any kind of major or minor changes in the environment and conditions which make the biogeography and health of the environment can be assessed and identified by bioindicators. Zooplankton are the best examples of bioindicators who provide information about their habitat and can screen the water quality to indicate the severity of water pollution (Ferdous & Muktadir, 2009). The zooplankton usually react to the slightest environmental changes which makes them good indicators of any pollution in the natural environment.

Zooplankton (rotifers, cladocerans, copepods (Bari *et al*., 2021) being a part of the planktonic population of the aquatic ecosystem, are considered to have a very crucial role in ecotoxicology and serve as an essential tool in understanding the different minute pollution cycle side effects on any habitat (Mani & Kumar, 2014) as they have the following features:

- They are the trophic connection between producers and higher-level consumers in the food chain, so they also transfer pollutants along with energy in any polluted site.
- They can identify the slightest changes in the surroundings they live since in most cases they usually are the first to get affected.
- The recycling of organic matter and toxic hazardous wastes are simultaneously done by the zooplankton only.

The zooplankton found in freshwaters are mostly microscopic protozoans, rotifers, cladocerans and copepods. The zooplankton assemblage is used for water quality assessment. They usually are the second or third trophic level of aquatic trophic web where they themselves feed on algae and bacteria and are consumed by the fishes and many other invertebrates. Hence, any deterioration in the zooplankton level will directly affect the fish population and in turn other higher trophic levels.

There are certain advantages of using zooplankton as indicators for water quality assessment:

- The optimum size of zooplankton makes them easy to handle and identify.
- Processing and sampling are also done very easily.
- Their life cycle can be easily studied in a small time since they have a very short reproductive span.
- Since they are quite abundantly found, working with them as specimen becomes easy.
- They can identify changes in water quality and nature faster than any other bigger biota of the aquatic ecosystem (Campos *et al*., 2022).

Figure 3.2 Zooplankton: indicators of water pollution: The urban waters have zooplankton as a biotic community, any adverse changes in them will be shown in the elevated nitrogen, phosphorus content balance which the zooplankton will increase their numbers through reproduction. The change will in course pollute the water and zooplankton being the intermediate link between aquatic trophic levels will affect the fish population which then in turn will harm human beings.

3.5 ZOOPLANKTON-ASSISTED BIOREMEDIATION IN WASTEWATERS

Bioremediation is an eco-friendly, cost-effective method which usually uses a group of harmless microbial communities to degrade pollutants in conditions where oxygen is used as an electron acceptor and water containing nutrients are infiltered.

Two types of bioremediations are witnessed—*in situ* and *ex situ*. The application of zooplankton to bioremediate urban wastewater shows a great potential to complement traditional wastewater treatment processes.

Due to anthropogenic activities, the toxic heavy metals accumulate in the soil and environment and finally into water where they enter the food chain and interfere with the normal biological mechanisms. Bioremediation is the best method to retrieve urban waters and to get rid of the harmful hazardous heavy metals that have polluted those waters. Toxic heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), zinc (Zn), uranium (Ur), arsenic (As), and selenium (Se) can disrupt metabolic processes and alter food chains. These metals enter ecosystems in small amounts initially and undergo biomagnification as they progress through successive trophic levels. This poses a threat to the health of living organisms in urban water bodies and can eventually affect humans through the food chain. Mostly reactive oxygen species (ROS) are formed due to heavy metal toxicity in organisms.

The ability of zooplankton to reduce/remove pollutants from waters depends on the suitability of aquatic conditions for their growth and metabolism which includes suitable temperature, pH, salinity, and so on. Zooplankton can degrade pollutants due to their metabolism. The bioremediation of industrial waste waters and municipal waste waters are done by a mixed community of microbes like zooplankton (protozoa) to form a high-quality effluent (Amin *et al.*, 2013).

The zooplankton activity gets inhibited in the presence of high concentration of organic matter. As a result of which the activity of the zooplankton is limited to tertiary treatment of wastes. Therefore, besides working with other natural treatments performing both primary and secondary treatments, wastewater clarification and nutrient polishing were done by zooplankton.

Daphnia is a Cladoceran plantonic crustacean which is very frequently used for ecotoxicological works. *Daphnia* usually remove small particles to aid in secondary systems, they also are known to reduce bacterial loss. Biological oxygen demand (BOD) is reduced by consuming the particulate organic wastes. Contaminants like ammonia, ammonium, nitrite, organic wastes, metals and so on also trigger daphnids. Studies show how *Daphnia* is suitable for tertiary wastewater treatment by reducing the suspended solid content (acting as clarifier), reducing the bacterial load (acting as a disinfectant). The BOD content is reduced by *Daphnia* via consumption of particulate organic matter.

3.6 PARAMETERS CONTROLLING BIOREMEDIATION IN WASTEWATERS BY ZOOPLANKTON

The periodic movement of the zooplankton and the parameters driving their erratic habits are prone to change under the varying ecological variables, especially in places where the water is connected to a very busy urban industrial shore and there is severe anthropogenic influence on the nutrient content affecting the aquatic ecosystem (Adhikari *et al.*, 2017).

The most essential parameters for bioremediation in waste waters are (Table 3.1):

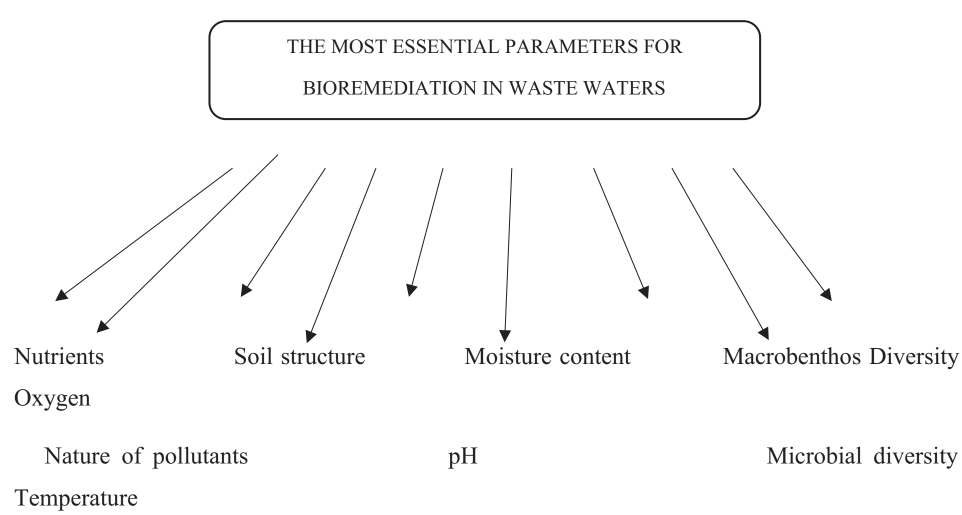

Table 3.1 Most essential parameters for bioremediation in wastewaters.

Nutrients	Contaminated areas do not possess enough nutrients for cellular metabolism and growth of microorganisms due to the presence of high amount of organic carbon which may be depleted during microbial metabolism. To enhance the bioremediation process, it is important to replenish any deficiencies in nutrients and supplement them accordingly. Nutrients such as nitrogen, phosphate, and potassium play a vital role in activating the cellular metabolism and promoting the growth of microorganisms involved in bioremediation.
Pollutants	Pollutants can be classified based on various characteristics. Here are some common types of pollutants: 1. **Physical State:** • Solid pollutants: Refer to particulate matter such as dust, soot, and debris. • Semi-solid pollutants: Include sludge, sediment, and other viscous materials. • Liquid pollutants: Refer to contaminated liquids like wastewater, oils, and chemical solutions. • Volatile pollutants: Refer to substances that easily evaporate into the air, such as volatile organic compounds (VOCs). 2. **Toxicity:** • Toxic pollutants: These substances have harmful effects on living organisms and ecosystems. • Non-toxic pollutants: Refer to substances that do not have significant toxic properties. 3. **Chemical Composition:** • Organic pollutants: Include substances derived from living organisms or containing carbon atoms. Examples include polycyclic aromatic hydrocarbons (PAHs) and pesticides. • Inorganic pollutants: Refer to substances that do not contain carbon atoms, such as heavy metals (e.g., lead, mercury) and chlorinated solvents. It is important to note that pollutants can exhibit multiple characteristics, and their impact on the environment can vary depending on factors such as concentration, exposure duration, and specific ecosystems involved.
Structure of soil	Different textures of sand, silt, and clay are present ranging from low-to-high contents. A well textured soil can facilitate aeration, nutrition and water to microorganism for bioremediation.
pH	The ideal pH range for microbial growth and the degradation of contaminants typically fall between 5.5 and 8.0. Within this range, microbial activity and enzymatic processes are most efficient, allowing for effective biodegradation of contaminants. However, it is important to note that the optimal pH range may vary depending on the specific microorganisms involved and the type of contaminants present. Some microbial species may thrive in more acidic or alkaline conditions, and certain contaminants may require specific pH ranges for effective degradation. Therefore, it is essential to consider the specific characteristics of the microbial community and contaminants when designing bioremediation strategies. Maintaining the pH within the optimum range can be achieved through various methods, including pH adjustment using suitable amendments, monitoring pH levels during bioremediation processes, and providing conditions favorable for microbial growth and activity.
Soil moisture content	The optimum soil moisture range is 25–28%. The soil water determines the dielectric constant of soil and other medium.

(*Continued*)

Table 3.1 Most essential parameters for bioremediation in wastewaters. (*Continued*)

Microbial diversity	The microbial species mentioned including *Pseudomonas*, *Aeromonas*, *Flavobacteria*, *Chlorobacteria*, *Corynebacteria*, *Acinetobacter*, *Mycobacteria*, *Streptomyces*, *Bacilli*, *Arthrobacter*, and *Cyanobacteria*, are known to play important roles in various environmental processes, including bioremediation. These microorganisms possess diverse metabolic capabilities and can contribute to the degradation and transformation of different contaminants in different environments. It is important to note that the effectiveness of these microbial species in contaminant degradation can vary depending on the specific contaminants, environmental conditions, and the presence of other microorganisms. Therefore, a comprehensive understanding of the specific contaminants and the microbial community present in each environment is crucial for developing effective bioremediation strategies.
Macrofauna diversity	An association of aquatic plants *E. crassipes, S. molesta, C. demersum* along with aquatic animals *A. woodiana* and *L. hoffmeisteri* degrades the ammonia content, nitrite, nitrate in domestic wastewaters. The association of aquatic plants such as *Eichhornia crassipes* (commonly known as water hyacinth), *Salvinia molesta* (commonly known as giant salvinia), *Ceratophyllum demersum* (commonly known as hornwort), along with aquatic animals like *Ampullariidae woodiana* (commonly known as golden apple snail) and *Limnaea hoffmeisteri* (commonly known as ramshorn snail) can contribute to the degradation of ammonia, nitrite, and nitrate in domestic wastewaters. Aquatic plants play a vital role in wastewater treatment by absorbing and utilizing nutrients like ammonia and nitrates as a source of growth. They help reduce nutrient levels in the water by assimilating these compounds into their tissues. Additionally, aquatic plants provide a suitable habitat for beneficial microorganisms, such as nitrifying bacteria, which aid in the conversion of ammonia to nitrite and nitrate. The presence of aquatic animals like *Ampullariidae woodiana* and *Limnaea hoffmeisteri* further contributes to the wastewater treatment process. These animals feed on organic matter, including decaying plant material and algae, promoting the breakdown of organic compounds, and reducing nutrient levels in the water. Overall, the combination of aquatic plants and animals in wastewater treatment systems can enhance the removal of ammonia, nitrite, and nitrate, leading to improved water quality. However, the specific effectiveness of this association may vary depending on factors such as environmental conditions, wastewater composition, and the presence of other organisms in the ecosystem.
Temperature	The biochemical reaction rates are affected by temperature, optimum temperature being 15–45°C.
Oxygen	The presence or absence of oxygen is a crucial factor in determining the type of bioremediation process: aerobic or anaerobic. In aerobic bioremediation, oxygen is available, and microorganisms utilize it as a terminal electron acceptor during the degradation of contaminants. Oxygen plays a vital role in the initial breakdown of hydrocarbons and other organic pollutants, as it serves as an essential component in the metabolic processes of aerobic microorganisms. These microorganisms, often referred to as aerobic bacteria, possess enzymes that require oxygen to efficiently oxidize and break down contaminants into less harmful by-products, such as carbon dioxide and water. On the contrary, in anaerobic bioremediation, the absence or limited availability of oxygen creates an environment suitable for anaerobic microorganisms. These microorganisms can still degrade certain contaminants in the absence of oxygen by utilizing alternative electron acceptors such as nitrate, sulfate, or carbon dioxide. Anaerobic bioremediation processes are particularly effective for certain types of contaminants, such as chlorinated solvents and heavy metals, which can undergo reductive transformations. It is important to consider the oxygen availability and the specific requirements of the contaminant and microbial community when designing and implementing bioremediation strategies. The initial breakdown of hydrocarbons and subsequent degradation processes can be significantly influenced by the presence or absence of oxygen in the contaminated site.

3.7 CUMULATIVE ROLE OF ZOOPLANKTON WITH OTHER ORGANISMS OF URBAN WATERS

The main aim of bioremediation is to discover a novel microorganism that can remediate pollutants from any polluted site. Different methods such as bioaccumulation, biodegradation pathways and different methods of biosorption are used to remove pollutants. The zooplankton resides in several other microorganisms some of which help the former to bioremediate. Daphnids, among other zooplankton, is very important since they improve the efficiency of wastewater treatment in ponds.

In several cases, zooplankton work with other microorganisms to be more potent at wastewater treatment. One of the examples is the natural-based alternative for treatment of waste waters by the cumulative effort of *Daphnia* (zooplankton) and bacterial/algal biofilm in a zooplankton-containing reactor. In this case, filtration capacity of the former acts with the nutrient removal capacity of the latter.

3.8 CONCLUSION

Therefore, it is quite evident that zooplankton are very crucial biotic members with respect to the proper health functioning of any aquatic ecosystem. They have a pivotal role in trophic chain as they transfer energy from planktonic algae to the upper levels such as larger invertebrates .The zooplankton are very quick in responding to the changes in the water they inhabit and their species composition indicate the water quality. Zooplankton themselves or with some other aquatic members are ecologically good at removing BOD, bacterial load, other organic matter, ammoniacal nitrogen, and so on from urban wastewaters. Being high in aesthetic value and low-cost maintenance requirement, zooplankton are the best bioindicators for isolated aquatic ecosystems. In the nature-based system where the integrated use of rotifer, cladocerans, with other zooplankton for treatment of wastewaters, sludge reduction is found to be very eco-friendly for the restoration of the environmental balance.

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Chapter 4

Carbon sequestration: principle and recent advances

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ABSTRACT

Cellular sequestration and conversion of CO_2 into biomass and chemical products can provide carbon neutral to negative alternatives to conventional fossil fuels. This chapter examines microorganisms and processes used for carbon sequestration and production of biofuels and other products. The benefits and challenges related to biological carbon sequestration from point sources, namely combustion-based power plants and wastewater treatment facilities, are addressed. Algal cultivation for synthesis of liquid biofuels offers an attractive, solar-power method for temporarily sequestering carbon plus nutrient pollutants from wastewater. Autotroph-derived biochar can provide for longer term carbon sequestration into soil. Microbial waste treatment can be paired to CO_2 reduction by methanogens for production of the gaseous biofuel methane. Emerging hybrid technologies enable biogas enhancement with further reduction of CO_2 and conversion into biomass. Other microbes assimilate carbon and release clean-burning hydrogen.

Keywords: carbon sequestration, algae, biodiesel, hydrogen

4.1 ATMOSPHERIC CARBON AND ITS SEQUESTRATION

Anthropogenic greenhouse gas (GHG) emissions have risen over the past century and today present an emerging climactic challenge for many regions of the planet (Mikhaylov *et al.*, 2020). Carbon dioxide is the most important carbon-based GHG released through human activity. Industrial combustion of fossil fuels including coal, natural gas, and oil for applications such as electrical power generation and internal combustion engine (ICE)-based transportation are society's leading sources of CO_2 emissions. With Earth's population surpassing 8 billion people in 2022, the demand for fossil fuels and petroleum-based products in energy and other sectors continues to accelerate. Concentrations of CO_2 in the atmosphere currently exceed 400 ppm and large developing nations, such as China, have emerged as the leading emitter of GHGs (Mikhaylov *et al.*, 2020).

GHG influenced climate change has adversely influenced many of Earth's natural processes. Sea ice coverage in the Arctic has diminished with rising temperatures as seawater levels have correspondingly risen, threatening many island and coastal communities (Mengel *et al.*, 2018). Atmospheric increases in CO_2 further elevate ocean acidity as CO_2 diffuses into sea water as carbonic acid (H_2CO_3), deleteriously impacting critical marine ecosystems like coral and oyster reefs (Doney *et al.*, 2020). As surface temperatures have increased so too have the frequency and intensity of storm events. It is broadly accepted that to avert the worst effect of climate change by mid-21st century, CO_2 emissions must be aggressively reduced. Carbon capture and sequestration from large point source emitters represents one attractive opportunity for reducing CO_2 emissions.

4.2 CONVENTIONAL CO_2 CAPTURE APPROACHES

Reduction of anthropogenic CO_2 emissions is a goal that can be achieved by various means, both natural and technological. Renewable energy systems, such as photovoltaics, wind turbines and hydroelectric methods, is a rapid growth area. Cost reductions in photovoltaics associated with economies of scale over the past two decades have enabled solar power plants to economically outcompete many polluting fossil plants, even in the absence of carbon taxes. Extended lifecycle operation of nuclear power plants ensures a low carbon source of baseload power in countries lacking ample fossil feedstocks. Some European nations have recently extended the operations of nuclear plants, despite concerns with radioactive waste storage, as natural gas supplies have been interrupted. Improved energy efficiency during electricity generation, transmission and use is critical for the reduction of carbon dioxide emissions.

Steel and concrete making are highly controlled, CO_2 intensive, heavy industries in which CO_2 reduction may be achieved using recent chemical advances. The so-called green chemical processes using novel catalysts to enhance mineral carbonization efficiency may offer advantages over more polluting traditional approaches (Hanifa *et al.*, 2023). For some carbon emission intensive industrial processes, like wastewater treatment, CO_2 production may be more difficult to avert chemically. At established wastewater treatment facilities, chemical capture of CO_2 can be challenging since the CO_2 is released over a broader area, rather than from just one flue stack or chimney. Carbon capture and sequestration describes processes used to avert increases in atmospheric CO_2 by recovering carbon from waste streams, typically process off gas. Existing CO_2 capture for sequestration technologies at power plants targets three main approaches: pre-combustion, post-combustion, and oxy-fuel.

Pre-combustion is typically used in industrial power plants where fossil fuel such as coal is converted into syngas composed of hydrogen gas (H_2) and carbon monoxide (CO), in the presence of water vapor, using a high-pressure gasifier (Ramesh *et al.*, 2021). The CO is run through a reaction that yields more CO_2, which is removed before combustion and captured (Ramesh *et al.*, 2021). The H_2 is then used to generate electricity as a fuel in a gas turbine (Molazadeh *et al.*, 2019). The pre-combustion process is typically used in integrated gasification combined cycle (IGCC) power plants. This method successfully sequesters CO_2; however, it comes with a high cost and added complexity which is a major limitation in this CO_2 removal approach. In post-combustion carbon capture, chemical absorption is employed to seize the CO_2 from flue gas after combustion has occurred (Prasad *et al.*, 2021). Fuel such as coal is burned with air to produce steam in a boiler that will power a turbine for electricity generation. In this approach, a chemical solvent, typically an amine, is used to react with the CO_2 in the flue gas at high temperature to create purified CO_2 that can be captured and stored (Erga *et al.*, 1995). Post-combustion can be used in powerplants other than IGCC and can be beneficial over a more diverse range of facilities (Erga *et al.*, 1995). Oxy-fuel combustion is a third widely used approach for carbon capture in which fuel is combined with pure oxygen to produce high yields of CO_2 ( ). Oxy-fuel combustion results in higher temperatures and is more favorable than other methods based on the higher concentration stream of CO_2 produced (Raho *et al.*, 2022). Chemical looping is a newer and potentially promising carbon capture method for in which carbon-based fuel is divided into separate oxidation and reduction reactions (Raganati *et al.*, 2021). In this approach, oxygen from air is taken by way of a carrier to the fuel (Raganati *et al.*, 2021).

4.3 CHEMICAL AND EMERGING CAPTURE METHODS

Chemical or physical CO_2 removal from industrial process off gases can occur through several methods. Adsorption and absorption are two commonly used approaches to capturing CO_2 from flue gas. In adsorption, CO_2 is collected onto an adsorber surface whereas in absorption, sometimes called chemical scrubbing, CO_2 is absorbed into an absorber medium. For effective adsorption, an adsorbent must adsorb the adsorbate with a regenerative ability based on a temperature or pressure condition (Raganati *et al.*, 2021). An adsorbent such as an amine reacts with the flue gas CO_2, capturing the CO_2 through intermolecular attraction between the molecules on the adsorbent and the CO_2 gas itself (Pires *et al.*, 2011). Physical adsorption, such as pressure and temperature swing adsorption (PTSA), can be employed by heating the adsorbent and regenerating CO_2 through depressurization (Ishibashi *et al.*, 1996). Chemical absorption of CO_2 into a liquid can be achieved through the temporary bonding of a liquid solvent such as monoethanolamine (MEA) to CO_2 through upward flow of gas through the packing of an absorber, providing a greater surface area for bonding (Raksajati *et al.*, 2018). The CO_2 enriched rich solvent leaves the bottom of the column, while the flue gas containing a lower concentration of CO_2 leaves from the top of the column (Raksajati *et al.*, 2013).

Emerging methods of CO_2 separation include membrane separation and cryogenic separation. Membrane gas separation (GS) is a means of removing CO_2 through employment of a membrane with specific permeability upon gas pressure application. Membrane GS technologies have certain drawbacks when treating low CO_2 concentration gas such that high quantities of flue gas need to be processed for efficient method optimization (Brunetti *et al.*, 2010). Potentially low CO_2 concentration coupled with low pressure of the flue gas require membranes with high selectivity to stay within International Agency Specifications, which tends to increase cost (Bounaceur *et al.*, 2006). Another consideration when using membrane GS is that the membranes must be resistant to harsh chemicals present in the flue gas so as not to disrupt the integrity of the membrane itself (Brunetti *et al.*, 2010). Periodic changing of membranes at the end of their life cycle could disrupt normal facility operations and lower the capacity factor of a power plant. In cryogenic separation, concentrated CO_2 is cooled and liquified through several steps to induce a phase change in the CO_2 from gas-to-liquid state (Meisen & Shuai, 1997). The low-temperature refrigeration equipment insulated piping and extra energy needed to operate cryogenic separation incurs additional cost. Disadvantages with many chemical and physical processes for carbon capture is that they are typically expensive and generally not environmentally sustainable (Molazadeh *et al.*, 2019). Captured carbon may be chemically or biochemically converted into different products or it can be stored underground in geologic formations (Moreira & Pires, 2016). A general overview of the possible fates of CO_2 produced through combustion at a large point source, such as a power plant, is shown in Figure 4.1.

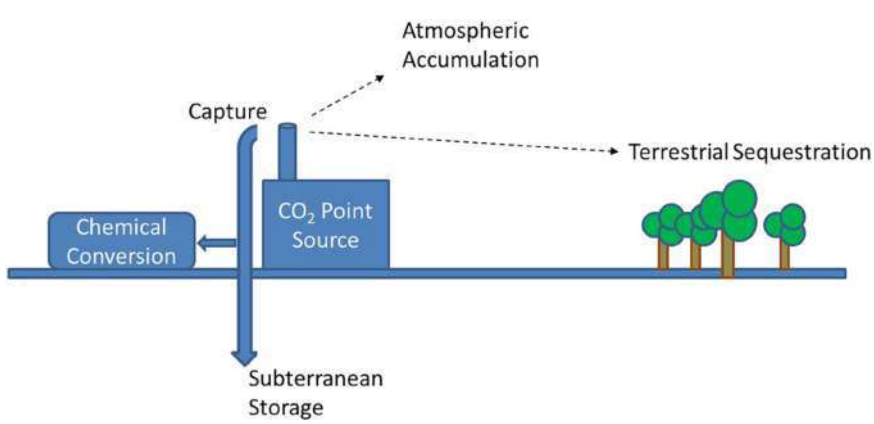

Figure 4.1 Overview of possible fates and disposition of CO_2 from a point source.

Emerging chemical CO_2 capture and sequestration methods show promise, but cost, complexity, and robustness are critical technoeconomic considerations with such industrial processes. One way to enhance the value proposition of a carbon sequestration process is to convert captured CO_2 into a more valuable product, such as biofuels or longer-lived bioplastics (Thevasundaram *et al.*, 2022). For this, some chemical or physical methods of CO_2 capture may be paired with carbon-fixing organisms, such as algae or cyanobacteria, for enhanced CO_2 sequestration and valorization (i.e., value upcycling) and/or water bioremediation. Biological carbon sequestration methods can also be used independently and may be particularly well suited for some industrial GHG emission sites, such as breweries and wastewater treatment plants where a constant stream of CO_2 is available under mesophilic conditions with few inhibitory or toxic chemicals. Wastewater treatment plants release copious CO_2 in addition to methane (CH_4), and nitrous oxide (N_2O), which are both potent GHGs (Asadi & McPhedran, 2021). Wastewater treatment plants contain diverse nutrients and other growth factors that can be used to promote the growth of CO_2 sequestering autotrophic cells. Even 100% unfiltered flue gas from coal-fired power plants can facilitate the cultivation of *Desmodesmus* spp. algae (Aslam *et al.*, 2017).

4.4 BIOLOGICAL CARBON CAPTURE AND SEQUESTRATION

Algae and higher plants naturally sequester inorganic carbon through photosynthesis. Algae is a general term that can be subdivided into three main groups: macroalgae, microalgae, and cyanobacteria. Macroalgae and microalgae are eukaryotic organisms whereas cyanobacteria are prokaryotes, many of which can survive extreme conditions. In marine environments macroalgae are informally referred to as seaweed for their rapid growth and apparent ubiquity. Macroalgae are more complex multicellular organisms ranging from tens of meters tall giant kelp of the Pacific to barely visible representatives of a few millimeters. Macroalgae include brown seaweed (Phaeophyceae), green seaweed (Chlorophyceae), and red seaweed (Rhodophyceae). Microalgae groups include diatoms, green algae (Chlorophyceae), and golden algae (Chrysophyceae) (Demirbas, 2010). Useful cyanobacteria, formerly referred to as blue-green algae, include members of the genera *Arthrospira* (*Spirulina*), *Nostoc*, and *Anabaena* (Li *et al.*, 2023). Some cyanobacteria are diazotrophic and can fix atmospheric nitrogen giving them an advantage in certain environments. Green and brown algae have most often been investigated for biofuel production. Novel animal feed applications for red algae have recently been described for inhibition of methanogens present in the gastrointestinal tracts of cows. Since ruminants are a source of the potent GHG methane, red algae can serve a role in addressing climate change by reducing emissions even though they are not commonly used to produce fuels directly.

Over 100 Pg of carbon is assimilated every year on Earth with around half by marine ecosystems (Beardall *et al.*, 2009). In the marine environment, around 75% is assimilated by marine phytoplankton and about 25% by algae and seagrasses (Beardall *et al.*, 2009). Algae are diverse, photosynthetic autotrophs that are critical to aquatic and marine ecosystems. They account for around 50% of the carbon assimilated into the biosphere each year (Field *et al.*, 1998). Algae use different methods of metabolism to assist in assimilation of CO_2. These including photoautotrophic metabolism as well as mixotrophic metabolism. In photoautotrophic species, light energy is harvested via photopigments to enable photosynthesis, transforming inorganic carbon into carbohydrates as CO_2 is assimilated via the Calvin–Benson (CB cycle).

In photoheterotrophic metabolism, organic carbon is used in the presence or absence of light. This method of metabolic activity can occur in certain algal species under dark conditions or in conditions of low-light where photosynthesis is not favorable. In these conditions, metabolism will typically occur through the pentose phosphate pathway (PPP) and organic compounds are assimilated from sources such as acetate, glucose, glycerol, and lactate, via different metabolic enzymes (Giordano *et al.*, 2005; Prasad *et al.*, 2021). Although this typically occurs in low-to-no light conditions, certain species can perform photoheterotrophy under normal light conditions (Ingram *et al.*, 1973; Prasad *et al.*, 2021). Interestingly, growth under low light conditions facilitates production of saturated fatty acids, which may be preferable as energy dense biofuel feedstocks (Constantopoulos, 1970). Certain photosynthetic bacteria are also capable of photoheterotrophic metabolism.

Mixotrophs are organisms, including certain algae that can metabolize using both photoautotrophic metabolism and heterotrophic metabolism. During mixotrophic growth, CO_2 and organic carbon are used together, in combination with both respiration and photosynthesis (Prasad *et al.*, 2021). Because this metabolic strategy permits the usage of both inorganic and organic carbon sources, high production of biomass is promoted (Venkata Mohan & Devi, 2014; Prasad *et al.*, 2021; Wang *et al.*, 2014). Aerobic respiration is employed to produce energy needed for organic carbon anabolism while photosynthesis is used for inorganic carbon assimilation into *de novo* organic material (Hu *et al.*, 2012; Prasad *et al.*, 2021). With multiple metabolic strategies employed within the organism, mixotrophs can produce higher yields per unit of energy compared to the other metabolic strategies independently (Prasad *et al.*, 2021; Yang *et al.*, 2000). Although mixotrophs show promise for carbon sequestration, they may entail a heightened cost of cultivation due to their higher carbon demands. However, mixotrophic cultivation of *Spirulina* using low-cost organic wastes, such as acetate, can increase growth rate and CO_2 assimilation by stimulating ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo) to enhance the overall utility of cyanobacteria for carbon sequestration (Li *et al.*, 2023). Geothermal emission off gases containing as high as 90% CO_2 have been used to cultivate cyanobacteria for carbon sequestration (Svavarsson *et al.*, 2017).

Oxygenic photosynthesis is the dominant mechanism algae use to convert light energy to chemical energy with water serving as the electron donor (Blankenship, 2010, 2014). Photopigments including chlorophyll and carotenoids enable algae to harness solar radiation (Blankenship, 2014). Chlorophylls absorb blue and red light, while carotenoids absorb blue-green wavelengths (Bari, 2004; Geider & Osborne, 1992). Certain cyanobacteria use phycobiliproteins such as phycoerythrin and phycocyanin to expand the fraction of the visible spectrum captured (Formighieri & Melis, 2015). Harnessed solar radiation triggers light-dependent reactions in the Z-scheme of oxygenic photosynthesis (Govindjee *et al.*, 2017).

Light energy absorbed by the photopigment antenna complex is transferred to photosystem II (PS-II), inducing water photolysis, which establishes a proton motive force (PMF) within the thylakoid lumen (Figure 4.2). For each water molecule oxidized, two light-energized electrons are carried through the photosynthetic electron transport chain (P-ETC) from PSII to cytochrome b6f via the lipophilic carrier plastoquinol. Additional protons are transported from the stroma into the lumen through Q-cycle proton pumping. Chemiosmotic dissipation of the PMF thus generated through the integral ATP synthase generates ATP through the overall process known as photophosphorylation. Electrons in the P-ETC are next transferred to photosystem I (PS-I) from cytochrome b6f via plastocyanin. At PS-I they are reenergized to a higher energy state by additional light enabling the reductive formation of NADPH (Bari, 2004). The precise amount of ATP to NADPH generated in many photoautotrophs depends on if the cell is utilizing non-cyclic (i.e., linear) versus cyclic photophosphorylation; the latter serving as a photoprotective mechanism during high light stress (Huang *et al.*, 2015).

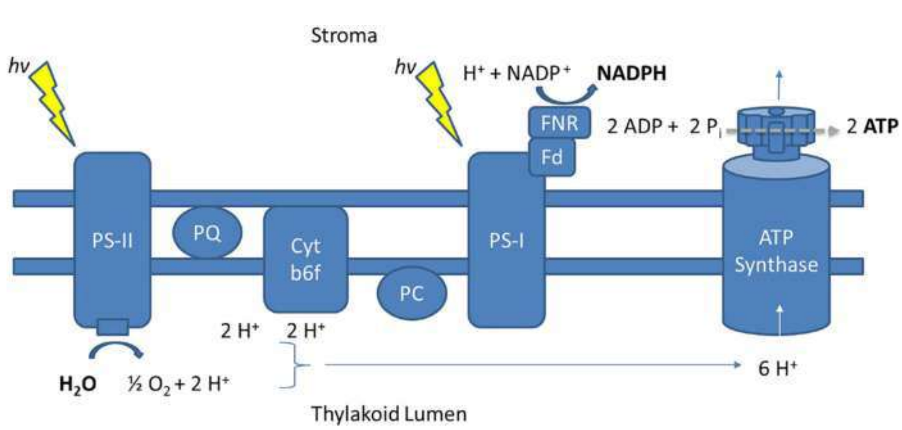

Figure 4.2 P-ETC showing conversion of light into chemical energy used to drive CO_2 fixation.

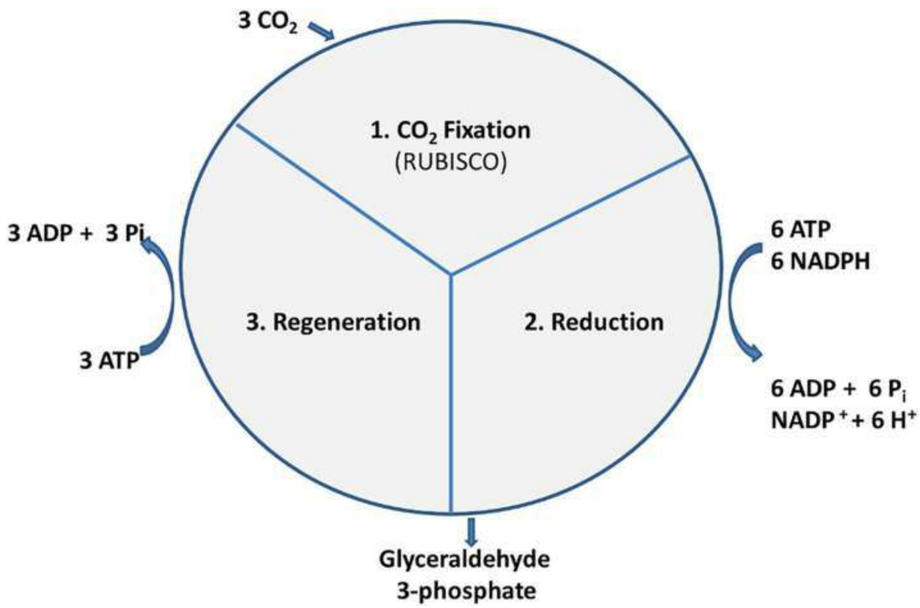

Figure 4.3 CB cycle-mediated biological CO_2 fixation.

CO_2 fixation during the light-independent (i.e., dark) reactions of the Calvin Benson cycle (CB cycle) is an anabolic process powered by the ATP and NADPH generated by the light reactions. The three primary stages of the light-independent reactions of CB cycle CO_2 assimilation involve CO_2 fixation, reduction and substrate regeneration (Nguyen & Hoang, 2016). In the CB cycle, carboxylation of ribulose-1,5-bisphosphate (RuBP) by RuBisCo produces two 3-phosphoglycerate (PGA) molecules (Furbank & Taylor, 1995). Energy from ATP and NADPH is utilized for subsequent conversion of PGA into glyceraldehyde-3-phosphate (G3P) as depicted in Figure 4.3 (Furbank & Taylor, 1995). Most of the G3P molecules are used for RuBP regeneration, but some G3P are used to synthesize other organic molecules, such as glucose that may be stored as starch (Furbank & Taylor, 1995). In contrast to many cyanobacteria, which tend to form starch as their energy storage material, oleaginous microalgae convert this fixed carbon into neutral lipids, namely triacylglycerides (TAGs), that can be converted to biodiesel (Li *et al.*, 2019).

4.4.1 Carbon capture mechanisms

CO_2 is essential for anabolism during photosynthesis, as it is the primary carbon substrate for microalgae to produce organic compounds using sunlight energy (Lam *et al.*, 2012). CO_2 for microalgae photosynthetic cultivation can be gained from the atmosphere at concentrations around 0.03–0.06% (v/v) or in concentrated form from point source flue gas of power plants in concentrations of approximately 6–15% (v/v) (Cheah *et al.*, 2015; Rahaman *et al.*, 2011). When considering microalgae as a means for carbon capture from point sources, pH, temperature, and algal stress must be considered. If the CO_2 concentration becomes too high, it becomes harmful to the algae by increasing the acidity of the local environment (Kumar *et al.*, 2011). In order to limit the negative effects associated with high CO_2 concentrations, microalgae contain a carbon capture mechanism (CCM) that allows for continued proliferation when photosynthesis may be limited.

Carbonic anhydrase (CA) activity can be limited in the presence of high CO_2 concentration. The effect on CA can be detrimental to the microalgae, as this mechanism is catalytically important for its part in alterations between CO_2 and HCO_3^- (Zhang, 2015). With high CO_2 concentration, the pH of the medium decreases due to increases in bicarbonate (HCO_3^-) and hydrogen ion (H^+) concentration.

The concomitant pH drop negatively affects the action of CA, resulting in a decrease in the CO_2 fixing abilities of the microalgae (Zhao & Su, 2014). Through different regulatory processes however, some algal species have adapted to the lower pH. These adaptations include the ability to increase the amount of ATP present while concurrently reducing organic carbon synthesis, increasing the ability of these cells to tolerate exceptionally high concentrations of CO_2 (Zhao & Su, 2014). Mechanisms involved in increasing tolerance to higher CO_2 concentrations include preventing acidification of the stromal compartment in the chloroplast and cytoplasm in order to keep RuBisCo functioning properly (Zhang, 2015).

Efforts to improve the efficiency of algal photosynthesis and thereby algal biofuel production have focused on genetic improvement of photopigments, components of the P-ETC and/or RuBisCo enzyme, which is inefficient due to its capacity for photorespiration in which O_2 rather than CO_2 is incorporated. CO_2 fixation in oxygenic phototrophs is reliant on RuBisCo and CA. Other important enzymes involved in carbon fixation process include phosphoribulokinase, phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxy-transfer enzyme, pyruvate carboxylase and malic enzyme (Bharti *et al.*, 2014). Similar to oleaginous microalgae, certain bacteria, including various *Rhodococcus* species, also synthesize fatty acids and triglycerides from acetyl-CoA using NADPH as a source for high energy reducing equivalents (Kumar *et al.*, 2018; Schirmer *et al.*, 2010).

Alkane fatty acid chain formation occurs by: (1) hexadecenoic acid elongation, (2) fatty acid reduction to aldehyde via fatty acid reductase, and (3) decarboxylation of aldehyde via aldehyde decarboxylates forming alkanes (Kumar *et al.*, 2018; Messner & Sleytr, 1992). *De novo* fatty acid biosynthesis in algae is similar to the process in plants (Rismani-Yazdi *et al.*, 2011; Tan & Lee, 2016). Fatty acid synthase (FAS) is the primary enzyme involved in the biosynthesis of fatty acids in many different algae, including model organism *Chlamydomonas reinhardtii* (Khozin-Goldberg & Cohen, 2011). The first step is the carboxylation of acetyl-CoA by CoA carboxylase (ACCase), forming malonyl-CoA (Reverdatto *et al.*, 1999). This is the rate-limiting step for fatty acid biosynthesis and genetic optimization for enhancement of ACCase activity can improve oil yield from algae (Post-Beittenmiller *et al.*, 1991, 1992). Next, malonyl-CoA binds acyl carrier protein (ACP) to form a metabolic scaffold malonyl-ACP via malonyl-CoA transacylase (Blatti *et al.*, 2013; Goncalves *et al.*, 2016). The chain is lengthened two-carbons at a time through integration of the acetyl group from acyl-ACP, then fully reduced by ketoreductase, dehydratase, and enoyl reductase (Blatti *et al.*, 2013). Once the chain is fully developed, the mature fatty acid, is transferred by acyl transferase onto glycerol-3-phosphate (Blatti *et al.*, 2013). A buildup of fatty acid acyl-ACP regulates the rate of fatty acid synthesis through ACCase feedback inhibition (Davis & Cronan, 2001; Tan & Lee, 2016). Thioesterases can reduce the inhibition caused by acyl-ACP build-up by converting acyl-ACP into acyl-CoA and releasing it into the chloroplast to become a component of TAGs (Chen & Smith, 2012). Acylation of glycerol-3-phosphate leads to production of diacylglycerol (DAG) in the chloroplast (Goncalves *et al.*, 2016). DAG is a precursor for membrane lipids, in *Chlamydomona*s (Goncalves *et al.*, 2016; Li Beisson *et al.*, 2015).

In microalgae, TAG neutral lipid biosynthesis occurs in the endoplasmic reticulum (ER)-derived sections of the chloroplast (Fan *et al.*, 2011). Thioesterase is responsible for the carbon flux between termination pathways, acting as a metabolic director for biosynthesis of lipids (Bonaventure *et al.*, 2003). The importance of thioesterase and its gatekeeping role for the destination of fatty acids has therefore made it a target for algal fatty acid bioengineering for improvement of biodiesel feedstock (Radakovits *et al.*, 2010). Genetic engineering has shown improvements in oil yields by modifying ACCase expression in plant cells, with similar findings in algae (Roessler, 1988). Genetic modification of thioesterases can prove valuable for fatty acid biosynthesis in microalgae since most synthesize C16–C22 chains with considerable saturation variation (Harwood & Guschina, 2009). These enzymes are responsible for chain termination, which determines the fatty acid end-product length. Since biodiesel production generally requires shorter-chain fatty acids, altering the chain length could prove valuable not only for biodiesel, but potentially for use in production of gasoline and jet fuel

(Radakovits *et al.*, 2010). Genetic engineering of thioesterases to select specifically for C8–C14 fatty acid products is one means of improving biofuel production from microalgae through modification of fatty acid biosynthesis (Blatti *et al.*, 2012).

4.4.2 Biological CO_2 sequestration from point sources

A consideration for algal technologies includes the use of microalgae for CO_2 capture from flue gas generated from power plants. In order to generate electricity, power plants will burn fossil fuels, which create the thick, heavy smoke that can be seen exiting stacks of different industrial plants. This flue gas contains high concentration CO_2 emissions that are being released directly into the atmosphere. Release of these chemical streams is adding to the GHG concentrations, and the greenhouse effect result from GHG increases. Coal is a primary fuel used in power plants. From combustion, this gas mainly contains CO_2, nitrogen (N_2), oxygen (O_2), and water vapor (Zhang, 2015). Concentrations of CO_2 up to 15% (v/v) are found in power plant flue gas, making it an excellent candidate carbon source for microalgae biomass production and coordinated CO_2 sequestration (Cheah *et al.*, 2015) The concentration being considerably higher than that of atmospheric CO_2 concentrations makes flue gas an excellent carbon source for the microalgae, creating a potential means of avoiding excess CO_2 emissions release. However, many microalgae will only grow at an optimal CO_2 concentration less than that of flue gas. The high concentrations of CO_2 in flue gas can inhibit growth of certain microalgae species. There are some species that are able to grow under the high CO_2 concentrations associated with flue gas; however, those species have been shown to have a decrease in CO_2 fixation and biomass generation (Zhang, 2015). This was also shown by Maeda *et al.* (1995) as optimal *Chlorella* sp. growth occurred around 10% CO_2 with tolerance possible at up to 100%. Due to these limitations, Zhang (2015) found that adaptation to the higher CO_2 concentrations is necessary for microalgae use in flue gas treatment and removal of CO_2, suggesting strains from lakes and ponds surrounding the power plants as a starting point for high CO_2-tolerating, combustion product exposed microalgae species. In addition to CO_2 concentration considerations, the additional products found in flue gas including NO_x and SO_x species along with certain heavy metals need to be analyzed further for their effects on microalgae. Recent research indicates optimal algal growth and productivity at 5% CO_2 and 30–100 ppm NO_x (Biscaia *et al.*, 2022).

Algal technologies have long been considered for usage in wastewater treatment. Algae are generally cultured during wastewater treatment in waste stabilization ponds (WSPs) or high-rate algae ponds (HRAP), typically in warmer climates (Molazadeh *et al.*, 2019). In a WSP, raw wastewater is fed into the pond and treated using algal and bacterial metabolic processes. The algae provide oxygen for the aerobic activity of the bacteria so that the bacteria can continue to metabolize the organic compounds in the wastewater. This system is much more cost effective than alternatives to treating wastewater, providing a continuous source of oxygen from the algae and the breakdown of organic matter from the bacteria. This process removes the biological oxygen demand (BOD) from such aerobic WSPs, and by heterotrophic bacteria in anaerobic WSPs (Molazadeh *et al.*, 2019). The HRAP in contrast use oxidation ponds and a photobioreactor with the algae providing oxygen for the bacteria, similar to a WSP and the bacterial ability to convert waste nutrients into usable forms for the algae (Molazadeh *et al.*, 2019).

Algae have been used in wastewater treatment, recovery of minerals and micronutrients and are being analyzed for usage in an integrated systems for and concurrent carbon sequestration. Considering the often-high nutrient concentrations in wastewater when combined with a CO_2 stream, algal-based systems are an attractive option for CO_2 capture and wastewater treatment as a synergistic system. With some intervention, these resource streams together can create an optimal growth medium for various algal species. To make such a system successful, innovations to optimize the C:N ratio in wastewater along with the need for a sourced carbon stream are necessary, as atmospheric CO_2 would not be able to adequately supply the necessary amount of CO_2 to the algae in such a system (Molazadeh *et al.*, 2019). One source being examined is the flue gas from power plants providing a continuous, concentrated CO_2 stream for ample algal carbon fixation. Woertz *et al.* (2009) found that adding a steady CO_2 stream to a wastewater supplied HRAP can successfully remove nutrients from the wastewater, while simultaneously building algal biomass that can subsequently be used as a feedstock for biofuels. Since many wastewater, treatment plants burn off their excess methane, the CO_2 thus generated from flaring of methane could serve as an on-site source of concentrated CO_2.

Flue gas from biogas flaring is an intriguing potential CO_2 source where the prevention of CO_2 from entering the atmosphere could be coupled with the treatment of wastewater to create a cohesive environmentally friendly clean-up mechanism. This can lower GHG emissions while simultaneously capturing nutrients like nitrogen and phosphorus from wastewater sources. Chaudhary *et al.* (2017) found a successful algal-based remediation system, with increased photosynthetic activity present in CO_2-fed municipal wastewater with algal cultures *C. vulgaris* and *S. obliquus*. Pond cultivation systems and flat panel bioreactors are the most cost-effective cultivation methods for growing algae on flue gas (Schipper *et al.*, 2021). The delivery method of CO_2 is an important consideration when optimizing maximum photosynthetic efficiency for cultivation of algae. Addition of CO_2 to the wastewater can cause pH alteration potentially affecting the growth rate of the microalgae. One means of containing the pH within the optimal level for illuminated, actively photosynthesizing, microalgae is periodic sparging with CO_2 from sources such as flue gas (Wang *et al.*, 2008). Using microalgae for wastewater treatment, CO_2 sequestering coordinated hybrid technologies must demonstrate cost-effective, environmentally friendly potential.

An advantage of algae-based technologies is that they are typically scalable. An algae–wastewater treatment model has shown promise for CO_2 assimilation and nitrogen uptake (Eze *et al.*, 2018). Up to 200 tons of CO_2 per ML of wastewater treated could potentially be captured (Green *et al.* 1995; Molazadeh *et al.* 2019) Adding flue gas can deliver needed CO_2 while addressing pH fluctuations that result from photoautotrophic growth of microalgae. Eze *et al.* (2018) found that phototrophs successfully assimilated nitrogen and CO_2 at pH of 8.1; however, as the pH increased CO_2 and nitrogen assimilation decreased. This pH increase was associated with ammonia volatilization and increased CO_3^- presence, decreasing autotrophic biomass production and nutrient usage as these forms of carbon and nitrogen are not readily utilized by microalgae (Eze *et al.*, 2018). The algal biomass may be used as a biofuel feedstock, neutralizing CO_2 emissions. Alternatively, the biomass could be buried or integrated as agricultural biochar for long-term carbon sequestration in soil (Mona *et al.*, 2021). The pairing of bioenergy production with longer term carbon storage for decades to millennia is sometimes called bioenergy with carbon capture and storage (BECCS) (Moreira & Pires, 2016). While around 99% of the roughly 2 billion tons of carbon sequestered annually is due to conventional land-use practices, like planting and encouragement of forests, there is a pressing need for scalable, cost-effective new methods (Naddaf, 2023).

4.5 ALGAL BIOFUELS

Microalgae have economic potential for environmentally favorable clean-up strategies of various waste streams. Microalgae are excellent candidates for CO_2 sequestration, and many can work synergistically with different bacteria. Using microalgae to perform multiple tasks, from CO_2 uptake to nitrogen recovery, in an integrated system could provide beneficial, cost-effective, and an environmentally friendly alternative to conventional methods of CO_2 capture and wastewater treatment. Once carbon fixation occurs in these organisms, the biomass has additional potential in that of biofuels. This not only allows for the reduction of CO_2 emissions into the atmosphere and potentially more efficient mechanisms for wastewater treatment, but also the ability to take that fixed carbon and use it for environmentally favorable biofuel production. Biodiesel is produced from algal neutral lipids when the fatty acids in TAGs are chemically converted into fatty acid methyl esters. Algal biomass can alternatively be converted into biocrude oil via hydrothermal liquefaction.

4.5.1 Biodiesel

Biodiesel can be produced from various neutral lipid feedstocks, including many fast-growing microalgae (Saad *et al.*, 2019). Conventional production of algal biofuel (i.e., biodiesel) requires destruction of the production cell during the extraction step. Newer processes have been developed that do not necessarily require destruction of the phototroph. Strategies using naturally occurring and genetically modified cyanobacteria are promising due to their ability to rapidly convert CO_2 into biomass and to produce alcohol (Zhang *et al.*, 2017). CO_2-concentrating cyanobacteria fix CO_2 into biomass and minimize rates of wasteful photorespiration attributable to the oxygenase activity of RuBisCo (Kamennaya *et al.*, 2012). The carboxysome of cyanobacteria concentrates CO_2 for RuBisCo, which has low carboxylation efficiency (Kamennaya *et al.*, 2012). The mechanism, which involves CA works by bicarbonate (HCO_3^-) symports and CO_2 transporters (Kamennaya *et al.*, 2012). Most of the CO_2 is converted into HCO_3^- via enzyme CA activities (Kamennaya *et al.*, 2012). Cyanobacteria are promising candidates for biofuels due to their high lipid content, efficient carbohydrate metabolism, mainly in the thylakoids, high photosynthetic capabilities, and growth rates, along with their low growth requirements (Quintana *et al.*, 2011). However, cyanobacterial lipids are mainly charged membrane phospholipids which are less suited for biodiesel production than neutral lipids.

The neutral lipid composition of many microalgae allows for the production of biodegradable biofuels, namely biodiesel (Saad *et al.*, 2019). The optimal growth conditions of algae are dependent on temperature, pH, CO_2 availability, light intensity, and nutrient availability (Li *et al.*, 2019). Most algal species have an optimum temperature between 20 and 30°C (Singh & Singh, 2015). Specifically, light, CO_2 and water are primary factors in biomass accumulation in algae and cyanobacteria (Khan *et al.*, 2017). Photoautotrophic systems are promising due to their usage of CO_2 as a sole carbon source and their minimal negative environmental impact (Mata *et al.*, 2010). The production strain screening process generally occurs in *in vitro* experiments, many turning to microfluidics or on-chip technology due to the higher costs of conventional experimental techniques (Saad *et al.*, 2019). Such techniques allow droplets to be captured and tested using many different configurations based on different culturing conditions along with DNA and oil extraction (Saad *et al.*, 2019). Selecting an appropriate cultivation system is equally important because it can have a direct impact on biofuel generation. Wild-type algae traditionally were used in algal biofuel production, but recent advances in genetic editing have led to the creation of novel algal strains with optimized carbon sequestration capabilities (Figure 4.4).

Processing of microalgae is simplified compared to macroalgae, but economical harvesting and dewatering of the cells can be difficult. Many microalgae such as *Chlorella pyrenoidosa*, accumulate significant intracellular neutral lipids that can be extracted and chemically converted into biodiesel. Nitrogen limitation provides one elegant means of inducing over three-fold lipid accumulation in *C. pyrenoidosa*, provided adequate light and carbon dioxide are available (Han *et al.*, 2013). Alternative approaches include genetic modification of components associated with both the light and light-independent catalysts of photosynthesis, an inherently inefficient process. Over 8 billion gallons of biodiesel is produced annually worldwide (Guo, 2020). Unfortunately, much of this is

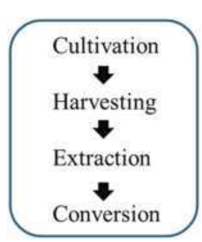

Figure 4.4 Flowchart overview of algal biodiesel production.

produced from lipids extracted from crop phototrophs used for foods, such as soybeans. Algal lipids can alternatively be used to produce algal biodiesel. The lipid content in many microalgae strains can be up to 50% of the organism's dry weight (Li *et al.*, 2019; Rawat *et al.*, 2013). The type of fatty-acid precursor a specific algal species produces affects various properties of biodiesel production including melting point, oxidative stability, cetane values and lubricity (Saad *et al.*, 2019). Biodiesel production is generally reliant on palmitoleic acid, palmitic acid, steric acid, linoleic acid, oleic acid, and fatty acids (Zheng *et al.*, 2013). Microalgae typically store solar energy as neutral lipids (i.e., TAGS) whereas many cyanobacteria accumulate starch which is more suited for ethanol production (John *et al.*, 2011; Möllers *et al.*, 2014). Accordingly, microalgae are considered useful feedstocks for biodiesel production. *Nanochloropsis* is a promising production strain capable of lipid accumulation of 47% of dry weight (Alishah Aratboni *et al.*, 2019; Pal *et al.*, 2011). *Chlorella* sp. BTA 9031 exhibited 25% of lipid accumulation per dry weight under 3% (v/v) CO_2 concentration (Mondal *et al.*, 2017), and *Chlorella vulgaris* SDEC-3M also accumulates significant neutral lipids (Qi *et al.*, 2019).

4.5.1.1 Cell cultivation and harvesting

Algal cultivation can be carried out in open system such as ponds or raceways or closed bioreactors (Chew *et al.*, 2018). Open systems are generally a closed-loop (raceway) or circular pond tank, a closed pond, or large shallow pond (Chew *et al.*, 2018). These ponds generally contain natural fresh or seawater, depending on the species being used (Anto *et al.*, 2020). Open systems are useful because they allow atmospheric CO_2 to be utilized and are generally lower cost than a closed system (Dharmaprabhakaran *et al.*, 2020). These open systems come with challenges such as contamination by grazers, bacteria, protozoa, or other microalgae species which can create toxic products that cannot be utilized (Narala *et al.*, 2016; Tan *et al.*, 2020; Ullah *et al.*, 2015). Open systems can be significantly affected by rainwater runoff which can cause alterations in salinity, pH, and increases in turbidity of water due to erosion (Tan *et al.*, 2020) along with other factors such as evaporation and temperature (Mata *et al.*, 2010; Narala *et al.*, 2016). A closed system can help to alleviate some of the challenges of an open pond such as contamination and evaporative losses (Carvalho *et al.*, 2006).

Closed systems employ photobioreactors which can be highly controlled allowing for ideal stirring and efficiency due to high light availability, delivering high-productivity yields (Lee & Lee, 2016; Liao *et al.*, 2018). Size and shape of the bioreactor can be flat-plate, tubular, vertical columnar, along with other configurations, providing more compact organization and more efficient land usage (Tan *et al.*, 2020). These systems can use acrylic or glass materials and can be constructed as bags, tanks, or towers (Anto *et al.*, 2020). Closed system bioreactors tend to have a higher productivity than open systems because of the controlled conditions used, but the overall cost of using an open system is more favorable (Leite *et al.*, 2013). For example, photobioreactors could be used for starting seed cultures that are subsequently scaled up into large ponds.

Harvesting of algae can be a challenge and care must be taken to efficiently remove the cells from the growth medium. Harvesting and dewatering of microalgae are energy-intensive processes. Methods such as flocculation, sedimentation, centrifugation, filtration, precipitation, sonication, and flotation have all been used with varying degrees of success. In many cases, dry biomass may be required, and dewatering must occur to remove the water content of the algal cells (Chen *et al.*, 2015). This process can occur through bulk harvesting and thickening (Alam *et al.*, 2017). In bulk harvesting, the biomass is separated from suspension, and the solid matter can be separated using gravity sedimentation, flocculation, or flotation (Lam *et al.*, 2019). Drying using sunlight may reduce input energy cost. Flocculation is a step that can be taken before using gravity sedimentation or flotation. Flocculants can be used to treat large quantities of microalgae, inducing flocculation (Alam *et al.*, 2017). In flocculation, aggregation of microalgae or other cells collide and adhere to one another into larger particles through charges on the cell surfaces (Khan *et al.*, 2017). This method is favorable due to the ability to harvest large quantities of cells from different taxonomic classifications (Wan *et al.*, 2015). Gravity sedimentation is used in species with high sedimentation rates or larger species such as *Spirulina* for harvesting in wastewater treatment applications (Brennan & Owende, 2010). This technique can be employed to separate algal cells of different types due to differences in settling velocity (Peperzak, 2003). Due to low microbial settling rates, it is not favorable for routine harvesting, but is a popular choice for wastewater treatment applications (Christenson & Sims, 2011; Greenwell *et al.*, 2009). In flotation, biomass is removed using gravity separation (Alam *et al.*, 2017). In this technique, gas bubbles are passed through a liquid suspension and attach to solid particles within the suspension (Nguyen & Hoang, 2016). This is an advantageous technique compared to others due to low microalgal density and buoyancy characteristics of certain species such as *Anabaena, Microcystis, Spirulina*, and *Nostoc* (Hanotu *et al.*, 2012; Laamanen *et al.*, 2016).

Thickening of the reduced volume cell slurry may be performed using more energy-intensive methods such as centrifugation, filtration, or ultrasonic aggregation (Brennan & Owende, 2010). Centrifugation is fairly cost-efficient and harvests many microalgal species rapidly (Khan *et al.*, 2017). It has proven 95% efficient at increasing the concentration of the cell slurry (Li *et al.*, 2017). Filtration can be used to harvest larger microalgae such as *Spirulina* and *Coelastrum* (Brennan & Owende, 2010), but it is not appropriate for large batch harvesting (Nguyen & Hoang, 2016). This allows for the collection of low-density algal cells using different membranes and pore sizes with the addition of a suction pump (Rastogi *et al.*, 2018). Flow filtration has shown a 70–89% efficiency for microalgae harvesting and cell structure preservation (Danquah *et al.*, 2009; Rastogi *et al.*, 2018). Ultrasonic aggregation employs acoustic force and heightened sedimentation for concentration of microalgal cells (Nguyen & Hoang, 2016).

4.5.1.2 Lipid extraction and conversion

Extraction of lipids from the slurry and dewatering must be carried out after harvesting to avoid decomposition (Kadir *et al.*, 2018). During dewatering, water is further removed from the biomass through a drying process either by use of sunlight, freeze-drying, fluidized drying, or spray drying, with spray drying being the most widely used technique (Khan *et al.*, 2017; Nguyen & Hoang, 2016). This allows the water content from the algae to be reduced, leaving dry biomass. Cell disruption is commonly employed during lipid extraction and is accomplished through mechanical, physical, chemical, and enzymatic phases (Lee *et al.*, 2012). Cell disruption is important when performing lipid extraction for biodiesel production (Johnson, 2008; Kadir *et al.*, 2018). Using a wet route to lipid extraction has been examined and has potential to improve production of valuable biofuel (Lakshmikandan *et al.*, 2020; Xu *et al.*, 2011). Using organic solvents and supercritical fluids are popular for high production yield in algal lipid extraction (Liu *et al.*, 2013; Rastogi *et al.*, 2018; Santana *et al.*, 2012; Soh & Zimmerman, 2011). Using solvents for extract on dry biomass is the most common method, but it can have a high-cost and high energetic requirement (Rastogi *et al.*, 2018). Because of this, wet biomass usage is an economically promising alternative (Chatsungnoen & Chisti, 2016; Grima *et al.*, 2012; Liu *et al.*, 2013). Lakshmikandan *et al.* (2020) found promising results using wet biomass of *C. vulgaris*, showing increased biomass and total lipid yield (22.5%) through extraction by mild pressure and heat shock (Lakshmikandan *et al.*, 2020).

Lipid extraction can be accomplished in four ways: using chemical solvents, supercritical CO_2, physiochemically, or biochemically (Nguyen & Hoang, 2016). Chemical solvents can be employed to break the chemical linkages within the cell, with minimal requirements for energy or heat (Nguyen & Hoang, 2016). Pressurized CO_2 can accomplish lipid extraction without utilizing toxic chemicals, but it is energetically demanding and expensive (Perrut, 2000; Tan & Lee, 2011). Various physical methods can be employed to cause cell disruption, but microwave is one promising technique (Lee *et al.*, 2012). The blending method is a simple method for fatty-acid extraction and separation of biodiesel and consists of the stages: filtration, drying, oil extraction, and biodiesel production (Khan *et al.*, 2017). Other methods that have been employed for biodiesel production include microemulsion and pyrolysis. Microemulsion involves using vegetable oils for biodiesel production, however, micro emulsification tends to have heavy carbon residues, inadequate combustion, random injector needle sticking, and high viscosity of lubricant oils (Tabatabaei *et al.*, 2019). Various impurities may need to be removed. Pyrolysis can also be employed using thermochemical mechanisms through application of heat to convert various species into chemical species in anoxic conditions (Tabatabaei *et al.*, 2019). This technique, however, has a high-cost due to complex equipment and the reaction conditions, and form a fuel more closely related to gasoline than to diesel (Tabatabaei *et al.*, 2019). Transesterification is the chemical conversion technique usually used in biodiesel production.

Transesterification occurs when short-chain alcohols (i.e., methanol, ethanol, propanol, or butanol) react with microalgae free fatty acids (triglycerides) using a catalyst (Tabatabaei *et al.*, 2019). Popular catalysts include acids such as HCl, H_2SO_4, H_3PO4, and sulfonic acid, alkali species such as KOH, NaOH, CH_3KO, CH_3ONa, or lipase enzymes (Demirbas, 2009; Fukuda *et al.*, 2001). Alkali species are favorable for use with methanol due to lower cost and higher reactivity than ethanol (Rastogi *et al.*, 2018; Robles-Medina *et al.*, 2009). Alkali-catalysts, such as KOH or NaOH, are important because they increase the rate of the reaction 4000 times over acid-based catalysts (Fukuda *et al.*, 2001). Alkali-catalysts can, however, cause some unfavorable issues including saponification (Tabatabaei *et al.*, 2019). In transesterification, TAGs combine with alcohol (methanol or ethanol), to form diglyceride followed by monoglyceride then glycerol, as three fatty acid methyl esters (FAME) are produced when methanol is used or three fatty acid ethyl esters (FAEE) when ethanol is used (Demirbas, 2010; Yusoff *et al.*, 2014). These FAME or FAEE biodiesel molecules can be combusted as fuel in regular diesel engines with certain benefits, like superior lubrication (Balat & Balat, 2010; Pragya *et al.*, 2013; Robles-Medina *et al.*, 2009). Large quantities of the glycerol waste product have accumulated as the biodiesel industry has matured. An example of the transesterification used for biodiesel production reaction is shown in Figure 4.5.

Ethanol and methanol are alcohols of choice for the transesterification process (Bouaid *et al.*, 2007). Methanol is the more widely used alcohol due to lower cost than ethanol, easy phase separation, mild conditions required for reactivity, along with easier extraction of methyl esters over ethyl esters (Yusoff *et al.*, 2014). Ethanol does have some advantages over methanol however, including easy attainment

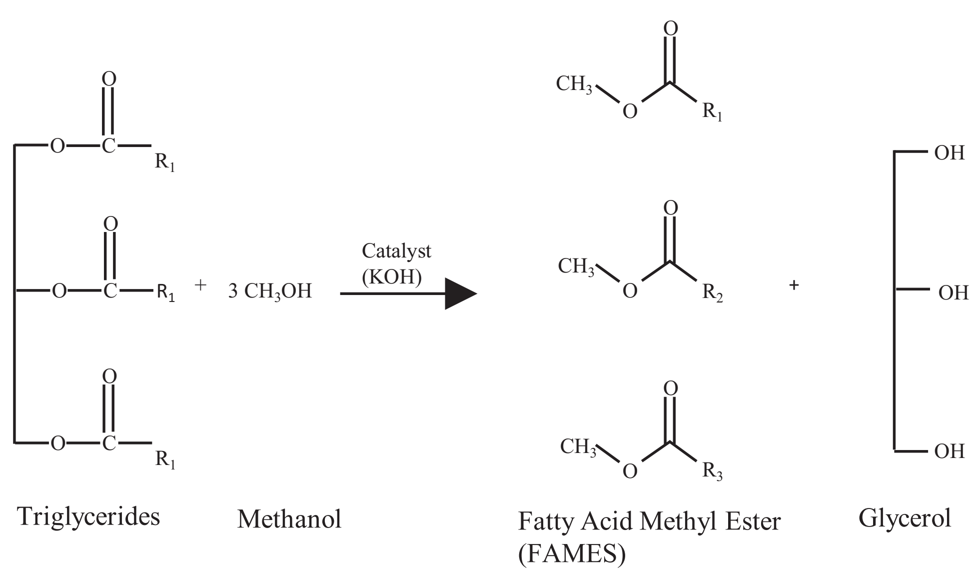

Figure 4.5 Depiction of the transesterification reaction used to produce fatty acid methyl esters (i.e., biodiesel) from algal TAG plus methanol in the presence of KOH catalyst. NaOH can alternatively be used to catalyze the transesterification reaction.

from agricultural sources such as sugar cane or corn, and produces cleaner products of ethyl esters opposed to methyl esters (Mendow *et al.*, 2011; Yusoff *et al.*, 2014). Ethanol has a lower safety risk and is easier to handle than methanol, making it a popular alcohol for transesterification (Yusoff *et al.*, 2014). Direct transesterification can be employed by combining oil extraction, esterification, and transesterification simultaneously, using alcohol for extraction (Liu *et al.*, 2017). Algal biodiesel has some drawbacks, such as low-temperature gelling during winter, capacity to degrade rubber seals and oxidation over time (Zuleta *et al.*, 2012). Ideally, synthesis and direct secretion of FAME could preserve the phototrophic producer cells.

4.5.2 Biocrude and triterpenes

Biocrude oil, alternatively called pyrolysis bio-oil, is produced through thermochemical conversion of organic material, such as algal cells. For this, biomass is thermally broken down and organic chemicals are recruited to restructure the products into biofuels (Naik *et al.*, 2010; Raheem *et al.*, 2018). This process can produce biochar, liquid fuel, or gaseous fuel (Adeniyi *et al.*, 2018). Pyrolysis is the first step in thermochemical conversion of solid fuel. In algal pyrolysis, algae decomposition occurs in anoxic conditions through thermochemical degradation causing the formation of gases and volatile products that condense to form liquid bio-oils at high temperatures (Aravind *et al.*, 2020). The solid product that remains is biochar, which can be used in gasification to form syn-gas which contains H_2 and CO (Saber *et al.*, 2016; Zainan *et al.*, 2015). This process may involve various mechanisms such as dehydration, decarboxylation, fragmentation, polymerization, and rearrangement reactions (Li *et al.*, 2019). Dewatering is required for pyrolysis because the feedstock must be dry in order for pyrolysis to occur, causing an increase in total energy consumption of the system (Saber *et al.*, 2016). Pyrolysis can occur by slow heating (10°C/min), fast heating (100°C/s), or by flash heating, generally done at 300–750°C/s (Li *et al.*, 2019). Slow pyrolysis generates larger quantities of solid char than fast or flash pyrolysis (Saber *et al.*, 2016). Fast or flash pyrolysis have up to an 80 wt.% dry feed yield of bio-oils, making them a promising pathway for bio-oil generation (Huber *et al.*, 2006). For bio-oil production, microwave-enhanced pyrolysis (MEP) is a popular method (Saad *et al.*, 2019; Zhang *et al.*, 2017). Microalgae pyrolysis-generated bio-oils have a composition mainly of aliphatic and aromatic hydrocarbons, along with oxygen-containing and nitrogen-containing compounds (Li *et al.*, 2019). The oxygen-containing and nitrogenous compounds are formed from carbohydrate and protein decomposition in the algal biomass (Li *et al.*, 2017). These oxygen-containing compounds can affect bio-oil by causing acidity, corrosion, and high viscosity and volatility, while nitrogen-containing compounds can contaminate catalysts and cause nitrogen-oxide formation during combustion (Czernik & Bridgwater, 2004; Oasmaa & Czernik, 1999; Wang *et al.*, 2013; Zainan *et al.*, 2015). The high oxygen content of pyrolysis oils causes them to have a lower heating value than oils generated through liquefaction (Duan & Savage, 2011; Peterson *et al.*, 2008). Oil products from pyrolysis are dark brown and viscous, containing over 300 different compounds including hydrocarbons, alcohols, acids, phenols, sugars, polyaromatics, nitrogenous compounds, and others, with straight-chain hydrocarbon and paraffin aromatics (up to C_{10}) preferred for transportation fuel (Li *et al.*, 2019). Catalysts can be recruited to lower the reaction temperature requirements and energy requirements in general (Hazrat *et al.*, 2015). Some catalysts that have been used in microalgae pyrolysis studies include zeolite, metal-loaded catalysts, and metal-organic frameworks (Yang *et al.*, 2019). The cost of pyrolysis is lower than that of liquefaction and many technologies are being commercially used (Yang *et al.*, 2019).

Hydrothermal liquefaction (HTL) can be used to process algal biomass waste into bio-oil. Essentially, biomass (algal slurries) is reacted in water at higher temperatures (200–400°C) and pressures (5–20 MPa) with or without the use of a catalyst to produce biocrude, gas, or char (Brand *et al.*, 2014; Chen *et al.*, 2015; Chiaramonti *et al.*, 2017; Ross *et al.*, 2010; Shuping *et al.*, 2010). Water is a popular solvent used in this technique, but the product is a very viscous bio-oil with a prominent oxygen content (Saber *et al.*, 2016). This has prompted the use of organic solvents such as ethanol, acetone, 2-propanol, methanol, among others, for a higher yield of bio-oil (Liu & Zhang, 2008; Ogi *et al.*, 1994; Yuan *et al.*, 2011). Catalysts, such as Na_2CO_3, KOH, CH_3COOH, and zeolite can be utilized to improve the bio-oil quality and yield (Duan & Savage, 2011; Jena *et al.*, 2012; Ross *et al.*, 2010). This process includes various mechanisms such as solvolysis, decarboxylation, depolymerization, hydrogenolysis, and hydrogenation (Demirbaş, 2008). HTL produces a higher yield of bio-oil than pyrolysis of up to 97% (Dote *et al.*, 1994; Saad *et al.*, 2019; Zou *et al.*, 2010). The main factors affecting HTL include temperature, loading, and residence time (Saber *et al.*, 2016). Bio-oil derived from this process is partially water soluble and of a higher energy content than pyrolysis due to lower oxygen content and water content, but also have a higher viscosity (Cheng *et al.*, 2017). Cost can be higher than that of pyrolysis due to the higher-pressure requirement (Huber *et al.*, 2006). It may be beneficial, however, in the use of wet biomass, eliminating the need for a drying step as in pyrolysis. Research by Jena and Das (2011) found HTL produced a higher bio-oil production with a lower energy requirement and solid char yield than pyrolysis, showing its potential advantages for bio-crude production.

Gasification can be used, in which case biomass is partially oxidated using a controlled quantity of air, oxygen, or steam at 700–1000°C, that produces a mixture of CO_2, H_2, CH_4, and CO (Demirbas, 2010; Naik *et al.*, 2010). The oxidized biomass can be used for heat production, electricity, or further processed (Li *et al.*, 2019). Direct combustion may be employed for the production of algal energy. In direct combustion, the algal biomass is first pre-treated through drying and mechanically grinding into smaller particles (Adeniyi *et al.*, 2018). Following pre-treatment, a furnace, boiler, or steam turbine is used for biomass oxygenation at ~1000°C (Saad *et al.*, 2019). Undesirable facets of bio-oil may include high oxygen, nitrogen, and water content, high viscosity and acidity, and thermal and chemical instability (Saber *et al.*, 2016). These characteristics are derived from the fundamental components of the algal biomass including carbohydrates, proteins, and fatty acids. Gasification of waste can facilitate CO_2 valorization with ethanol production through the process of microbial syngas fermentation (Stoll *et al.*, 2020). LanzaTech is one company that is currently commercially developing microbial syngas fermentation (Stoll *et al.*, 2020).

Triterpenes are chemical components of certain algae that can be converted into drop-in biofuels without necessarily destroying the cells. Triterpenes are relatively large organic molecules composed of six isoprene rings that are produced by various bacteria, fungi, animals and various phototrophic microorganisms, including some algae. They may serve as steroid precursors and are involved in the synthesis of biologically important molecules like cholesterol or ergosterol in fungi. Triterpenes have a wide variety of functions in microorganisms as well, some of which can factor towards the production of biofuels (Chacko *et al.*, 2019). For example, the hopanoid triterpenes present within the cell membrane of *Z. mobilis* help confer tolerance to high concentrations of ethanol by this ethanologenic microbe (Bringer *et al.*, 1985; Hermans *et al.*, 1991). Elevated ethanol tolerance might accordingly be conferred to other biofuel producing microorganisms, such as yeast, via the genomic modification with genes and pathways needed for triterpene biosynthesis. Triterpene molecules are energy dense hydrocarbons that can additionally serve as biofuels, or biofuel precursor. Triterpenes produced by microalgae or other photosynthetic microbes are of particular interest as potential biofuels.

Botryococcene is a leading algal triterpene biofuel candidate. Botryococcene is synthesized by *Botryococcus braunii,* with the *B. braunii* race B strain recognized as a particularly productive isolate since it can accumulate 89% botryococcene by dry weight (Brown *et al.*, 1969). This microalga synthesizes botrycoccene via an unusual biochemical mechanism distinct from the mechanism other eukaryotes utilize to synthesize triterpenes such as squalene (Niehaus *et al.*, 2011). One of the advantages of this green microalgae is that the botryococcene is secreted from the cells into the surrounding media (Suzuki *et al.*, 2013). This could potentially simplify collection or avert the need to sacrifice the producer strain which is normally necessary for TAG producers during the extraction step. Another in key advantage of botryococcene is that it can easily be processed in existing oil refineries and converted into jet fuel or a drop in 'green gasoline' biofuel replacement capable of powering conventional gasoline-powered trucks and automobiles (Chacko *et al.*, 2019).

One major disadvantage of *B. braunii* is that it has an exceptionally slow growth rate with a maximum doubling time of around a day and a half for one strain investigated under a variety of conditions (Yoshimura *et al.*, 2013). Related to its slow growth rate is the presence of a thick cell wall that may pose challenges if extraction is utilized. Different strategies have been taken to overcome this, one approach cultivates the microbe in a trickle-film photobioreactor for optimized metabolic production rates of botryococcene (Khatri *et al.*, 2013). Genetic modification of other phototrophs with the metabolic machinery akin to *B. braunii* is an alternative approach to circumvent the slow growth rate. Genetic modification of *Synechocystis* allowed this cyanobacterium to produce terpene hydrocarbon (Formighieri & Melis, 2014).

Microalgae may hold promise for various stages of environmental cleanup and CO_2 capture and sequestration (Bahr *et al.*, 2013). Although some limitations may be present, a cohesive system might be used on a large scale to clean up the areas impacted by nutrient pollution while addressing, on a limited scale, the effects of GHG emissions and climate change. The treated liquid effluent of wastewater treatment plants is often associated with downstream environmental issues, including phytonutrient pollution and eutrophication. Nutrient-fed blooms of macroalgae can disrupt coastal waterways, cause dead zones and hinder industries such as fishing and tourism. Clean up and removal of the carbon-rich algae waste material could be economically incentivized if it were to serve as a biofuel feedstock. One challenge associated with macroalgae like *Sargassum* seaweed is that it is relatively low in neutral lipids; however, HTL technology can convert *Sargassum* into bio-crude oil (He *et al.*, 2020). The large size of macroalgae simplifies harvesting, but low lipid content and specialized structures, such as recalcitrant holdfasts can present a processing challenge. Even without harvest, some macro algae can promote long-term carbon sequestration into the environment. Seagrasses and possibly macroalgae support complex microbial communities that influence long-term carbon sequestration in marine sediments (Mohapatra *et al.*, 2022).

4.6 BIOGAS

Wastewater and municipal solid waste contain carbon in varied organic forms that can be catabolized to produce methane microbiologically through a process called anaerobic digestion (AD) (Gijzen, 2002). A portion of the carbon is biologically sequestered as biomass with a high degree of biochemical efficiency while the rest is released as methane which can be used as a gaseous biofuel (Lemaire *et al.*, 2020). Diverse microbial communities naturally found with input waste establish a food web inside of the anaerobic digester, essentially an airtight tank, to break down the mixture of waste lipids, proteins, and carbohydrates into biogas plus a residual liquid called digestate (Chen *et al.*, 2020). Liquid digestate is a useful, phytonutrient-rich coproduct of biomethane production that can be used in place of nitrogen-rich chemical fertilizers in agriculture (Gijzen, 2002). Cultivation of phototrophs, such as oleaginous algae, on digestate and CO_2 from AD is a useful way to recover nutrients from wastewater while producing algal biomass as a biodiesel feedstock (Gijzen, 2020). Biogas produced from AD is not pure methane, but rather a mixture of microbially produced gases that contains a significant fraction of carbon dioxide. Depending on the organic waste material being processed and incubation conditions, the composition of biogas is typically about 60% methane and 40% CO_2 with trace amounts of ammonium and hydrogen sulfide (H_2S) (Liu *et al.*, 2020).

4.6.1 Anaerobic digestion

Bacteria, eukaryotic microbes, and methanogenic archaea all work together in syntrophic connection to metabolize the many polymers in waste into first CO_2, H_2, and volatile fatty acids (VFAs), eventually forming methane. Hydrolytic enzymes including proteases, lipases, cellulases, and amylases break down proteins, lipids, and carbohydrates, respectively. This initial polymer hydrolysis and fermentation phase represents the rate-limiting step of biogas formation. Metagenomic community analysis indicates *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* as the most numerous bacterial groups, with *Methanosaeta* and *Methanosarcina* conspicuous among the archaea (Guo *et al.*, 2015). Polymer hydrolysis is considered the rate-limiting step during biogas formation. Much of the CO_2 and H_2 produced during fermentation is subsequently metabolized by acetogenic bacteria. These acetogens use the reductive acetyl coenzyme A pathway or Wood–Ljungdahl pathway to form acetate that is released into the digester (Müller, 2003). Bacteria do not directly produce methane as they breakdown organic wastes. Rather, they produce molecules that are consumed by two groups of methanogenic archaea. The methane produced during anaerobic digestion is created by archaea. Acetoclastic methanogens, like members of the genus *Methanosarcina*, consume the acetate secreted by acetogenic bacteria and convert the methyl group into methane (Ferry, 1997). Acetoclastic methanogens produce most of the methane released because relatively few other microorganisms can compete with them for acetate. The second group involved in CH_4 production in digesters are the hydrogenotrophic methanogens. They consume and oxidize hydrogen gas as their electron and energy source as they reduce carbon dioxide into methane in accordance with the following equation (4.1) (SanchoNavarro *et al.*, 2016).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{4.1}$$

Acetogenic bacteria, namely the homoacetogens, within the digester compete for substrate CO_2 and H_2 with the hydrogenotrophic methanogens. This substrate competition tends to reduce the amount of methane produced via the hydrogenotrophic route. In contrast, acetate produced by the homoacetogens serves as the substrate for acetoclastic methanogens which produce most of the methane in wastewater treatment anaerobic digesters (Guo *et al.*, 2015). Hydrogenotrophic methanogens such as *Methanococcus maripaludis* are capable of rapid autotrophic growth as they assimilate CO_2 into biomass using energy released from the oxidation of H_2 (Lyu *et al.*, 2016).

In the future, hydrogenotrophic microorganisms could prove useful for the direct biological sequestration of CO_2 into long-lived consumer goods, like bioplastics, using H_2 produced from renewable energy sources, such as can be generated using wind turbines or solar photovoltaic panels (Ayol *et al.*, 2021; Pisciotta & Blessing, 2022). Recent genetic modification of *M. maripaludis* has resulted in autotrophic conversion of CO_2 into the bioplastic polyhydroxybutyrate (PHB) (Thevasundaram *et al.*, 2022). Conversion of CO_2 into long-lived consumer could help to offset the demand for petroleum-based plastics while slowing the carbon return rate back into CO_2. While methanogenic archaea appear to hold considerable promise for carbon capture, sequestration, and valorization, at present, methanogens are primarily used in mixed communities for the synthesis of biogas. The ordered sequence of waste conversion steps that take place within an anaerobic digester are shown in Figure 4.6.

Many types of anaerobic digesters have been developed over the past century. Mesophilic digesters operate at ambient temperatures and are the most common form but may suffer from a long hydraulic retention time (HRT). HRT is described as the amount of time the waste must pass through the digester for effective treatment and energy extraction with conversion to biogas. Thermal hydrolysis as a means of pretreatment of the input organic waste can substantially reduce HRT (Chen *et al.*, 2020). Thermophilic anaerobic digesters operate at higher temperatures and have shorter HRTs for untreated wastes but require an input of heat energy. Mesophilic digesters feature more ecologically diverse

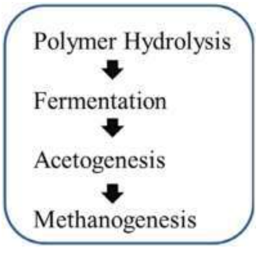

Figure 4.6 Stages of organic waste catabolism form biogas that occurs within an anaerobic digester.

microbial communities as compared to thermophilic digesters (Chen *et al.*, 2020). A key industrial advantage of thermophilic digesters for wastewater treatment over mesophilic digesters is enhanced pathogen inactivation by the former (Xu *et al.*, 2016). The resulting liquid digestate can be used as agricultural fertilizer. Solid municipal waste can also be anaerobically digested for biogas production at landfills for bioenergy production (Gijzen, 2002).

4.6.2 Biomethane enhancement

Several approaches, such as processing biogas with phototrophs using hybrid carbon sequestration systems, can elevate methane levels while reducing CO_2. Such hybrid approaches bolster conversion of diverse biomass wastes into useful methane concurrently removing CO_2 and H_2S from the product biogas (Bahr *et al.*, 2013; Nagarajan *et al.*, 2019). Since CO_2 is not an energy carrier, this increases, or rather enhances, the energy density of the biogas product. Application of electrical energy can increase methane production in a connected digester while concurrently altering the microbial community composition (Lee *et al.*, 2017). This relatively recently discovered bioelectrochemically driven phenomenon is referred to as electro-methanogenesis (Logan *et al.*, 2019). Bioelectrochemical technologies may be used to sequester CO_2 and improve the treatment of various waste and gas streams, including different types of refinery wastewaters (Ren *et al.*, 2013). Addition of the calcium-based mineral wollastonite was recently found to enhance CH_4 production while concurrently chemically sequestering CO_2 (Zhang *et al.*, 2019). Increases in CH_4 concentration to over 95% of biogas are possible through pairing wollastonite addition into bioelectrochemical digester systems (Zhang *et al.*, 2020).

4.7 BIOHYDROGEN

Carbon sequestered by autotrophic microbes is converted to cell biomass that can generate useful H_2 gas either directly by the autotroph or indirectly through biomass fermentation (Kumar *et al.*, 2013). Hydrogen presents an attractive fuel for multiple reasons. It is an energy-dense molecule that can be produced using a number of chemicals as well as biological methodologies. It is widely used in industry today for applications ranging from welding to the production of fertilizer from atmospheric nitrogen via the Haber–Bosch process (Rapson *et al.*, 2020). It can be combusted directly to power engines, including jet and rocket engines, or to provide heat (Baroutaji *et al.*, 2019). Another advantage of H_2 is that it can be converted into direct current (DC) electricity using fuel cells, such as are used in fuel cell vehicles. Since H_2 does not contain carbon, its oxidation either through combustion or using a fuel cell produces only water without climate warming carbon dioxide. For these reasons, governments are demonstrating heightened interest in renewably produced 'green' H_2 as a tool for the decarbonization of human society (Galvin, 2020). Microbiologically produced hydrogen can be produced through three main routes: dark fermentation, photofermentation, and biophotolysis.

4.7.1 Dark fermentation

Dark fermentation occurs under anaerobic conditions in sealed fermenters and is catalyzed by various anaerobic or facultative anaerobic heterotrophic bacteria. Members of the genera *Bacillus*, *Clostridia*, and *Enterobacter* have all been examined for their capacity to produce H_2 gas through dark fermentation (Sharma *et al.*, 2020). In most organisms, including *Clostridium* and *Bacillus* hydrogenase enzymes are employed to generate H_2 (Arunasri *et al.*, 2016). Hydrogenases catalyzed proton reduction as a means of oxidizing electron carriers resulting in H_2 formation. While loss of H_2 represents a net loss of energy from cells, over-reduction of the pool of electron carriers, such as NADH and ferrodoxin, could inhibit cell metabolism (Schut & Adams, 2009). Various types of hydrogenases have been identified including [FeFe]-type hydrogenases and [NiFe]-hydrogenase that can be genetically engineered into more robust bacterial species (Lamont & Sargent, 2016).

Hydrogenases are typically highly sensitive to atmospheric O_2 and are irreversibly inhibited by it. The Knall gas bacterium *Cupriavidus necator*, formerly known as *Ralstonia eutropha*, provides an interesting exception in that this microbe synthesizes an oxygen tolerant, membrane-bound [NiFe] hydrogenase which provides for sustained hydrogen production, even in the presence of oxygen (Goldet *et al.*, 2008). While not autotrophic, certain facultatively anaerobic Enterobacteriaceae family members, such as *Escherichia coli* and other mixed acid fermenters, are capable of producing hydrogen gas using a different enzyme, formate hydrogen lyase, which oxidizes formic acid into CO_2 plus H_2 (Yoshida *et al.*, 2005). During dark fermentation, carbohydrates are catabolized to CO_2 plus H_2. Dark fermentation requires no input of light energy and tends to decrease media pH through release of organic acids along with H_2. The process generally does not result in complete substrate catabolism and mineralization to inorganics, so the H_2 yields is suboptimal. Glycerol waste from biodiesel production can be further processed microbiologically to form hydrogen gas biofuel through fermentation (Rastogi *et al.*, 2018; Selembo *et al.*, 2009). Production of a mixture of fermentation end products such as volatile organic acids (VOAs) alongside H_2 by many dark fermenting microbes means a less optimal hydrogen yield is achievable by this method. Hybrid approaches are being investigated in which VOAs are used as substrates by other microbes for additional H_2 production via a light-stimulated photofermentation (Silva *et al.*, 2019).

4.7.2 Photofermentation

Certain anoxygenic phototrophic microorganisms create hydrogen gas through a light-driven process called photofermentation. Here, organic molecules, typically short-chain VOAs, including those produced by dark fermenters, serve as the electron donors with excitation energy provided by light (Silva *et al.*, 2019). The VOA-rich effluent from dark fermentation can be used to feed illuminated photofermenters for sustained and enhanced hydrogen production in a dual stage hybrid H_2 production system (Silva *et al.*, 2019). The most prominent group of microbes capable of carrying out photofermentation include the purple non-sulfur bacteria (PNS) with members of the genera *Rhodobact*er or *Rhodopseudomonas* most often evaluated for their ability to form H_2 biofuel by photofermentation (Sharma *et al.*, 2020). Their distinctive purple color is attributable to photopigments including carotenoids and bacteriochlorophyll which allows for the absorption of long wavelength, infrared radiation (Fowler *et al.*, 1997). Many PNS bacteria are diazotrophic and can produce H_2 by a second mechanism.

Nitrogenase is yet another enzyme capable of generating H_2 as a coproduct, along with ammonia in diazotrophic bacteria which facilitate biological cycling of nitrogen from the atmosphere. All of life requires bioavailable nitrogen for amino acid synthesis, mainly in the form of ammonia, which today comes from (1) atmospheric discharge, (2) biological binding, and (3) chemical synthesis via the Haber–Bosch process (Wolinska *et al.*, 2016). Conversion of gaseous N_2 into ammonia accessible to microorganisms and plants is the process of nitrogen fixation (N_2) and can only be used by certain microorganisms containing an enzymatic nitrogenase system (Wolinska *et al.*, 2016). The cellular energy required to drive nitrogenase activity can be provided by light-dependent as well as light-independent processes. When diazotrophs are grown in an inert atmosphere lacking dinitrogen, such as argon, H_2 production is enhanced. Much like most hydrogenases, nitrogenase is irreversibly inhibited by O_2 and sophisticated physiological mechanisms exist in microbes to separate O_2 from nitrogenase. Under anaerobic conditions, hydrogen gas is produced by nitrogenase alongside ammonia by way of the following biochemical reaction (Gu & Milton, 2020).

$$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi. \tag{4.2}$$

4.7.3 Biophotolysis

Biophotolytic H_2 production is carried out by certain photoautotrophs capable of concurrently sequestering carbon as biomass. As such, biophotolysis holds the greatest promise as a means of cellularly sequestering substantial CO_2 while producing clean burning H_2. Provided the biomass was disposed of properly, for instance as agricultural biochar, this could enable a shift from net carbon neutral biofuels to net negative H_2-based biofuels (Mona *et al.*, 2021). Biophotolysis is similar to photofermentation in that both are light-driven, microbiological processes useful for generating H_2. However, in biophotolysis, an inorganic molecule, water, rather than an organic acid, serves as the primary source of electrons utilized for biological H_2 formation (Kosourov *et al.*, 2020). Biophotolysis and photofermentation are not mutually exclusive and some phototrophs, such as *Chlamydomonas reinhardtii*, can carry out both processes (Scoma *et al.*, 2014). In algae, under illuminated conditions, photosystem II-mediated water photolysis provides electrons to the P-ETC and under illuminated anaerobic conditions the green microalgal *C. reinhardtii* produces H_2 gas using primarily water-derived electrons (Scoma *et al.*, 2014). Cyanobacteria such as diazotrophic *Anabaena* spp. are capable of producing H_2, though this may generate from biophotolysis and as an end product of nitrogenase activity (Kosourov *et al.*, 2014). Hydrogen production appears to be one-way photoautotrophs shed excess electrons from the P-ETC under high light conditions or when CO_2, the usual electron acceptor, is limited (Cinco *et al.*, 1993). Downregulation of RuBisCo gene expression in the green algae *C. reinhardtii*, significantly increased H_2 production by 10-fold (Pinto *et al.*, 2013). This indicates when the normal electron acceptor is lacking or biochemically inaccessible, an alternative electron path must be provided via proton reduction to sustain metabolism. Hydrogenases catalyze the proton reduction reaction and formation of H_2 gas according to the following equation:

$$2e^- + 2H^+ \rightleftharpoons H_2 \tag{4.3}$$

Hydrogenases, in most cases, are reversible yet are usually exceptionally susceptible to irreversible oxygen-mediated inactivation (Fakhimi *et al.*, 2020). This presents something of a quandary precluding sustained biophotolytic H_2 production in wild-type algae when using H_2O as electron source. This is because oxygenic phototrophs generate O_2 as a metabolite from light-driven photolysis of water. A variety of approaches have been taken to minimize O_2 formation for sustained biophotolytic H_2 formation by algae. One method seeks to reversibly downregulate PS-II activity via sulfur limitation since this element is needed for this photosystem (Zhang *et al.*, 2002). While this approach can temporarily boost hydrogenase activity and thereby H_2 formation, it is not a long-term solution as exhaustible internal starch reserves are catabolized to supply electrons rather than water. Furthermore, sulfur limitation would tend to restrict cell growth, consistent with Liebig's law of the minimum. Since over 90% of the electrons used for proton reduction during H_2 formation may derive from water, sustained PS-II activity is an essential requirement for industrialization of this means of green hydrogen generation (Kosourov *et al.*, 2020). Co-culturing highly aerobic bacteria such as *Pseudomonas* alongside *Scenedesmus* or *Chlorella* green algae is one simple yet elegant method for quickly consuming O_2 through respiration to sustain H_2 production (Ban *et al.*, 2018). *Scenedesmus* is commonly found in wastewaters suggesting water treatment and biophotolytic H_2 could be industrialized in clear, covered tanks at treatment plants with the algae later harvested for lipid conversion to biodiesel or assimilated carbon and cellular nitrogen used as biochar and field fertilizer in agriculture for longer term carbon sequestration (Mona *et al.*, 2021). Recent research indicates biochar amended into some soil types can sequester the buried carbon for hundreds of years while conferring certain agricultural benefits to the land (Yin *et al.*, 2022).

4.8 CONCLUSION

Industrial emissions are recognized as key contributors to the enduring rise in atmospheric GHG levels and associated ecological issues, including global climate change. Carbon dioxide is the most important anthropogenic GHG because of the vast and growing amounts released from sources ranging from centralized electric power generation stations to distributed transportation in developed and developing nations (Kumar *et al.*, 2011). International political and scientific consensus has reaffirmed a commitment that CO_2 levels must be reined in to avert a worsening of the climate crisis in the 21st century (Mikhaylov *et al.*, 2020). Carbon capture and sequestration offers one attractive avenue by which the rise in CO_2 levels might be alleviated.

Point source emitters, such as fossil fuel-fired power plants and wastewater treatment plants, are foremost targets for CO_2 mitigation efforts through carbon capture and sequestration. Chemical and physical carbon capture methods ranging from chemical absorption to cryogenic separation have been or are currently being developed (Meisen & Shuai, 1997). Unfortunately, many such capture methods are prohibitively expensive and or unsustainable. Long-term carbon sequestration by pumping captured carbon into subterranean geologic formations is also costly and may not be a viable option in some locations or with all sediment types. Hybrid technologies that combine chemical, physical, and biological methods may be used to lower costs. Biological carbon capture using organisms, such as microalgae, for temporary or long-term sequestration occur naturally, are scalable, and can be used independent of chemical or physical CO_2 capture methodologies.

Biological carbon fixation offers an intriguing alternative since fast-growing autotrophs, such as cyanobacteria and microalgae, already possess effective solar-powered CO_2 fixation mechanisms, grow rapidly, and form many useful products and product precursors (Lam *et al*., 2012). Carbon-neutral biofuels, like biodiesel made from the transesterification of extracted algal TAGs, can serve to temporarily sequester carbon up until the point of combustion. The use of such carbon neutral to near-neutral biofuels could help society to reduce the amount of CO_2 released from the combustion of petroleum and other fossil fuels as we transition to more renewable sources of energy.

Methanogenic archaea are mainly useful during waste and wastewater treatment for biogas methane production. In this regard, methanogens can help lower human dependence on fossil fuel natural gas. This is a critical consideration throughout Europe and other areas afflicted by natural gas supply disruptions. However, since methanogens can assimilate and reduce CO_2 into bioplastics, even in complete darkness, these archaea are emerging as potential living biological catalysts for cell-mediated CO_2 sequestration (Thevasundaram *et al*., 2022).

Carbon negative and carbon-free biofuels can enable long-term carbon sequestration for an enhanced impact on CO_2 mitigation efforts. Certain microorganisms, including various algae, assimilate CO_2 and release clean-burning hydrogen gas formed through disparate biological routes (Kumar *et al*., 2013). Some algae-derivative products, like agricultural biochar, can be employed in a carbon-negative manner to sequester biologically fixed carbon into amended soil over long periods. Future efforts should seek to optimize the carbon capture efficiency of biological processes while decreasing the cost of implementation of carbon sequestration technologies.

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Chapter 5

Exploiting hydrocarbon-degrading bacteria for reclamation of petroleum hydrocarbon polluted sites

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ABSTRACT

Petroleum hydrocarbon pollution has emerged as one of the major problem worldwide. Indiscriminate activities of petrochemical industry and accidental spills have contaminated both land and water bodies. Cleaning of hydrocarbon contaminated site and refurbishing it to uncontaminated condition is a challenging task. Bioremediation is often employed for restoration and reclamation of petroleum hydrocarbon-contaminated site as it is an environment-friendly technology. In bioremediation, metabolic capabilities of hydrocarbon utilizing microorganisms particularly bacteria are exploited to remove pollutant. In recent past, bacteria-assisted reclamation process has advanced to a new level and achieved several goals with the aid of extremophiles. However, there are several environmental factors that hinder practical application of this technology. This chapter provides an overview of bacteria-assisted reclamation of petroleum hydrocarbon-polluted sites. It also discusses chemistry of petroleum hydrocarbons, sources of contaminations, toxicity, fate of petroleum hydrocarbons in the environment, microbial hydrocarbon degradation pathways, bioremediation technologies generally employed for reclamation and different factors influencing reclamation.

Keywords: biodegradation, biosurfactant, hydrocarbonoclastic bacteria, extremophiles, enzymes

5.1 INTRODUCTION

Petroleum hydrocarbons are important global resources for human society. It fulfills a major portion of global energy demands and serve as a raw material for several industries. The global policies and strategies are often framed keeping in view of the petroleum hydrocarbon resources. Large demand for petroleum hydrocarbons has helped petrochemical industry to burgeon globally. Relentless expansion and activities of petrochemical industries added hydrocarbons to soil and water bodies as a toxic environmental pollutant. Petroleum hydrocarbon spills are very common in the marine environment. The Exxon Valdez oil spill in Alaska (1989) and the Deepwater Horizon oil spills in Gulf of Mexico (2020) are two famous industrial accidents that became the source of hydrocarbon pollution. A number of small incidences of petroleum hydrocarbon pollution are often not reported and ignored, particularly in the regions having minimal or unenforceable environmental laws.

Petroleum hydrocarbon is a mixture of simple and complex organic compounds. Majority of organic compounds present in the petroleum hydrocarbons are enlisted in persistency organic pollutant (POP) list and considered as priority environmental pollutant. Contamination of soil and water by petroleum hydrocarbons cause extensive undesirable impact on the environment. Pollution due to hydrocarbons renders adverse effects on humans as well as on livestock, wild animals, microorganisms and plants. The recalcitrant nature of hydrocarbons make it a difficult pollutant for natural degradation. Gradual accumulation of petroleum hydrocarbons in the environment alters population dynamics and disturbs interaction of organisms at the tropic level. This further aggravates to disturb the natural community structure of polluted ecosystem. Reclamation of contaminated site is a difficult assignment due to recalcitrant nature of petroleum. This has drawn attention of scientific community to study the chemical properties and environmental fate of petroleum hydrocarbons so as to strategize cost-effective and environment-friendly methods for restoration and reclamation of contaminated sites. Reclamation of petroleum hydrocarbon-contaminated sites and enhanced oil recovery are two main issues facing petrochemical industries.

The term reclamation of petroleum hydrocarbon-contaminated sites refers to the methods used for transforming toxic hydrocarbon-polluted site to non-hazardous or less hazardous site approximately to pre-polluted conditions. Properly reclaimed site must support growth of living organisms. The methods selected for remediation purpose play a crucial role in complete removal, cleaning, reclamation, and restoration of petroleum hydrocarbon-contaminated sites. A successful and effective reclamation must refurbish the natural flora and fauna of contaminated site.

Decision with selection of remediation method is influenced by environmental condition of contaminated sites, cost, and time constrains. Conventionally, remediation of petroleum hydrocarbon-contaminated sites can be achieved by physical, chemical, and biological processes. Very often physical and chemical processes fail to achieve desirable level of toxic neutralization due to generation of toxic intermediates. Transportation of toxic intermediates to surrounding areas further aggravates the contamination problem. Use of biological systems like microorganisms, plant and their products for reclamation purpose is a sustainable and cost-effective method. Reclamation of petroleum hydrocarbon-contaminated sites by biodegradation and bioremediation techniques uses metabolic capabilities of living organisms for rendering hazardous pollutant to non-hazardous form.

This chapter focuses on microorganism-assisted reclamation of petroleum hydrocarbon-polluted sites. It discusses the chemistry of petroleum hydrocarbons, toxicity and the fate of petroleum hydrocarbons in the environment, type of bioremediation technologies generally employed for reclamation, different factors influencing reclamation and microbial metabolic pathways of petroleum hydrocarbon degradation.

5.2 CHEMICAL NATURE OF PETROLEUM HYDROCARBONS

Fossil fuel, petroleum (in Latin means 'crude oil') is a dark viscous liquid produced by thermal decomposition of buried organic material over millions of years. Petroleum hydrocarbons are complex substances consisting of different proportions of hydrogen and carbon. Sometimes, they also contain other impurities such as sulfur, nitrogen, and oxygen (Varjani, 2017). Total petroleum hydrocarbon (TPH) is a term used for any mixture of hydrocarbons that are found in crude oil. There are several hundreds of these compounds, but not all occur in any one sample. Composition of crude oil may differ with location and age, as well as with depth of oil well (Varjani, 2017).

Crude oil can be categorized as heavy, medium, and light oil based on the relative proportion of the present molecular weight constituents (Varjani, 2017). Generally, crude oil is classified in four broad categories according to their polarity and polarizability: (1) saturates (aliphatics), (2) aromatics (ringed hydrocarbons), (3) resins and (4) asphaltenes (Chandra *et al*., 2013). Saturates are the simplest hydrocarbons. They constitute the maximum fraction of crude oil constituents and consist of non-polar linear, branched, and cyclic hydrocarbons without double bonds. Ethane, methane, propane, and cyclohexane are common saturates present in petroleum hydrocarbons.

Aromatic hydrocarbons are slightly more polarizable than saturated and consist of aromatic rings, mostly substituted with different alkyl groups. The simplest known aromatic hydrocarbon is benzene (C_6H_6). The aromatic name refers to '*Aroma*', fragrance of compounds. Other common examples of aromatic hydrocarbon include toluene, ethylbenzene, and xylene.

Alternatively, resins and asphaltenes contain polar non-hydrocarbon compounds and have highly complex structure consisting of carbon, nitrogen, oxygen, and sulfur atoms (Chandra *et al*., 2013). Resins consist of fused aromatic rings with branched paraffin. The resins are soluble in lighter alkanes such as pentane, but are insoluble in liquid propane. The resins get adsorbed on solids such as alumina, clay, or silica, and subsequently removed by using more polar solvents. The composition of resins varies depending upon the nature of precipitating liquid. There are three main types of resins: (a) C5 aliphatic, (b) C9 aromatic, and (c) dicyclopentadiene (DCPD) cycloaliphatic resins. The C5 aliphatic hydrocarbon resins are made from C-5 piperylene. The important aliphatic resins include 2-methyl-2-butene, *cis/trans* 1,3-pentadienes, cyclopentadiene, cyclopentene, and DCPD. Aromatic hydrocarbon resins such as indene, styrene, alpha-methylstyrene, methyindenes and so on are important C9 hydrocarbons.

Asphaltenes are large dark-brown complex molecules occurring as a colloidal dispersion in saturates and aromatics. They are soluble in light aromatic hydrocarbons such as benzene and toluene (Chandra *et al*., 2013). Asphaltenes may also contain trace amounts of vanadium and nickel along with carbon, oxygen, hydrogen, nitrogen, and sulfur. Carbon:hydrogen ratio in asphaltenes is approximately 1:1.2. Distillation products of bitumens are more likely to have asphalt-like properties, so it gained the name asphaltene.

5.3 SOURCES OF PETROLEUM HYDROCARBON POLLUTION

Crude oil is present in underground pockets called oil reservoirs. Drilling is employed to extract crude oil from reservoirs. Drilled-out crude oil is transported to oil refineries for separating different fractions of petroleum hydrocarbons. Petroleum exploration, extraction from reservoirs, transportation to refineries and hydrocarbon processing are largely associated with petroleum hydrocarbon pollution (Ossai *et al*., 2020). Accidental spillage of petroleum hydrocarbon during transportation, loading, and discharging of oil from tanks are a common cause of environmental pollution. According to the EPA Toxic Release Inventory report (2005), crude oil industry is one of the major sources of petroleum hydrocarbon pollution. More than 1.7–8.8 million metric tonnes of petroleum hydrocarbon is released annually into the marine environment globally due to human-made accidents and spills from oil tankers. Recently, Russia reported the worst accidental fuel leakage from Norilsk-Taimyr Energy Thermal Power Plant on May 29, 2020. Damage occurred due to poor maintenance of a fuel storage tank that resulted in escape of nearly 20 000 tonnes of diesel oil into the Ambarnaya River. Greenpeace Russia compared the potential environmental effects of the Norilsk spill to that of the 1989 Exxon Valdez oil spill (Khurshudyan, 2020).

The Niger Delta is considered as one of the most heavily petroleum hydrocarbon-contaminated regions in the world (Sam *et al*., 2017). The Nigerian delta has over five decades of oil exploitation history with poor management practices. There are more than 2000 land-based petroleum hydrocarbon-contaminated sites in the Nigerian delta. According to Ambituuni *et al*. (2014) sabotage, pipeline vandalism, well blowout, and engineering failures are the main contributors of petroleum hydrocarbon pollution in the Nigerian Delta.

5.4 TOXICITY OF PETROLEUM HYDROCARBONS

Petroleum hydrocarbons are composed of a wide range of compounds with varying degree of toxicity for living organisms and the environment (Varjani, 2017). Hazardous effects of petroleum hydrocarbons are primarily dependent on the chemical nature, composition, and properties of the constituting compounds. Mode, intensity, and duration of exposure also have profound influence on the environment. Petroleum hydrocarbons have toxic effect on all known living organisms.

Plants exposed to hydrocarbons exhibit stunted growth and death under extreme exposure. Hydrocarbons either reduce or obstruct water and mineral uptake by plants (Nie *et al.*, 2011). Plants experiencing stress due to hydrocarbons have chlorophyll-deficient leaves and deformed roots. Such plants are more susceptible to pest and diseases. Generally, leaves and flowers exhibit chlorotic and necrotic spots. Petroleum hydrocarbons are known to kill or inhibit growth of many microbial species. Hydrocarbon toxicity changes composition of microbial communities. This change alters functionality and subsequently the ecosystem. The absence of microbial population or diversity is known to adversely influence biogeochemical networks in nature (Truskewycz *et al.*, 2019).

Human and animal exposure to petroleum hydrocarbon causes severe health issues depending upon the nature and duration of contact. Direct contact may cause eye irritation, skin irritation, blisters, headaches, dizziness, nausea, vomiting, and lung infections (Ossai *et al.*, 2020). Long-term exposure has more drastic effect on humans and animals. They are potentially known to be toxic to genetic, immune, and endocrine systems (Figure 5.1).

5.5 FATE OF PETROLEUM HYDROCARBON IN NATURE

Petroleum hydrocarbon undergoes slow weathering in nature (Figure 5.2). Weathering includes combination of physical, chemical, and biological processes (Varjani, 2017). Weathering converts

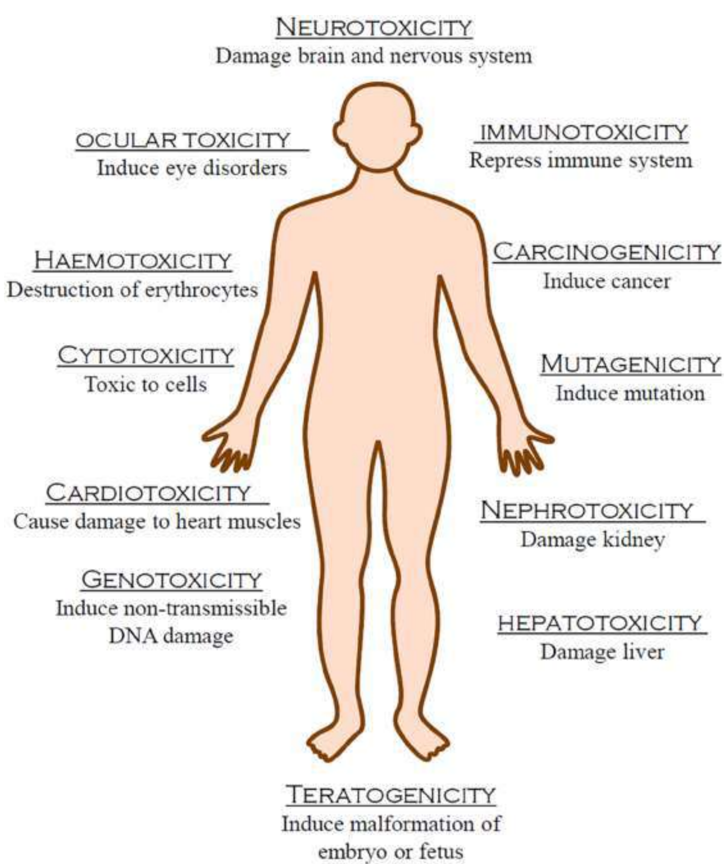

Figure 5.1 Toxicological effects of petroleum hydrocarbons on human health.

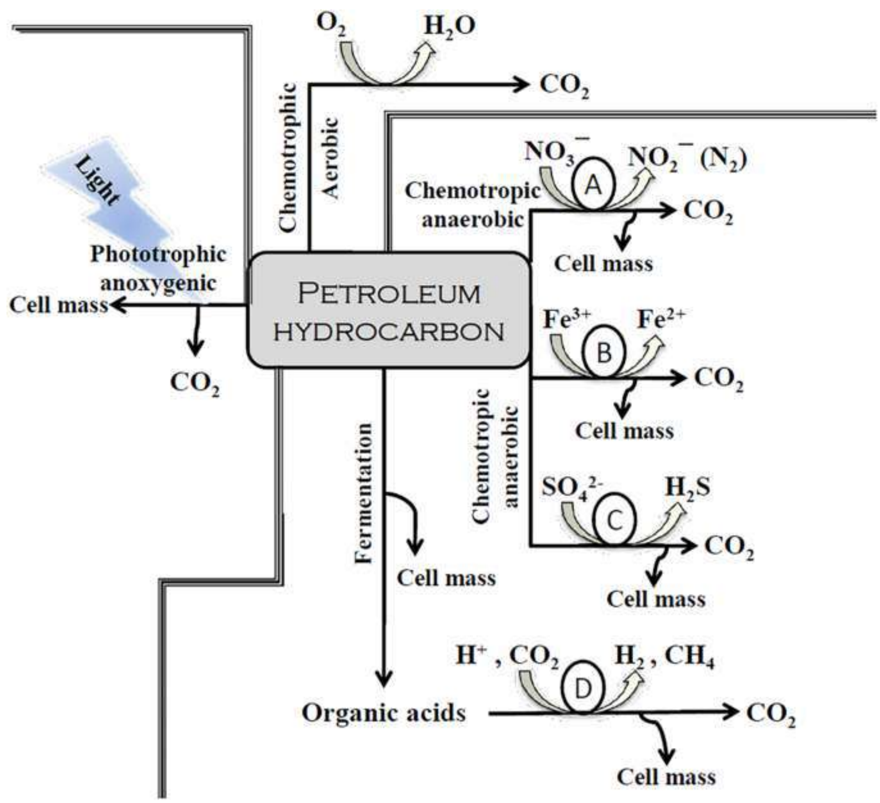

Figure 5.2 Degradation mechanisms for petroleum hydrocarbons in nature.

complex petroleum hydrocarbons to simpler forms or modifies it to less toxic forms. The ability of petroleum hydrocarbons to degrade under natural conditions is highly dependent on the surrounding environmental conditions (Widdel & Rabus, 2001). The nature, composition, physical, and chemical characteristics of petroleum hydrocarbons have significant influence on the weathering process (Varjani, 2017). Non-biological weathering of petroleum hydrocarbons occurs largely through hydrolysis and oxidation–reduction reactions. Hydrolysis of petroleum hydrocarbon requires H_3O^+, H^+, and OH^- ions. Metal ions present in loose association with soil catalyze hydrocarbon transformation and mineralization during oxidation–reduction reactions (Ossai *et al.*, 2020). Fraction of petroleum hydrocarbon with high dissolution exhibits more mobility in the environment. Dissolution is directly influenced by molecular size, structure, and polarity of hydrocarbons (Ossai *et al.*, 2020).

Aliphatic hydrocarbons are more volatile than aromatic hydrocarbons. Hence, aliphatic hydrocarbons are lost from the environment by a physical process called volatilization. Exposure to higher temperature converts lower molecular weight aliphatic hydrocarbons to vapors (Maletic *et al.*, 2013). These vapors enter atmosphere causing air pollution at the contaminated site. Among all weathering processes, volatilization is the rapid, immediate, and most effective process for removing lower molecular weight aliphatic hydrocarbons from contaminated sites (Ossai *et al.*, 2020). Light is also known to play an important role in petroleum hydrocarbon weathering process. Petroleum hydrocarbon absorbs light energy and undergoes photolytic splitting to form low molecular mass molecules (Widdel & Rabus, 2001).

Weathering by biological processes is the most prominent natural means of degrading petroleum hydrocarbons. Microorganisms carry out enzyme-specific degradation of petroleum hydrocarbons either in aerobic or anaerobic environmental conditions (Díaz, 2004). Very often, biological degradation is limited by bioavailability of hydrocarbons. Microorganisms produce surface-active substances like biosurfactant and bio-emulsifier to increase bioavailability of hydrocarbons. Microbial enzymatic activity converts hydrocarbon to less-hazardous or benign form that can be directly assimilated into biogeochemical cycles.

Petroleum hydrocarbon utilization by various microorganisms present in nature. Methanogenesis has to be coupled with fermentation reactions for complete hydrocarbon degradation (A, denitrification; B, ferric ion reduction; C, sulfate reduction; D, methanogenesis).

The presence of petroleum hydrocarbons in water bodies results in the formation of thin surface films or slicks that float on the surface of water. Marine aquatic environment often favors the formation of oil–water emulsion commonly called '*mousse*'. Weathering of petroleum hydrocarbons in aquatic environment includes processes such as spreading, evaporation, dissolution, dispersion, and emulsification (Rodriques & Totola, 2015). Heavy molecular fraction of petroleum hydrocarbon generally sinks to the bottom of water bodies. The movement of petroleum hydrocarbons from water bodies to coastal regions may expand the area under pollution (Rodriques & Totola, 2015).

Contamination of terrestrial environment by petroleum hydrocarbons has more serious impact on surrounding environment. Hydrocarbon gradually percolates through soil and reaches underground water resources. Petroleum hydrocarbons present in ground water moves later to increase the polluted area (Ossai *et al.*, 2020). Few fractions of petroleum hydrocarbon undergo hydrophobic interactions with soil organic matter and consequently get adsorbed on the soil particles (Ukalska-Jaruga *et al.*, 2019). Adsorption on soil particle decreases mobility of petroleum hydrocarbons. Volatile hydrocarbons become part of the soil gases (Ossai *et al.*, 2020). Hydrophobic nature of petroleum hydrocarbon implies it to form non-aqueous phase liquid (NAPL). NAPL tends to contaminate both soil and ground water (Logeshwaran *et al.*, 2018).

5.6 HYDROCARBON DEGRADING BACTERIA

Microorganisms are ubiquitous with diverse metabolic capability. However, only few microorganisms have ability to use petroleum hydrocarbon as carbon and energy source (Abbasian *et al.*, 2015). General ability of a microorganism to use petroleum hydrocarbon may be related to their encounter with these compounds or similar compounds during recycling of plant-derived material (Harwood & Parales, 1996).

In natural environment, microorganisms detoxify petroleum hydrocarbons by mineralization, transformation, and immobilization. However, among all microorganisms, bacteria are widely studied for hydrocarbon degradation. At any site, certain indigenous bacteria can successfully degrade or utilize certain components of petroleum hydrocarbon, while others fail to utilize available hydrocarbons (Varjani, 2017). This can be attributed to the fact that catalytic enzymes required for degradation of hydrocarbons is not available with all indigenous bacteria. Effective remediation of petroleum hydrocarbon-contaminated site requires joint action of multiple efficient bacteria. Several microorganisms like *Achromobacter*, *Acinetobacter*, *Alkanindiges*, *Alteromonas*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Dietzia*, *Enterobacter*, *Kocuria*, *Marinobacter*, *Mycobacterium*, *Pandoraea*, *Pseudomonas*, *Staphylococcus*, *Streptobacillus*, *Streptococcus*, and *Rhodococcus* are known as petroleum hydrocarbon degraders (Sarkar *et al.*, 2017; Xu *et al.*, 2018). Some microorganisms like *Alcanivorax, Cycloclasticus, Marinobacter, Oleispira,* and *Thallassolituus* are referred as obligate hydrocarbonoclastic bacteria (Naether *et al.*, 2013; Yakimov *et al.*, 2007). Hydrocarbonoclastic bacteria are a group of bacteria that are undetectable or exhibits low abundance in microbial community before hydrocarbon contamination (Radwan *et al.*, 2019). After experiencing hydrocarbon in there vicinity, hydrocarbonoclastic bacteria becomes dominant and play a crucial role in the degradation of petroleum hydrocarbons (Yakimov *et al.*, 2007). Hydrocarbonoclastic bacteria are believed to have noticeable influence on transformation and fate of petroleum hydrocarbons in the environment (Xu *et al.*, 2018).

Aerobic and anaerobic bacteria are known to play an important role in petroleum hydrocarbon removal from the contaminated site. Aerobic microorganisms are efficient in removing petroleum hydrocarbon from the environment. Oxygen is essential for aerobic respiration. In aerobic respiration, oxygen acts as final electron acceptor. Oxygen also activates substrate degradation by oxygenation reaction (Díaz, 2004). However, availability of oxygen for hydrocarbon degradation is not a ubiquitous phenomenon. Hydrocarbon present in aquatic sediments and submerged soils do not have enough oxygen concentration to support strict aerobic microorganisms. Strict anaerobes and facultative anaerobes bring about microbial degradation of hydrocarbon in the absence of oxygen. Anaerobes use alternative electron acceptors such as nitrate, sulfate, ferric ion (Fe-III), carbon dioxide, and so on (Díaz, 2004; Ossai *et al*., 2020).

In terms of energy, aerobic degradation of hydrocarbon generates more energy than anaerobic mode of degradation. Anaerobic hydrocarbon degradation with nitrate and Fe-III as terminal electron acceptors is nearly as efficient as aerobic respiration. Contrary, sulfate reducers and methanogenic conditions are comparatively less efficient owing to less generation of energy (Field *et al*., 1995). Fermentation of petroleum hydrocarbons results in the formation of intermediates. The presence of methanogens or sulfate-reducing bacteria aid in further degradation of intermediates generated during petroleum hydrocarbon fermentation (Gibson & Harwood, 2002). Hence, syntrophic existence of hydrocarbon-fermenting microorganisms and methanogens or sulfate-reducing bacteria is essential for complete degradation of petroleum hydrocarbon. Anaerobic degradation of petroleum hydrocarbon can also be carried out by photosynthetic bacteria. Energy assimilated from light is used for anaerobic degradation of petroleum hydrocarbon (Gibson & Harwood, 2002).

Bacteria exhibiting chemotaxis toward hydrocarbon plays a key role in remediation process by bringing cells into contact with degradation substrates (Parales *et al*., 2008). Lanfranconi *et al*. (2003) reported a bacterium *Flavimonas oryzihabitans* chemotactic to gas oil and hexadecane. Another study by Smits *et al*. (2003) shows *Pseudomonas aeruginosa* PAO1 is chemotactic to hexadecane.

Petroleum hydrocarbon contains more than one toxic substance. Single bacterial species may not hold ability to degrade all toxic substances to benign form. Under these conditions, syntrophic bacterial consortia may provide suitable strategy for complete degradation (Díaz, 2004). In one of the study, Rizzo *et al*. (2018) reported that *Pseudomonas* strain A6, *Joostella* strain A8 and *Alcanivorax* strain A53 exhibited 38.6%, 26.8%, and 52.7% biodegradation efficiency of diesel oil, respectively. However, bacterial consortium of *Joostella* – *Alcanivorax* and *Joostella* – *Pseudomonas* exhibited biodegradation efficiency of 99.4% and 99.2%, respectively. Thus, highlighting the importance of bacterial consortium in biodegradation of petroleum hydrocarbons.

5.7 DEGRADATION PATHWAY OF PETROLEUM HYDROCARBON

Petroleum hydrocarbon is a complex mixture of alkanes, alkynes, cycloalkanes, and aromatic compounds. The aliphatic hydrocarbons are more easily degraded by the bacteria whereas the long chain and the branched or cyclic chain hydrocarbon are more difficult to degrade (Ossai *et al*., 2020). Among all known hydrocarbons, aromatic compounds with benzene ring are more recalcitrant to microbial degradation. Benzene rings are thermodynamically stable, hence some of the aromatic compounds with benzene rings are persistent in the environment (Díaz, 2004). The bacterial degradation of aromatic hydrocarbons has more complex pathways as compared to alkanes, cycloalkanes, and alkynes (Varjani & Upasani, 2016). Bacteria assimilate and degrade petroleum hydrocarbon to generate ATP for fulfilling the daily energy requirements (Gibson & Harwood, 2002). The complete degradation of petroleum hydrocarbon in aerobic condition results in the formation of CO_2 and H_2O as by-products. The degradation tendency of the hydrocarbons is reported in the order of n-alkanes $>$ branched alkanes $>$ monoaromatics $>$ cycloalkanes $>$ polyaromatics (Tyagi *et al*., 2011).

As evident from figure 9, microorganisms can utilize petroleum hydrocarbon by three possible ways namely, (a) phototrophic, anoxygenic; (b) chemotrophic, aerobic; and (c) chemotrophic, anaerobic.

Microbial degradation pathway of petroleum hydrocarbons can be grouped into two categories namely peripheral and central pathways (Figure 5.3). Peripheral pathways degrade or transform structurally diverse compounds into intermediates that can become part of central metabolic pathways (Harayama & Timmis, 1992). Generally, several peripheral pathways end up in a common product that further enters the central pathways. Peripheral pathways are often referred to as ‘funnel’ compounds into central pathways. Central pathways consist of a series of reactions leading to the formation of intermediates that can become part of Krebs cycle (Harayama & Timmis, 1992).

Aerobic reactions are mainly involved in bacterial degradation of alkanes. Aliphatic hydrocarbon degradation is catalyzed by monooxygenases and/or dioxygenase enzymes (Tyagi *et al.*, 2011). The degradation of aliphatic hydrocarbons begins by adding an oxygen atom to the terminal or sub-terminal carbon (Abbasian *et al.*, 2015). Oxygenation ultimately converts aliphatic hydrocarbons to primary or secondary alcohols. The enzyme alcohol dehydrogenase converts alcohol to aldehyde. Aldehydes are further converted to fatty acids. The fatty acid passes through β-oxidation pathway to produce acetyl CoA (Abbasian *et al.*, 2015). The process of converting alkane into acetyl CoA is often known as carboxylation reaction (Abbasian *et al.*, 2015). The acetyl CoA generated during carboxylation reaction enter the central metabolic pathway such as the Krebs cycle (Díaz, 2004). Degradation of n-alkanes and iso-alkanes follows similar pathways before becoming a part of the bacterial central metabolic system (Abbasian *et al.*, 2015; Varjani, 2017).

Degradation pathways of cycloalkanes are slightly different and complicated as compared to alkanes (Abbasian *et al.*, 2015). Generally, complete degradation of cycloalkanes is achieved through co-metabolism by bacterial consortium (Abbasian *et al.*, 2015). The degradation of cycloalkanes starts with oxygenation of alkyl side chain by cycloalkanes monooxygenase enzymes. Oxygenation of alkyl side chain of cycloalkanes results in the formation of cycloalkanols. The enzyme cycloalkanols dehydrogenase converts cycloalkanols to cycloalkanone. The cycloalkanone is acted upon by enzyme cycloalkanone monooxygenase to produce ε-caprolactone. Adipic acid is produced from ε-caprolactone by a reaction catalyzed by ε-caprolactone dehydrase. Adipic acid enters β-oxidation pathway to produce acetyl-CoA. Acetyl-CoA is utilized in the Krebs cycle (Abbasian *et al.*, 2015).

Degradation of alkynes by single bacterial species is very rare due to the presence of triple bonds between the carbon atoms. The degradation of alkynes starts by adding water in the presence of enzyme alkynes hydratase. Hydration of alkynes requires strong acid and Hg^{2+} ions. Hydration of alkynes produces ketone, which further gets converted to carboxylic acid (Abbasian *et al.*, 2015).

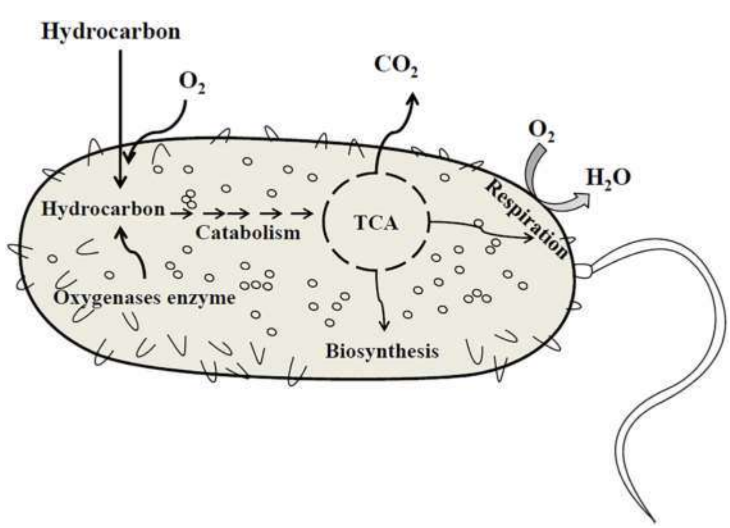

Figure 5.3 Schematic overview of potential pathways for petroleum hydrocarbons utilization by bacteria.

Formation of carboxylic acid requires strong permanganate oxidizing agent and carboxylase enzyme. The carboxylic acid is oxidized to produce acetyl-CoA which is further consumed in the Krebs cycle (Abbasian *et al.*, 2015).

Degradation of aromatic hydrocarbons is more complex due to the involvement of numerous enzymes. Most of the peripheral pathways of aromatic hydrocarbon degradation involve oxygenation reaction catalyzed by monooxygenases and/or hydroxylating dioxygenases (Abbasian *et al.*, 2015; Truskewycz *et al.*, 2019). Oxygenation results in generation of dihydroxy aromatic compounds. These aerobic degradation intermediates undergo ring cleavage to open the aromatic ring between hydroxyl groups or proximal to one of the two hydroxyl groups (Abbasian *et al.*, 2015). Ortho-cleavage or intradiol pathway (cleavage between two hydroxyl groups) is catalyzed by ring-cleavage enzymes intradiol dioxygenases (Peng *et al.*, 2008). Meta-cleavage or extradiol pathway (cleavage near one of the two hydroxyl groups) is catalyzed by extradiol dioxygenases. Ring cleavage reaction result in the formation of liner hydrocarbons like carboxylic acids and/or aldehydes (Abbasian *et al.*, 2015; Truskewycz *et al.*, 2019). These compounds are further degraded by pathway similar to aliphatic hydrocarbons degradation pathway. Peripheral pathways of anaerobic catabolism of aromatic compounds results in the formation of benzoyl-CoA. Under certain anaerobic conditions resorcinol or phloroglucinol may be formed during anaerobic catabolism of aromatic compounds (Heider & Fuchs, 1997).

5.8 GENETICS OF PETROLEUM HYDROCARBON BIODEGRADATION

Bacteria capable of degrading petroleum hydrocarbon have catabolic genes, transport genes, and regulatory genes, usually arranged together in a cluster (Meckenstock *et al.*, 2016). Catabolic genes encode for enzymes involved in catabolic pathway. The catabolic gene clusters are generally present on the mobile element like transposons and plasmids (Wilkes *et al.*, 2016). Mobile elements facilitate horizontal transfer of catabolic genes among microorganisms. Catabolic genes mobilization helps in rapid adaptation of microorganisms to new toxic environment. Product of transport genes help in uptake of petroleum hydrocarbon from the surrounding environment. Regulatory genes regulate expression of catabolic genes and transport genes under the influence of petroleum hydrocarbons present in the surrounding environment (Wilkes *et al.*, 2016; Varjani, 2017). Bacteria experiencing petroleum hydrocarbons in their vicinity receives external signals for activating degradation pathway. Depending upon physiological capability, efficiency of metabolic return and experienced toxicity, bacteria may initiate process of petroleum hydrocarbon degradation (Wilkes *et al.*, 2016).

Bacteria have evolved mechanism to exploit petroleum hydrocarbon as a source of energy and carbon. However, this mechanism is not always efficient enough to bring about bioremediation and subsequent reclamation of polluted sites. Bacteria have acquired degradation ability to gain in ecological advantage for survival under hostile conditions (Truskewycz *et al.*, 2019). Bioremediation of polluted site for reclamation purpose requires bacteria to have wide catabolic activity at optimal rate (Varjani, 2017).

Designing of recombinant bacteria for bioremediation purpose is often seen as a method to overcome the aforementioned hurdles. An efficient recombinant bacterium can be designed by manipulating the host cell and specific catabolic pathway. The rate of hydrocarbon removal as well as the range of substrate degraded by a catabolic pathway can be enhanced by manipulating the key enzymes and regulatory mechanism that control expression of catabolic genes (Timmis & Pieper, 1999).

Engineering metabolic pathway allows generation of novel hybrid pathways by combining catabolic components from different origins into the same host cell (Pieper & Reineke, 2000). Rational engineering of metabolic pathway may result in construction of bacteria with multiple pathways for degrading large number of toxic hydrocarbons and also to complete the incomplete pathways without generation of toxic intermediates (Díaz, 2004).

Bacteria surviving in petroleum hydrocarbon have to cope up with hostile environment. Extremophiles have inherent ability to thrive in hostile conditions like extreme pH, high temperature, high ionic strength, toxic hydrocarbons and heavy metals (Pieper & Reineke, 2000). Engineering extremophiles for creating hydrocarbon degrading bacteria can be an effective strategy for remediating a contaminated site (Díaz, 2004). For instance, consider a petroleum hydrocarbon-contaminated site with high salinity. Thus, engineering saline bacteria for hydrocarbon degradation can be a good option for remediating such contaminated site (Díaz, 2004). Several petroleum hydrocarbon sites contain a mixture of heavy metals and radioactive substances. In this condition, radiation-resistant bacteria can be engineered with hydrocarbon degradation genes to make them suitable for remediating such sites (Daly, 2000).

Biosafety is a major concern with release of recombinant microorganisms into the natural environment. Several microorganisms engineered for petroleum hydrocarbon degradation can thrive and survive only till pollutants are present. Once the pollutants have been consumed, there will be no carbon and energy source for survival of engineered microorganisms (Díaz, 2004). Transfer of genes from engineered microorganisms to native microbial population can be prevented by gene-containment circuits based on a toxin and its equivalent antidote (Torres *et al.*, 2004).

5.9 RECLAMATION OF PETROLEUM HYDROCARBON-CONTAMINATED SITES

Exploiting hydrocarbon-degrading bacteria for biodegradation of contaminates is simplest, environmentally safe, sustainable, and cost-efficient method for reclaiming site. Remediation of contaminated sites by petroleum hydrocarbon-degrading bacteria is slow process and often requires months to several years for successfully completing the process. Bacterial treatment for remediation can be *in situ* and *ex situ*.

Bioremediation is traditional and most sorted biological methods for cleaning and reclamation of contaminated site. Bioremediation of petroleum hydrocarbon-contaminated site has been known since 1940. However, it gained recognition after remediating the Exxon Valdez spill site in 1980s (Hoff, 1993). In recent past, bioremediation with selected hydrocarbon-degrading bacteria has attracted attention of researchers as well as policy makers. It exploits metabolic capability of petroleum hydrocarbon-degrading bacteria for neutralizing the toxic contaminates (Ossai *et al.*, 2020). The hydrocarbon-degrading microorganisms assimilate contaminates as energy source and exhibits growth and reproduction. Ample nutrient supply and overcoming all bacterial growth-limiting factors are essential for accomplishing remediation and reclamation of the site. Successful bioremediation oxidizes contaminates to CO_2 and H_2O while converting the site to a benign status (Yanti, 2018).

Bioattenuation exploits a variety of chemical, physical, and biological processes to remove, neutralize, transform, and reduce toxicity or concentration of petroleum hydrocarbons at the contaminated sites (Ossai *et al.*, 2020). Bioattenuation is accomplished through several processes like advection, dispersion, sorption, dissolution, stabilization, volatilization, abiotic, and biological transformation of petroleum hydrocarbons (Abatenh *et al.*, 2017). Indigenous microbial population degrades or transforms petroleum hydrocarbon to less toxic form depending upon their metabolic capabilities. Bioattenuation is useful at contaminated sites having low concentration of petroleum hydrocarbon, where adopting other remediation method is not feasible and time is not a limiting factor (Vásquez-Murrieta *et al.*, 2016).

Biostimulation is the addition of materials to support bacterial growth and enhance activity of hydrocarbon degrading enzyme for accomplishing the remediation of contaminated sites (Sarkar *et al.*, 2016). It involves addition or optimization of various parameters that limits remediation of hydrocarbon. Amending material may be biosurfactants, biopolymers, macro- and micro- nutrients. Cleaning petroleum hydrocarbons-contaminated site by biostimulation is the most successful and efficient '*in situ*' remediation method in comparison with other documented methods (Ossai *et al.*, 2020). Sarkar *et al.* (2016) enhanced degradation activity of indigenous microorganism by adding nitrate to the growth medium. Indigenous microorganism preferred utilization of both higher and middle-chain length hydrocarbons, and degraded 80% of TPH within 90 days (Sarkar *et al.*, 2016).

Bioaugmentation involves the addition of exogenous bacterial cultures, autochthonous bacterial consortium or genetically engineered bacteria for remediating petroleum hydrocarbon-contaminated site (Nwankwegu & Onwosi, 2017). Microorganisms selected for bioaugmentation must be adapted to the environmental site and have proven record to degrade hydrocarbon contaminants. Szulc *et al.* (2014) observed that bioaugmentation with consortium consisting of *Aeromonas hydrophila*, *Alcaligenes xylosoxidans*, *Gordonia* sp., *Pseudomonas fluorescens*, *Pseudomonas putida*, *Rhodococcus equi*, *Stenotrophomonas maltophilia*, and *Xanthomonas* sp. increased degradation efficiency by 89% in a 365-day treatment of diesel oil-contaminated soil.

Bioventing is a process of enhancing the rate of *in situ* remediation of petroleum hydrocarbon-contaminated site by injecting air into the soil (Thomé *et al.*, 2015). The addition of air stimulates and increases aerobic conditions for the growth of indigenous microorganisms and augments the catabolic degradation of the contaminants. Recently, Thomé *et al.* (2015), conducted bioventing for 60 days on diesel-contaminated soil and observed 85% remediation of the contaminated site. In another study by Agarry and Latinwo (2015), bioventing resulted in removal of 91.5% of the contaminants from brewery effluents amended diesel-contaminated soil within 28 days of remedial period.

Biosparging is *in situ* remediation process of increasing biological activity of indigenous microorganisms by injecting nutrients and air into the saturated zone (Azubuike *et al.*, 2016). Nutrients and air support growth of microorganisms and enhances degradation of petroleum hydrocarbon contaminates. Biosparging is effective in reducing the hydrocarbon adsorbed on the soil particles or trapped between capillary spaces of soil particles (Kao *et al.*, 2007). Biosparging at a petroleum oil spill site removed 70% of BTEX within 10 months of the remedial period (Kao *et al.*, 2007).

Bioslurry is an *ex-situ* method of remediation where excavated contaminated soil is treated in controlled bioreactors (Tuhuloula *et al.*, 2014). In the treatment process, nutrients are added to excavated soil present in the bioreactors. Nutrients support growth and enhance microbial degradation of petroleum hydrocarbons. The bioreactor used for treatment has means to monitor and regulate various essential parameters such as temperature, air flow and nutrients mixing rate. Remediation by bioslurry method is relatively faster with controlled emission. Major drawback with bioslurry method is longer treatment period, soil excavation, transportation facility, and pretreatment of soil (Ossai *et al.*, 2020). Tuhuloula *et al.* (2014) reported 79.35–99.73% of naphthalene removal from petroleum hydrocarbon-contaminated soil obtained from oil drilling site of Pertamina Petrochina in Indonesia with the help of microbial consortia (*Bacillus cereus* and *Pseudomonas putida*) in the slurry bioreactor.

Biotransformation is the process of modifying structure of hydrocarbon contaminants from one form to another with less toxicity or reduced persistence (Smitha *et al.*, 2017). Transformation can be carried out by bacteria, fungi, yeast, or genetically engineered microorganisms (Abatenh *et al.*, 2017). In natural environment, the process of transformation is very slow, nonspecific, and less productive. However, microbial biotransformation process is rapid and specific (Ossai *et al.*, 2020). Størdal *et al.* (2015) reported biotransformation of crude oil in the presence of marine copepods feces. In another study, Brakstad *et al.* (2004) reported depletion of n-alkanes and 4–5 ring PAH hydrocarbons from mineral oil due to biotransformation processes. Denaturing gradient gel electrophoresis bands indicated microorganisms responsible for transformation are related to the α- and γ-subdivisions of proteobacteria and to the chloroflexus–flavobacterium–bacteroides group (Brakstad *et al.*, 2004).

Landfarming is a remediation method that applies tilling, ploughing, and spreading of contaminated soil into a thin layer on the land surface (Brown *et al.*, 2017; Guarino *et al.*, 2017). Surface layering of contaminated soil increases and stimulates aerobic microbial activity. Efficiency of landfarming can be increased by the addition of oxygen, water, and nutrients (Brown *et al.*, 2017). Landfarming has been reported to be suitable for soil with low molecular weight contaminants and volatile organic compounds (Guarino *et al.*, 2017). Brown *et al.* (2017) reported that landfarming removed 53% of TPH from contaminated soil within 6 weeks. Combination of natural attenuation, landfarming, and bioaugmentation was applied by Guarino *et al.* (2017) to remove 86% of TPH from contaminated soil within 90 days. Landfarming practice alone achieved 70% reduction in TPH remedial period (Guarino *et al.*, 2017).

Windrow is an *ex-situ* method of remediation aiming to increase aeration and distribution of nutrients by periodic tilling and turning of hydrocarbon-contaminated soil with the addition of water

(Azubuike *et al.*, 2016). Aeration and hydration increases indigenous microbial activity and consequently degradation of contaminants. Indigenous microorganisms bring about cleaning by biotransformation, assimilation, and mineralization of petroleum hydrocarbons (Azubuike *et al.*, 2016). As compared to biopiling, windrow method exhibits higher rate of hydrocarbon removal. Windrow treatment is not suitable for remediating contaminated site that release volatile compounds. Toxic volatile compounds released during periodic turning and tilling can harm workers as well as surrounding atmosphere (Azubuike *et al.*, 2016). Al-Daher and Al-Awadhi (1998) performed remediation of petroleum-contaminated soil with windrow soil system. Windrow system was subjected to regular watering, tilling, and turning to increase aeration and microbial activities. After a period of eight months, nearly 60% reduction in the TPH was observed (Al-Daher & Al-Awadhi, 1998).

Compositing is the process of adding suitable microbial consortia and organic waste as soil supplement for enhancing the rate of bioremediation (Ossai *et al.*, 2020). Controlled addition of nutrients, periodic tilling and watering is also performed during compositing. Microbial activity during compositing increases soil temperature from 50°C to 65°C. High soil temperature aids in thermal degradation of petroleum hydrocarbon (Atagana, 2008). Petroleum hydrocarbon-contaminated soil was inoculated with sewage sludge and incubated in compost heaps for 19 months (Atagana, 2008). After incubation, petroleum hydrocarbons concentration got reduced to 99% and 17% in the sewage sludge compost and in the control lacking sewage sludge, respectively (Atagana, 2008).

Biopiling is an *ex-situ* method of remediation involving pilling of an excavated contaminated soil along with soil amendments to perform biostimulation. The basic biopiling system consists of treatment bed, aeration system, irrigation system, nutrient system, and leachate collection system (Kim *et al.*, 2018; Ossai *et al.*, 2020). Generally, piles of hydrocarbon-contaminated soil are covered with sheets to control runoff, evaporation, and volatilization. Covering of piles also increases solar heating. Important factors namely moisture, heat, nutrients, air, and pH are controlled for enhancing the microbial degradation. This method is suitable for treating a large volume of contaminated soil within limited space (Ossai *et al.*, 2020). Crude oil-contaminated soil of Kuwait was cleaned using sequential biowashing–biopile processes (Kim *et al.*, 2019). Biowashing was performed by an enrichment culture of the indigenous soil bacterial community.

Hemoglobin-catalyzed oxidation was used in biopile process to remove TPH from contaminated soil. The sequential biowashing and biopile process removed 86% of TPH (Kim *et al.*, 2019).

5.10 FACTORS INFLUENCING RECLAMATION OF PETROLEUM HYDROCARBON-CONTAMINATED SITE

Biological cleaning of petroleum hydrocarbon-contaminated site is influenced by several biotic and abiotic factors. Bacteria employed for cleaning of contaminated site is the most important biotic factor influencing the reclamation process. In the natural ecosystem, microbial community grows together in a synergistic relationship and produces bioactive metabolites (including oxidative and hydrolytic enzymes) to implicate the mineralization of petroleum hydrocarbons (Truskewycz *et al.*, 2019). The selection of bacteria or bacterial consortia is decided keeping in view the environmental conditions and available resources (Haghollahi *et al.*, 2016). Abiotic factors that influence remediation are summarized in Figure 5.4. Factors influencing bacterial growth are important abiotic factors that influence the reclamation process.

5.10.1 Bioavailability

Bacteria can easily utilize water-soluble compounds as they are easily bio-available. Petroleum hydrocarbon has low bio-availability due to poor water solubility. Few bacteria have ability to increase hydrocarbon solubility by producing surface-active agents (like biosurfactant or bioemulsifiers) in the surrounding environment. A combination of biosurfactant-producing bacteria and hydrocarbon-utilizing bacteria improves remediation efficiency. Mnif *et al.* (2017) reported that the addition

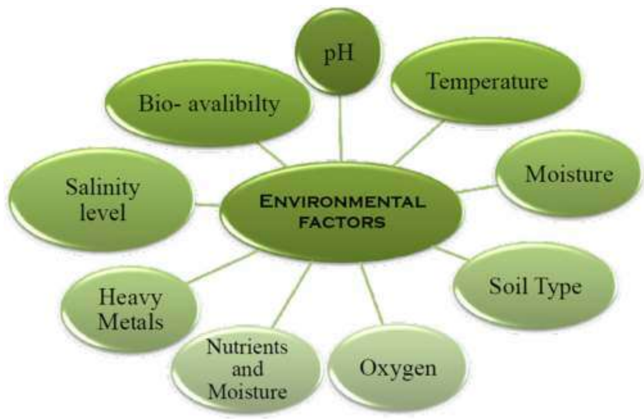

Figure 5.4 Environmental factors influencing reclamation of petroleum hydrocarbon contaminated site.

of biosurfactant-producing bacteria (*B. subtilis* SPB1) and hydrocarbon-degrading bacteria (*A. radioresistens* RI7) to diesel-contaminated soil improved the remediation of the contaminated soil by 32.67%.

5.10.2 pH

The pH of surrounding environment is an important factor influencing growth and enzyme activity. Each enzyme exhibits optimum activity at specific pH. Generally, neutral pH and slightly alkaline pH is known to support degradation of petroleum hydrocarbon. Neutral to slightly alkaline pH is known to support carboxylation reaction (Varjani, 2017).

5.10.3 Temperature

Temperature influences petroleum hydrocarbon degradation by affecting the rate of microbial metabolism and composition of the microbial community. Optimum temperature is essential for good bacterial growth and consequently degradation of petroleum hydrocarbons. Temperature has profound effect on microbial enzyme activity (Varjani, 2017). Extreme temperature (too high or too low) retards and in some scenario even inhibits growth of bacteria. Higher temperature increases the rate of hydrocarbon metabolism to a maximum, particularly in the range of 30–40°C. Generally, above 40°C, bacterial degradation of hydrocarbon is inhibited as it has adverse effect on growth. However, thermophilic hydrocarbon utilizing bacteria are known in literature that can accomplish cleaning activity even at higher temperatures (Nzila, 2018).

Temperature also influences degradation of hydrocarbons by altering physical nature and chemical composition of petroleum hydrocarbons. High temperature decreases viscosity, increases solubility, increases mobility, and enhances volatilization (Nzila, 2018). High temperature has a significant influence on the bioavailability of petroleum hydrocarbons, especially PAHs, by decreasing their viscosity, leading to an increase in their diffusion coefficients, therefore, improving their availability to microorganisms (Nzila, 2018).

5.10.4 Oxygen

Aerobic degradation of petroleum hydrocarbons is more effective in cleaning of contaminated site than anaerobic degradation (Mille *et al.*, 1988). The initial step in degradation of hydrocarbon under chemotrophic aerobic condition involves oxidation of substrate by oxygenases (Fritsche & Hofrichter, 2000). Molecular oxygen is also utilized to produce alcohol during peripheral degradation pathway (Varjani, 2017). Oxygen concentration is the rate-limiting variable in the biodegradation of petroleum hydrocarbon in the soil. Mille *et al.* (1988) reported that hydrocarbon degradation occurred in sediments where the dissolved oxygen concentration was 8 ppm and the redox potential was nearly +150 mV. The degradation rate decreased nearly 2–3 times when the dissolved oxygen concentration was 2–3 ppm and the redox potential was +30 mV. No degradation was observed under suboxic conditions having dissolved oxygen of 0.2–0.3 ppm and redox potential between −180 and −200 mV (Mille *et al.*, 1988).

5.10.5 Nutrient and moisture

Microorganisms require proper nutrition and moisture for growth. Petroleum hydrocarbons provide carbon, oxygen, and hydrogen to bacteria, but the availability of additional elements such as nitrogen, phosphorus, sodium, and so on is limited in contaminated sites (Ossai *et al.*, 2020). Biostimulation is the best example of nutrients-mediated enhancement of hydrocarbon degradation. Petroleum hydrocarbon-contaminated marine sediments having limited availability of nitrogen and phosphorus generated 1.10 ± 0.03 μmol CO_2/g wet sediment/day. The addition of inorganic nitrogen and phosphorus increased CO_2 production to 18.40 ± 1.04 μmol CO_2/g wet sediment/day (Singh *et al.*, 2014).

The moisture content of soil should be within an optimum range for accomplishing hydrocarbon degradation (Truskewycz *et al.*, 2019). The decrease in moisture concentration impacts soil microbial communities by hampering their interactions with each other and the environment, which may lead to a reduction in abundance, diversity, and structure. Excess moisture hinders oxygen transfer and retards hydrocarbon degradation (Truskewycz *et al.*, 2019).

5.10.6 Salinity

Most of the petroleum degradation is reported for non-saline environmental conditions. High salt concentration in the environment causes osmotic stress on bacteria and inhibits synthesis or activity of several enzymes involved in the degradation of hydrocarbon (Truskewycz *et al.*, 2019). High salinity is also known to reduce oxygen concentration in the environment, thus hindering bacterial growth. Degradation of petroleum hydrocarbons present in the marine environment requires halo-tolerant bacteria. Cao *et al.* (2020) reported that *Exiguobacterium* sp N4-1P exhibited maximum degradation efficiency at 15 g/L NaCl concentration.

5.10.7 Soil type

Soil type, pore-size distribution, and soil texture are some less investigated factors affecting remediation. Soil type affects remediation by influencing moisture, oxygen, and nutrient level of the soil. Fine-grained soils like clay have low permeability and retarded oxygen and nutrients mobility in the soil. Maintaining optimum soil moisture is also difficult in fine-grained soils due to small pore sizes and high surface areas (Haghollahi *et al.*, 2016). Hydrocarbon gets strongly adsorbed on the surface of clay soil particles, thus decreasing bioavailability of hydrocarbons to bacteria (Haghollahi *et al.*, 2016). Haghollahi *et al.* (2016) reported that remediation of hydrocarbon-contaminated sandy soil is more feasible than clay soil. Soils containing both clay and sand have good remediation feasibility than soil containing only clay.

5.10.8 Heavy metal contamination

Very often, hydrocarbon-contaminated site is co-contaminated with a toxic level of heavy metals. Osuji and Onojake (2006) reported the presence of heavy metals (Pb, Cd, V, Cu, and Ni) at Ebocha-8 oil spill site located in the Niger Delta region of Nigeria. The presence of heavy metal retards and sometimes even inhibits mineralization of hydrocarbons. Heavy metals are known to inhibit several metabolic pathways, such as the enzymatic and respiratory processes of many bacteria, and create additional stress to hydrocarbon-degrading microorganisms (Khudur *et al.*, 2019). The presence of lead at contaminated site decreases the rate of petroleum hydrocarbon degradation by reducing the number of hydrocarbon-degrading bacteria and activities of dehydrogenase enzyme. Khudur *et al.* (2019) observed that lead restricted microbial remediation of hydrocarbon-contaminated soils.

5.11 CONCLUSIONS AND FUTURE DIRECTION

Petroleum hydrocarbons are toxic pollutants that have contaminated both soil and water resources. Cleaning of petroleum hydrocarbon-contaminated site is of utmost importance for researchers due to its hazardous and persistent nature. Applying petroleum hydrocarbon-degrading bacteria for remediating contaminated site is widely considered as an environment-friendly approach of reclamation. A large number of hydrocarbon-degrading bacterial species have been reported for cleaning of contaminated sites. However, global success of bacterial-assisted reclamation is curtailed during practical applications. Several strategies have been proposed and formulated to make the process effective and efficient under all possible environmental conditions. Still there are lots of hurdles that need to be overcome for making bacterial-assisted restoration of petroleum hydrocarbon-contaminated site a global success.

Designing of novel and biocompatible surface-active agents for enhancing the bioavailability of hydrocarbon for bacteria is the primary requirement for on-field success. Modern omics approaches (genomics, transcriptomics, proteomics metabolomics, and fluxomics) can increase our understanding of bacterial metabolism in polluted environment and help in designing or formulating effective and efficient remediation strategies. Synthetic biology can be utilized to construct genetically engineered bacteria with broad hydrocarbon degradation ability. Exploring new and extreme location with high-throughput screening method can increase and enrich functional bacterial resources of petroleum hydrocarbon- assimilating bacteria.

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Chapter 6

Recent advancement in microbial remediation of heavy metals from industrial effluents

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ABSTRACT

Currently, heavy metal contamination has become a greatest challenge because, they are not degradable. They get accumulated in biotic and abiotic environment. Different strategies are being tested to remediate heavy metal pollution; however, an ecofriendly approach to remove them from the environment is much needed. Bioremediation is one such alternative to the conventional method. Among the utilization of plants and microbes for the heavy metal remediation, microbial remediation is found to be cost effective and low waste generating strategy. Many microorganisms acquired different mechanisms to adapt themselves and remove or detoxify the heavy metals. Besides, many biological agents like biosurfactants, can also be utilized for metal removal from an aqueous environment. This chapter focuses on the impact of heavy metal on the environment, different microbes involved in detoxification process, and biotechnological solutions of heavy metal contamination.

Keywords: heavy metals, bioremediation, microbial system, bioactive molecules, detoxification

6.1 INTRODUCTION

Due to population growth and economic development, pollution levels are rapidly rising. Due to the considerable increase in the quantity of industrial waste that is released into the ecosystem, especially soil and water, heavy metals have accumulated, particularly in urban areas. Because they cannot be converted into nontoxic forms and have a huge detrimental effect on the environment, heavy metals are a major health problem for everyone. Additionally, they are dispersed carelessly into the ground and rivers. There is very little risk to the public or security when heavy metals or their by-products are removed naturally. The body can store heavy metals, which are persistent. They might also bio-magnify and accumulate further up the food chain. To create a better environment for people, contaminated land and water bodies must be cleaned up to get rid of trace elements and heavy metals. The removal of heavy metal ions from liquid waste has lately been made possible by a number of novel methods. Reverse osmosis, ion-exchange, chemical precipitation, electrochemical treatment, and electrochemical processing are some of these procedures. These methods aim to decrease the bioavailability of metals by toxicizing heavy metals, although most of these methods are useless at doses below 100 mg. However, the majority of techniques are costly, using living, green plants or microorganisms, whereas bioremediation is a promising technique that cleans up soil, surface water, and groundwater of pollutants. Metals can also be detoxified by microbes through bio-sorption, bioleaching, bio-mineralization, and so on. The term 'bio-sorption/bioaccumulation' refers to the most common biotechnological technique for removing metal ions from wastewater. Pollutants may adhere to the cellular walls of microorganisms. The biosorption process looks to be more feasible for large-scale application than the bioaccumulation process since the addition of nutrients is necessary for bacteria to actively take up heavy metals, which increases the waste's biological or chemical oxygen need. In addition, heavy metal toxicity and other environmental variables make it particularly challenging to maintain a healthy population of bacteria. While poor nations appeared to focus more on sporadic, more expensive ex-situ treatments, developed economies appeared to embrace environmentally benign in-situ bioremediation techniques, such as monitored natural attenuation, more frequently.

6.2 TOXICITY OF HEAVY METALS

6.2.1 Arsenic

Drinking water may become contaminated using arsenical insecticides, naturally occurring mineral deposits, or the erroneous removal of arsenical synthetics. If arsenic is actively swallowed during suicide attempts or accidentally consumed by children, it can also result in acute poisoning episodes (Mazumder, 2008). Arsenic is a protoplastic toxin because it fundamentally damages cells in the sulfydryl bunch and prevents mitosis, cell enzymes, and respiration (Gordon & Quastel, 1948).

6.2.2 Lead

The main industrial processes, food and tobacco products, drinking water, and home sources are where lead exposure occurs. Plumbing fixtures, pewter pitchers, storage batteries, toys, and faucets are other sources of lead in addition to lead paint and gasoline (Thurmer *et al.*, 2002). Lead metal poisoning in living cells is caused by ionic and oxidative stress processes. Oxidative stress in living cells is caused by an imbalance between the production of free radicals to detoxify reactive intermediates and the production of antioxidants to stop damage or repair it. The key contributor to lead toxicity's ionic mechanism is the capacity of lead metal ions to displace other bivalent cations like Ca^{2+}, Mg^{2+}, and Fe^{2+} as well as monovalent cations like Na^{+}. This eventually causes disruptions in cell metabolism. The ionic mechanism of lead toxicity has a substantial impact on a number of biological processes, including cell adhesion, intra- and intercellular communication, protein folding, maturation, apoptosis, ionic transport, neurotransmitter release, and enzyme control. Lead can have an impact on protein kinase C, which regulates brain excitation and memory storage, even at pico molar quantities (Flora *et al.*, 2008).

6.2.3 Mercury

Mercury is highly toxic and bioaccumulative. The distribution of mercury in aquatic habitats is the subject of numerous studies since it has a negative impact on marine ecology. According to Chen *et al.* (2012), mining, waste incineration, municipal wastewater discharges, agricultural, and industrial wastewater discharges are the main man-made sources of mercury pollution. Methylmercury is a neurotoxin that accumulates neurotoxic compounds, including serotonin, aspartate, and glutamate in addition to damaging microtubules, mitochondria, lipid peroxidation, and microtubules (Patrick, 2002). The brain continues to be one of mercury's main targets, despite the fact that it can harm any organ and cause problems with nerves, organs, and muscles. It's possible to disturb both the membrane potential and the calcium balance within cells.

6.2.4 Cadmium

According to the ATSDR classification, cadmium is the sixth most dangerous heavy metal. It may come into contact with animals or people while working or in the environment because it is a by-product of the manufacture of zinc. People will continue to consume this metal throughout their lives, causing it to accumulate in their bodies. It was first used as a pigment and tin substitute in the paint industry during World War I. When cadmium attaches to metallothionein or another protein high in cysteine, its concentration rises three times. The accumulation of cysteine–metallothionein complex produces hepatotoxicity in the liver and nephrotoxicity in the renal tissue of the kidney (Lin *et al.*, 2005).

6.2.5 Chromium

Chromium is the eighth most common element in the cosmos. Chromium can be present in the environment in oxidation levels ranging from Cr^{2+} to Cr^{6+}, according to Rodríguez *et al.* (2007). Some examples of chromium's natural sources are coal and oil combustion, ferro chromate-derived petroleum, pigment oxidants, catalysts, chromium steel, fertilizers, oil well drilling, and tanneries used for metal plating. Because of its low membrane permeability, trivalent chromium Cr(III) is typically safe to breathe in. Hexavalent chromium, or Cr(VI), on the other hand, is more successful at rupturing cell membranes through openings for isoelectric and isostructural anions, such as SO_4 $^{2-}$ and HPO_4 $^{2-}$ channels, and these chromates are subsequently taken up through phagocytosis. Strong oxidant Cr(VI) is capable of being reduced to yield the temporary, distinct forms of pentavalent and tetravalent chromium (III). It is believed that intracellular reduction of Cr[VI] is a detoxifying process when reduction occurs somewhere other than the target site because glutathione stabilizes the pentavalent form. When the biological reductants thiols and ascorbate react with the metal Cr(VI), reactive oxygen species such the superoxide ion, hydrogen peroxide, and hydroxyl radical are produced. As a result, the cell experiences oxidative stress, which damages proteins and DNA (Hasin *et al.*, 2010).

6.2.6 Aluminum

According to Pazirandeh *et al.* (1995), aluminum is the third most common element in the planet's crust. Aluminum is a naturally occurring element that can be found on the earth, in water, and air. Mining and processing of aluminum have a greater negative impact on the environment. High concentrations of aluminum can result in osmoregulatory failure by depleting plasma and hemolymph ions, which is extremely detrimental to aquatic life, especially gill-breathing fish. Fish contain monomeric aluminum, which prevents an enzyme needed for ion absorption from working (Mishra *et al.*, 2017). Seaweed and crawfish are just two examples of the aquatic life that is affected by aluminum toxicity. Aluminum inhibits various enzymes that are active on DNA and RNA because it has a stronger affinity for these molecules than hexokinase, phosphodiesterase, alkaline phosphatase, and phosphoxidase. The metabolism of iron, calcium, phosphorus, fluorine, and other elements in living beings is impacted by aluminum. Aluminum has been found to be particularly harmful to hemopoietic, osseous, and neurological cells (Flora *et al.*, 2008).

According to the iron is the second most prevalent metal on Earth's surface. When the ingested iron does not attach to the protein, a large number of dangerous free radicals are created. This has a detrimental effect on the amount of iron in mammalian cells and bodily fluids. The digestive system and the circulating free iron corrodes bodily fluids. The body is saturated with iron once the rate-limiting step of absorption has been passed. These flexible irons can reach the cells in the heart, liver, and brain. Free iron raises the acidity of the metabolism by generating hydrogen ions, changing ferrous iron to ferric iron, and interfering with oxidative phosphorylation. The lipid peroxidation caused by free iron causes severe harm to microsomes, mitochondria, and other cellular organelles (Li, 2010). Iron-mediated tissue damage has been linked to cellular oxidizing and reducing processes as well as their toxicity towards intracellular organelles like mitochondria and lysosomes. A high iron intake produces several different free radicals, some of which are thought to have the ability to damage cells.

6.3 IMPACT OF HEAVY METALS ON SOIL

Heavy metals damage the soil biota by obstructing crucial microbial processes and lowering the number and activity of soil microorganisms. However, the ability of bacterial populations and fungi, notably arbuscular mycorrhizal (AM) fungi, to tolerate heavy metals over a long term may be crucial for the restoration of a damaged ecosystem (Mora *et al*., 2005). The toxicity of heavy metals was influenced by their chemical compatibility with the enzymes of soil organisms. The microbial community in soil will suffer as a result. Important indicators of soil quality include the diversity and activity of soil microbes, which are essential for the detoxification of harmful compounds, the maintenance of soil structure, the management of plant pests, and the recycling of plant nutrients. The microbiological properties of the soil, such as respiration rate and enzyme activity, which seem to be highly effective markers of soil pollution, are generally adversely affected by an increase in metal content. In lead-contaminated soil (Pb), there was a slight change in the microbial composition of the soil.

6.4 IMPACT OF HEAVY METALS ON PLANTS

Because they interact with food chains and are absorbed by plant roots, heavy metals constitute a major threat to both animal and human health. Temperature, moisture, organic matter, pH, and the availability of nutrients are only a few of the numerous elements that affect how easily heavy metals are absorbed and deposited in plant tissue. Chlorosis, restricted plant development, low yields, reduced nutrient uptake, abnormalities in plant metabolism, and a reduced capacity of leguminous plants to fix molten nitrogen are all effects of heavy metal buildup. Low Pb concentrations may inhibit some crucial plant processes as photosynthesis, mitosis, and water absorption. Toxic warning signs include dark green leaves, fading older leaves, stunted foliage, and brown short roots.

6.5 IMPACT OF HEAVY METALS ON AQUATIC SYSTEMS

Aquatic species that are exposed to heavy metals, which are toxic in small doses, will experience oxidative stress. As a result, these pollutants have a big impact on ecotoxicology. Furthermore, since microbes cannot degrade metals, they stay in the marine ecosystem forever. When levels of heavy metal contamination rise, the diversity of aquatic life is restricted, and the ecological balance of the aquatic ecosystem may suffer. Particulate matter that finally settles down and becomes a part of the sediment is usually responsible for retaining heavy metals that are deposited into aquatic systems. Therefore, in aquatic ecosystems, surface sediment acts as the main reservoir or sink for metals and other contaminants.

Bioremediation is regarded as a significant, practical, eco-friendly, and cost-effective technology for the removal of heavy metals in contaminated sites in order to address the serious health consequences on humans caused by the accumulation of heavy metals in water and other sources. Both aerobic and anaerobic methods of bioremediation can be used to convert or degrade harmful components into harmless materials using microbial enzymes.

6.6 BIOREMEDIATION

Through a metabolic response, biological organisms are used in the bioremediation process to remove or neutralize an environmental contaminant. The term 'biological' in this sense refers to both the 'remediation' (the process of fixing the issue) and microscopic organisms like fungi, algae, and bacteria. Microbial enzymes can be utilized in both aerobic and anaerobic bioremediation processes to transform or degrade hazardous components into innocuous ones. The microorganisms utilized in bioremediation may be imported and introduced, or they may exist naturally in the contaminated area. Bioremediation is the most widely used technique for lowering, detoxifying, mineralizing, or changing more dangerous contaminations into less deadly toxins. Pesticides, agrochemicals, heavy metals, xenobiotics, hydrocarbons, atomic waste, colors, polymers, and slime are a few of the toxins in this group (Figure 6.1).

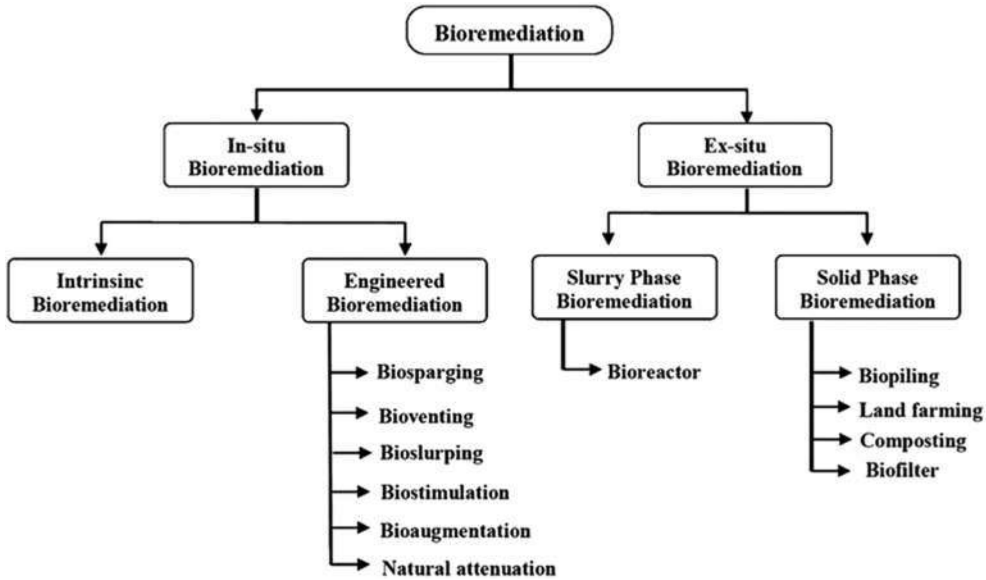

Figure 6.1 Classification of Bioremediation

Various methods are employed during the bioremediation process. The fundamental steps in the bioremediation process include bio-stimulation, attenuation, augmentation, and venting.

6.6.1 Bio stimulation

To encourage the activity of nearby microorganisms, this type of method entails adding specific nutrients to the area (soil or ground water). Its primary focus is on bacterial and fungal communities that are local or spontaneously occurring by initially supplying trace minerals, development aids, and fertilizers. Additional environmental requirements including pH, temperature, and oxygen are also addressed in order to accelerate their metabolic rate and pathway (Adams *et al.*, 2015; Kumar *et al.*, 2011). A modest amount of pollutant can also act as a stimulant by turning on the operons for the bioremediation enzymes. In order to support local microorganisms, this form of strategic corridor is typically maintained by supplying nutrients and oxygen.

6.6.2 Bio attenuation (natural attenuation)

The process of removing pollution concentrations from the environment is known as bio attenuation or natural attenuation. Advection, dispersion, dilution, diffusion, volatilization, sorption/desorption, chemical reactions (ion exchange, complexation, abiotic transformation), as well as biological processes like aerobic and anaerobic biodegradation, animal and plant absorption, and sorption/desorption, combine physical and biological processes to achieve this. Natural attenuation has a broader definition that encompasses ideas like intrinsic repair and biotransformation (Mulligana & Yong, 2004). According to Li *et al.* (2010), nature can remediate the environment following chemical pollution in four different ways.

(1) Certain chemicals are consumed by tiny insects or bacteria that live in soil and groundwater. Once the chemicals have been completely absorbed by the organism, they can be transformed into safe gases and water.
(2) Chemicals can adhere to dirt and retain it there by binding or sorbing to it. This method does not clean up the pollutants, but it may stop them from leaking out of the site and poisoning the groundwater.

(3) Pollution can contaminate clean water as it passes through land and groundwater. As a result, the pollution is diminished.
(4) Some chemicals, like oil and solvents, have the ability to evaporate, or change from liquids to gases inside the soil. If these gases are permitted to leak into the air close to the ground's surface, sunlight might kill them.

If the rate or degree of natural attenuation is insufficient, bioremediation will be hastened through bio stimulation or bio augmentation.

6.6.3 Bio augmentation

Bio augmentation is the manual addition of an organic culture to an environment, such as a bioreactor, in order to handle sewage or other contaminated pollutants. In most instances, this environment already has a microbial population, but bio augmentation is intended to enrich this population and increase its capacity to lower the level of contamination. Pre-grown microbial cultures can be added to make bioreactors and other treatment equipment available to immediately reach their peak levels of efficiency. The apparent justification for this microorganism addition is to boost the efficacy and efficiency of the processes taking place inside the bioreactor or in another industrial application. At a lower cost, the contaminants are eliminated more swiftly and efficiently. This makes it simpler for organizations to keep that crucial equilibrium, preserving profits while pursuing ecological sustainability. Increased levels of live microbes and microorganisms in the treatment area are how bio augmentation works. Each microorganism can only manage this transformation up to a certain rate, but these microorganisms are already working to convert contaminants into less harmful compounds.

6.6.4 Genetically engineered microorganisms in bioremediation

Genetically engineered (GE) bacteria, recombinant DNA, and RNA technology have all been used for efficient bioremediation. To improve bioremediation procedures, microbial genes have been changed to produce new metabolic pathways. GE microorganisms might be the preferred technique because of the distinctive features of their metabolic pathways. The use of GE bacteria, a cutting-edge technology, to remove heavy metals and toxic waste from contaminated places has attracted public attention. Additionally, it has aided in the detoxification of chemicals like heavy metals that are resistant to treatment. With the help of their metal regulatory genes, bacteria can convert hazardous forms of heavy metals into less toxic ones. The production of metallothioneins (MT) by GE microorganisms can speed up the accumulation of heavy metals. A possible technique for preparing GEO for bioremediation that might successfully remove environmental contaminants is recombinant DNA technology. Chakrabarty gave a description of the development of the first DNA technology for the bioremediation of contamination from petroleum. This method can be used to clean up regions that have become contaminated as a result of heavy metals, chlorinated hydrocarbons, pesticides, petroleum hydrocarbons, and explosives. DNA shuffling, a potent mutagenesis method can produce novel enzyme activity and biocatalysts that can degrade polyaromatic hydrocarbons and chlorinated ethane more quickly.

6.6.5 Bioventing

Bioventing is a technique for in-situ remediation that employs microbes to break down organic pollutants that have been adsorbed on soil in the unsaturated zone. Bioventing improves air or oxygen flow into the unsaturated zone and, if necessary, supplies nutrients by boosting the activity of local bacteria and replicating in-situ biodegradation of hydrocarbons in soil. During bioventing, direct air injection may be utilized to provide oxygen into dirt containing any lingering contaminants. Bioventing primarily aids in the breakdown of adsorbed fuel residuals, but it also aids in the degradation of volatile organic compounds (VOCs), as vapors move slowly through biologically active soil. Instead of a lack of nutrients, a lack of oxygen and other electron acceptors (i.e., substances that gain electrons during biodegradation) (i.e., electron donors) often limits the rate of natural deterioration. In conventional bioventing equipment, oxygen is delivered to underground wells by an electric blower. Bioventing, as opposed to soil vapor vacuum extraction, only uses modest airflow rates to supply the oxygen necessary to sustain microbial activity. Bioventing wells are used in passive bioventing systems to supply the subsurface with oxygen through spontaneous air exchange. When the pressure inside a vent well is lower than the atmospheric pressure, a one-way valve fitted on the well allows air to enter. The valve closes, trapping the air in the well and improving soil oxygenation when atmospheric pressure drops below the subsurface pressure (due to a shift in barometric pressure).

6.6.6 Biopile

In a bio-pile, excavated soils are enclosed for treatment after being mixed with soil additions and shaped into compost piles. The basic bio-pile system consists of an irrigation/nutrient system, an aeration system, a leachate collection system, and a treatment bed. To facilitate biodegradation, temperature, nutrients, oxygen, and pH are all regulated. To move air and nutrients through the earth, an irrigation/nutrient system is buried beneath the surface. The height of a soil mound can reach 20 feet. To regulate runoff, evaporation, and volatilization as well as to encourage sun heating, they may be covered with plastic. If the soil includes VOCs, the air leaving the soil may be treated to eliminate or destroy them before they are released into the atmosphere. Normally, the excavated material is processed for three to six months before being either put back where it came from or disposed of.

6.7 THE SEVERAL SPECIES OF ORGANISMS UTILIZED IN BIOREMEDIATION

The core of bioremediation is the co-metabolism of a single or a collection of microorganisms. Numerous investigations revealed that a wide variety of organisms were capable of biosorbing heavy metals. Prokaryotes are less sensitive to heavy metals than eukaryotes are, and whether an organism is a eukaryote or a prokaryote affects how it reacts to heavy metals. Active metal extrusion, intracellular chelation (in eukaryotes) by a variety of metal-binding peptides, and transformation into less dangerous chemical species are the three potential interaction pathways. Microorganisms must enzymatically attack the pollutants and change them into harmless chemicals for bioremediation to be successful. Aerobes, anaerobes, and fungi are a few of the microorganisms that participate in the enzymatic degradation process.

For remediation to be successful, a contaminated site may contain several different types of contaminants, necessitating the use of numerous microorganisms. Some bacteria can degrade the molecules of petroleum and use the carbon and energy they contain as a source. However, the organisms utilized must be carefully selected because they can only survive in the presence of a particular group of chemical pollutants, which varies based on the chemical makeup of the polluting agents. The ability of the particular microbe to combine the hydrocarbon with molecular oxygen and create the intermediates that eventually enter the cell's main metabolic route for generating energy determines the efficiency of the degradation process.

Numerous bacteria can consume oil, and many of them produce potent surface-active chemicals that can emulsify oil in water and facilitate its removal. Among the bacteria that can degrade petroleum compounds are *Pseudomonas, Aeromonas, Moraxella, Beijerinckia, Flavobacteria, Chlorobacteria, Nocardia, Corynebacteria, Modococci, Streptomyces, Bacilli, Arthrobacter, Cyanobacteria,* and various yeasts. *Pseudomonas putida* MHF 7109 may be isolated from microbial consortia present in cow dung and is capable of biodegrading a number of petroleum hydrocarbons, including benzene, toluene, and o-xylene (BTX) (Figure 6.2).

A quick and reversible passive adsorption mechanism is biosorption. The physical interactions between the metals and the functional groups on the cell surface, such as ion exchange, adsorption, complexation, precipitation, and crystallization, keep the metals in place. A few factors that can

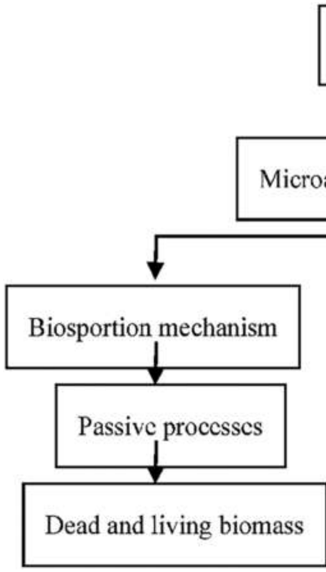

Figure 6.2 Microorganisms' function in the bioremediation process.

affect the biosorption of metals are pH, ionic strength, biomass concentration, temperature, particle size, and the presence of other ions in the solution. Biosorption is a process that can take place with both living and dead biomass since it is not dependent on cell respiration. Passive absorption, on the other hand, plays a much smaller and less clear-cut role in bioaccumulation, which includes both intra- and extracellular processes. Therefore, the only purpose of living things is bioaccumulation.

Bioremediation can be carried out using microbial bioreactors, which are also employed in a number of other processes, such as the management of solid waste, the treatment of waste water and industrial effluent, the treatment of soil and land, and the reduction of air pollution. A number of environmental parameters, including temperature, pH, moisture, the mix and concentration of pollutants, influences the growth and activity of microorganisms in bioreactors and macronutrients. Environment-related factors such as temperature, pH, oxygen availability/electron, and salinity have an impact on growth, the metabolic activities of bacteria, and to a lesser extent, the behaviour of the pollutant, such as solubility and volatility. When maximizing a process for biodegradation, it is crucial to take into account the effects of environmental factors and adapt the process in response to the relevant environmental conditions.

6.7.1 Temperature

Microorganisms can survive in a wide range of temperatures, including their lowest, optimal, and maximum temperatures. Additionally, every microorganism always performs the bio therapy it requires at the temperature that is optimal for its biochemistry. Both microbial development and enzyme-catalyzed processes in microorganisms are impacted by temperature extremes (too high or too low) (Sharma, 2011). When the temperature rises within a reasonable range, the rate of the bioremediation processes accelerates due to an increase in microbial metabolism. Many chemicals become more soluble at higher temperatures, increasing fluidity, and diffusion rates. For instance, warming increases both the solubility and subsequent bioavailability of contaminants like PAHs and heavy metals. Temperature is therefore an essential component for bioreactors to run as effectively as possible and deliver the greatest outcomes for bio treatment. Temperature regulation is usually considered when designing specialized bioreactors.

6.7.2 pH

pH has an impact on microbial growth and metabolic functions similarly to temperature. pH affects the ionic properties of microbial cells and subsequently microbial development. Microorganisms have a minimum, maximum, and optimal pH range in which they can develop. For example, most bacteria grow best at a pH of 6–7.5, though some (acidophiles) prefer an acidic or alkaline pH. (alkaliphiles). In general, fungi thrive at pH levels lower than bacteria do. The operating pH of the reactor must be adjusted to offer the ideal pH range for growth and enzyme activities. pH has an impact on the bioremediation of contaminants by altering their behaviour. For instance, pH has an impact on the redox and solubility of metals, and different forms and valences have different impacts on microbes. Alkaline pH favors metal ion precipitation, and metal solubility rises as middle pH falls (Gargouri *et al.*, 2011; Liu *et al.*, 2017). Metal attachment to the exterior of microbial cells frequently requires lower pH values. The metal ions are more soluble when acid-producing microorganisms are present. Buffers are used in the media preparation process as well as the bioreactor process to achieve the ideal pH conditions.

6.7.3 Nutrients

Microorganisms require nutrients for their growth and metabolism. The creation of energy and the process of biosynthesis both need several components. More than any other, carbon is the most crucial component of all living things. Depending on the type of microorganism, other components required for a healthy nutritional bioreactor environment include hydrogen, oxygen, nitrogen, sulfur, phosphorus, iron, calcium, and magnesium (Srivastava *et al.*, 2014). Reactor medium has every macro- and micronutrient need. When pollutants are co-metabolized, microorganisms get a major source of energy in addition to the pollutants they are currently utilizing as a source of energy.

6.7.4 Moisture

To enable microbial growth and catalysis, water is necessary. Since cellular chemical reactions take place in aquatic environments, water is required to maintain the right osmotic pressure for microbial development. The amount of water that can promote the growth of microorganisms is referred to as (aW). Osmotolerant bacteria can develop at a range of low aW, but most bacteria need water activity of 0.98 or higher to flourish (Naik & Duraphe, 2012).

6.7.5 Electron acceptors

Electron acceptors, such as oxygen in aerobic bacteria and NO_3^{1-}, SO_4^{2-}, and Fe (III) oxides in anaerobic microbes, also have an impact on the biodegradation processes.

6.7.6 Factors related to the reactor design

Bioreactors must create the right environment for microbial growth and metabolic activities to occur. Reactor design must take into account the reactor's size, configuration, and mode of operation. The reactor should provide optimal physical, biological, and physical–chemical conditions in order to achieve the best biological remediation processes. To ensure that the physical entity of the bioreactor is advantageously suited to the biological system that conducts the bioreactions, it is essential to construct the bioreactor under favorable physical conditions for the transit of gases, liquids, and solids throughout time (Mandenius, 2016). On the contrary, it is crucial to guarantee that the biophysical and biological processes that are taking place are doing so in settings that are representative of real-world conditions at their peak efficiency.

Both dry and slurry materials can be used to feed polluted samples for remediation into the reactor (Azubuike *et al.*, 2016). Hydrophobic pollutants are frequently resistant to microbial oxidation, especially if they are bonded to soil substrate. Therefore, their transition to liquid is what prevents them from degrading too much. Hexachlorocyclohexane (HCH) was discovered to degrade in slurry batch bioreactors with the least amount of mass transfer resistance (Quintero *et al.* 2007). Due to their extensive use in biotechnology, bioreactors have developed quickly, but there are still issues that need to be resolved regarding the safety and speed of bioprocesses.

The stability and efficiency of the process could be compromised by poor bioreactor design and building that results in insufficient mixing. In addition to keeping the conditions in the reactor consistent and ensuring adequate contact between the media reactants and the microbial culture, mixing prevents thermal stratification. It is impossible to overstate how crucial mixing is in bioreactors; inefficient mixing reduces the effectiveness of the bacteria process. It is essential to identify and optimize the hydraulic retention times (HRT) needed in the bioreactor to accomplish the required remediation goals. Shorter HRTs prevent microorganisms from successfully degrading the pollutant and may cause microbial washout from the system, whereas longer ones cause low substrate concentration that reduces the population of microbes.

6.7.7 Organism-related factors

Variables that affect organisms include population size, composition, and interactions between and within species. The most diverse living things on earth, microbes, have developed a wide range of metabolic pathways that enable them to endure in a wide range of ecological settings, including those that can expose them to xenobiotics. Numerous habitats have been used as sources of microorganisms for bioremediation, including aerobic, anaerobic, acidic, alkaline, and low-to-high temperature (Tekere *et al.*, 2005). Only a few types of microbes and fungi have demonstrated that they are effective pollutant degraders. In the natural world, complex interactions between microbial populations are frequently used to degrade pollutants. For pollutant remediation in bioreactors, either a single or a combination of microbial colonies is used. When bioagumentation is used, the newly imported organisms must get along with the local population. Because different microorganisms frequently have varying metabolic capacities, to find the best degraders, it is required to compare multiple strains of different microbial players.

The ability of newly identified white-rot fungal strains to digest PAHs was examined and compared. It was shown that these fungi did not accumulate the metabolite quinone, which builds up as a dead end metabolite in *P. chrysosporium*, and that they exhibited degradation capacities that were higher or equivalent to the model, highly regarded *P. chrysosporium*.

Environments, which are polluted, are a source of microbes that have developed a tolerance for or adaptation to the contaminant. However, it is also feasible to choose microbes that have been shown to possess particular innate physiological traits, such as the capacity to degrade well-known substrates that resemble significant xenobiotics structurally or the capacity to adapt to particular environmental conditions. This has been demonstrated in numerous experiments that used microorganisms to degrade pollutants.

6.7.8 Pollutant-related factors

Physical and chemical characteristics of the pollutant, such as its solubility, volatility, molecular complexity, concentration, and toxicity, might influence bioremediation in bioreactors. Most studies on pollutant biodegradation have focused on how various concentrations, combined pollutants, solubility, and molecular structure can affect microbial bioremediation. Alkanes degrade more slowly than PAHs than branched chain alkanes, low molecular weight aromatics, and cycloalkanes (Tekere *et al.*, 2005). The fact that some toxins are refractory, or resistant to degradation, and only degrade gradually even in the correct microbial population and environmental conditions, should also be kept in mind.

6.7.9 Mechanism of bioremediation

The process of bioremediation involves a wide range of mechanisms, including electrostatic attraction, redox reactions, adsorption, complexation, ion exchange, and precipitation. Microorganisms may start redox reactions that lead to metal movement or immobilization, affecting the bioremediation process.

Fe, As, Cr, and Hg are examples of heavy metals that go through stages of oxidation and reduction. When an element is converted from its fixed, soluble form in sediments to its mobile, soluble state, bioremediation is considerably simpler. Mobilization can also have detrimental impacts when harmful metal ions are redistributed and discharged from the solid phase of the sediments into the solution phase (Fomina and Gadd 2014). Heavy metals can now enter microbial metabolic pathways and are more bioavailable. The crucial process of heavy metal bio methylation in soil and water can change the toxicity, volatility, and mobility of heavy metals. By allowing the elimination of cells' volatile methylated species, it also significantly contributes to detoxification (Bolan *et al.*, 2014).

The microbial breakdown of organic molecules, which hastens the release of these ions, is another secondary form of metal mobilization. According to research by Wengel *et al.* (2006), *Schizophyllum commune* emits both dissolved organic waste and heavy metals. A vital technique for chelating metal ions is the bacteria's excretion of metabolites like carboxylic acids and amino acids.

To survive, bacteria have evolved defences against metal ions and methods of remediation (Mustapha & Halimoon, 2015). Metals including Cu, Zn, Pb, Cd, and Cr can be swiftly removed using bacterial detritus (Özer & Özer, 2003). The amount of heavy metal ions and the kind of bacteria have an effect on the efficacy of bio sorption because different bacteria have different cellular structures in terms of peptidoglycans such as poly-*N*-acetylglucosamine and *N*-acetylmuramic acid (Hassan *et al.*, 2010). The primary physical interaction between metal ions and bacterial material takes place through the bacterial cell wall. The ability to bind metals to or within the cell wall is conferred by the overall negative charge produced by anionic functional groups (such as amine, hydroxyl, carboxyl, sulfate, and phosphate) found in both Gram-positive and Gram-negative bacteria (in peptidoglycan, teichoic acids, and teichuronic acids) (Sherbet 1978).

Marine ecosystems have a wide variety of different types of algae. The three species of algae – Phaeophyta, Rhodophyta, and Chlorophyta – brown, green, and red algae – have the highest bio sorption capacity, according to studies on phytoremediation. Charge, chemical composition, and algal biomass type and form all have an impact on metal ion biosorption in different ways (Brinza *et al.*, 2007; Oyedepo, 2011). Whether they are in live or dead form, different algae have been used singly or in combination, in batches or columns, in in-situ remediation (Abbas *et al.*, 2014; Romera *et al.*, 2007). Numerous algal proteins have the potential to contain metal sites, such as amine, hydroxyl, carboxyl, sulfate, and phosphate, which work in complex ways to remove heavy metals. During ion exchange, heavy metal ions take the place of the calcium, magnesium, and sodium ions in the cell membrane. Mycoremediation is a reasonably priced method that does not generate any hazardous waste. The proper fungal species must be found and used for the target heavy metal or other pollutants for mycoremediation to be successful. Fungi can efficiently collect heavy metals in their fruit bodies in order to reduce or entirely eradicate their presence in the environment (Ogbo & Okhuoya, 2011). The bio sorption process is mediated by adsorption, ion-exchange, complexation, and interactions with the cell walls of fungal organisms, which contain chitin, proteins, glucans, lipids, pigments, and polysaccharides as well as functional groups like hydroxyl, carboxyl, amino, sulfate, or phosphate. The longevity of the fungi, the chemical composition of the elements, and the presence or absence of the fungi after sequestration all affect how easily accessible heavy metals and other contaminants will be in the media in the future. According to research by Damodaran *et al.* (2011), *Saccharomyces cerevisiae* can absorb up to 65–79% of the Pb and Cd found in polluted soil.

6.8 CONCLUSION

Globally, heavy metal poisoning has serious negative effects on the environment. Several specialized techniques – microorganism-based and hybrid – currently employed to lessen heavy metal pollution have been assessed in this study. Because it is dedicated to adhering to the biogeochemical cycle's principles, which have an effect on the environment, soil structure, and fertility, the ecological microbiome is significant. The future tendency is to promote the treatment of heavy metals by combining multiple strategies to make use of the best exploratory convention, which maximizes the benefits and minimizes the drawbacks. Similar to this, there have not been many heavy metal bioremediation field applications. Research for future innovation upgrade should be directed in this direction to analyse the viability of contemporary waste treatment on an overall scale and with the use of on-site treatment frameworks. Then try to pinpoint any field designing application issues.

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Chapter 7

Clean production approaches in industries: a case study on pulp and paper production facility applications

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ABSTRACT

The sustainability of life and economy is directly dependent on natural resources, and the effects of the use of these resources are felt on both ecology and the economy. As scarce and valuable resources such as raw materials, energy, and water constitute the basic inputs of the economy as well as life. Resource use not only causes increasing pressure on natural resource reserves and adversely affects the environment, but also affects national and international trade and market prices. The efficient and sustainable use of raw materials, energy, and water by both reducing the impact on the environment and producing more value by using less input also contributes to the sustainability of production and therefore the economy. Especially in the second half of the 20th century, the destruction of environmental values brought about by rapid technological and industrial developments and the rapid decrease of non-renewable resources continue to accelerate today.

The rapid industrialization that started with the Industrial Revolution in the 18th century and the unstoppable increase in the world population caused production and consumption to increase at the same rate. In parallel with the changes in industrialization and lifestyles, the wastes have shown a logarithmic increase over time and the local environmental problems due to these wastes have gained a global dimension. The uncontrolled discharge of these wastes continued for years until it was understood that there was a limit to the capacity of nature to accept the wastes generated after production and consumption. Thinning of the ozone layer, global warming, acid rain, toxic and hazardous waste discharges in quantities far above the absorption capacity to various natural receiving environments can be counted within this scope. One of the reasons for the increase in environmental problems has been the very high cost of investments based on the end-of-pipe approach to environmental protection. For this reason, over time, industrial organizations have focused on 'clean production techniques and practices,' which is an approach to prevent waste at its source. It has been observed that the industries that have started *Clean Production Practices* and allocate a budget for investments when necessary, amortize this cost in a short time as it saves water and energy from the cost of waste treatment, and therefore the application area is expanding day by day. It has been understood that *clean (sustainable) production* can be applied to all type of industries, including small and large, regardless of material, energy and water consumption levels. Observations show that this approach offers the potential to reduce resources by an average of 10–15% without costly investments.

Therefore, in this study, the clean production (CP) approaches in different industries will be discussed with details and CP practices of the pulp and paper production facility will be evaluated in this context.

Keywords: clean production and clean technologies, sustainability, waste, waste reduction

7.1 INTRODUCTION

Especially in the second half of the 20th century, the destruction of environmental values and the rapid decrease of non-renewable resources, brought about by technological and industrial developments, are accelerating today. Wastes that emerged in parallel with industrialization and changes in lifestyles showed a logarithmic increase over time and local environmental problems caused by these wastes have gained a global dimension. Thinning of the ozone layer, global warming, acid rains, various natural receiving environments – toxic and hazardous waste discharges in quantities far above their absorptive capacity can be counted in this context.

The first approach developed to prevent the destruction of environmental values and which has been used intensively until today was the elimination of pollutants after they have emerged. This approach, referred to as pollution control (or 'end-of-pipe'), can be defined as the elimination or removal of pollutants using various environmental technologies after they emerge. The removal of the pollutant with such an approach brings with it a high investment requirement. The financial burdens that conventional treatment and disposal facilities bring to the investor cause people or institutions to refrain from environmental investments in some areas and countries. One of the reasons why environmental problems are increasing is the very high cost of investments in environmental protection.

As a result of the continuous increase in the amount of waste generated in the process and the cost of treatment, the continuous decrease in the discharge standards of the receiving environment in parallel with the increasing environmental awareness in the public has led the institutions and sectors that produce products and services to seek cheaper solutions to this problem. In addition, increasing environmental awareness in the last 20–30 years has led consumers living in developed countries to increasingly prefer products and processes that are less harmful to the environment in production, use, and post-use processes.

The studies that started after this new orientation have revealed that even with simple precautions to be taken, these losses can be prevented and waste generation can be reduced as a result of the more effective use of raw materials that become waste before they can be turned into a useful product in the production process. This was followed by approaches such as increasing efficiency in the production and service sectors, replacing the raw materials used for production with those that are less harmful to the environment, and reducing the water and energy needs required in the production and usage processes. The result is waste reduction, recycling, reuse, more environmentally responsible design of products and services, and so on based on several research studies and 'pollution control' approaches have been replaced by 'clean production' approaches (Demirer, 2003).

For all these reasons, many countries have started to focus on clean production (CP) practices compared to the pollution control (end-of-pipe) approach. In a study conducted in 2004 by the Center for European Economic Research active in Germany, the current situation of end-of-pipe and CP approaches in the Organization for Economic Cooperation and Development (OECD) countries was compared (Figure 7.1). As can be seen in Figure 2.1, it is seen that the CP approach is at the forefront especially in developed countries, especially in Japan and France.

7.1.1 Clean (sustainable) production concept and approach

The concept of 'clean production' is defined by the United Nations Environment Program (UNEP) as 'reducing the risks on people and the environment through the continuous application of a holistic and preventive environmental strategy to products and processes' (UNEP (United Nations Environment Programme), 1996).

Contrary to the 'pollution control' approaches that try to solve environmental problems after they arise, CP approaches require that environmental issues be included in the planning processes as a parameter in the design phase of industrial, urban, agricultural, and all kinds of human activities. Contrary to conventional pollution control approaches, the CP approach aims to prevent/reduce pollution before it occurs. Pollution control approaches adopt production and design stages as constant

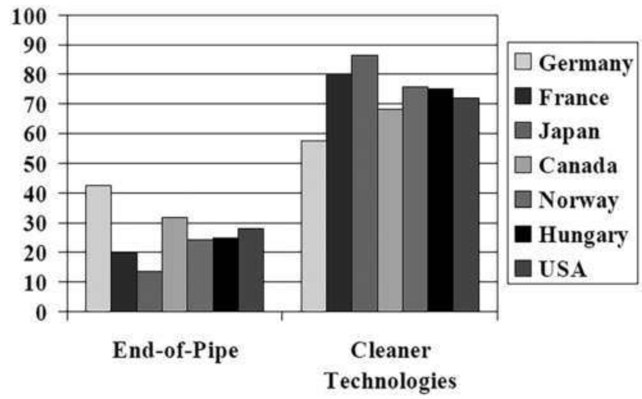

Figure 7.1 Current state of end-of-pipe and CP approaches in some OECD countries. *Source*: Frondel *et al.* (2004).

factors, and see pollution as an inevitable result of these stages. After the pollution has emerged, it tries to find a solution to this problem. Therefore, these approaches focus on better identification of pollution and treatment and disposal of waste, bringing significant additional costs to organizations. Contrary to conventional pollution control approaches, the CP approach aims to prevent/reduce pollution before it occurs (Table 7.1).

On the contrary, CP approaches see pollution and waste as a result of inadequacy, inefficiency, and ineffectiveness in the stages of design, resource use, and production processes and it aims at solving the problem by providing the necessary developments at these stages. Therefore, it not only reduces waste generation, but also provides economic benefits. CP is closely related to the concept of sustainability, as well as the development of environmental-friendly new products, processes, systems, and services that are compatible with natural processes (Glavic & Lukman, 2007; Kjaerheim, 2005).

In addition, UNEP states that the CP approach has an important role in fulfilling the responsibilities of countries related to international agreements (http://www.unep.org/pdf/dtie/CP_MEA_and_Cleaner_Production.pdf).

Since pollution control approaches aim only to comply with the laws and regulations in force, the attempts of the organizations to contribute to their environmental performance are limited to a change in the laws and regulations. Such an approach not only ignores many potential developments, but also causes unpreparedness in planning and implementing control and prevention practices in the event of radical changes in environmental pollution, and environmental improvements are only possible with high-cost initiatives. On the contrary, CP approaches are the environmental initiatives of the enterprises themselves. It aims to continuously monitor and increase its performance, and thus prevents these improvement requirements from being limited to static facts such as laws and regulations. Organizations that adopt and implement pollution prevention approaches will not have difficulty in complying with stricter laws and regulations that will come into force in the future, as they will increase their environmental performance to a much higher level than those required by these laws and regulations.

7.1.2 Development of CP concept

The United Nations Environment Program – Department of Technology, Industry and Economics (UNEP-DTIE) started the Clean Production Program in 1989 and took the first important step towards raising awareness on the issue, establishing an institutional structure and promoting sustainable development efforts by demonstrating its benefits. Since then, the concept of ‘clean production’, which has been adopted by many countries, institutions, and organizations, has gained a global character. For example, many references were made to the concept of CP, which is mentioned as an important

Table 7.1 Differences between approaches to prevent environmental pollution.

Conventional Pollution Control Approach	Pollution Prevention/CP Approach
Adopts the classical end-of-pipe treatment approach. It focuses not on the problem itself and its source, but on the removal of the resulting pollution.	Based on prevention at source approach: Deals with the problem itself and its source and tries to eliminate the root cause
Environmental improvement practices for pollution control require additional costs.	Pollutants and wastes are considered as potential sources that can be converted into useful products or by-products and are tried to be managed within the framework of circular economy.
Identification and application of pollution control technologies, waste managers, and so on is the responsibility of environmental experts.	All employees, including design and process engineers responsible for production, play an active role and take responsibility in all processes related to the improvements planned for pollution control, prevention or elimination.
Environmental improvements are achieved by carrying out technological applications that provide pollution control at the technical level.	Planned improvements to prevent pollution require the integration of non-technical and standardized managerial practices (ISO 9001, ISO 14001, ISO 45001, ISO 50001, etc.) with the use of technology.
Improvements in pollution control are primarily determined by taking into account the needs of customers, suppliers, or consumers.	The development or optimization of production processes is based on minimizing the effects on human health and the environment in producing products that will respond to the needs of customers, suppliers, or consumers by multi-dimensional analysis.

strategy to implement the concept of sustainable development in the Agenda 21 Program at the Rio Summit in 1992 (UNEP (United Nations Environment Programme), 2002).

When the examples of various countries are examined, the development of the concept of CP in a country generally started with raising awareness on the subject and continued with capacity building studies including exemplary practices in the production and service sectors. Efforts were made to spread CP practices through partnerships and information sharing networks created, followed by the establishment of financial mechanisms and the implementation of necessary policy reforms. It has been reported by UNEP that the typical process of the development of the concept of CP in a country can take place from the bottom up, but it can sometimes be realized from the bottom up or in different ways due to local, cultural, and similar reasons (UNEP (United Nations Environment Programme), 2002) (Figure 7.2). As a result of CP initiatives and efforts initiated by UNEP/United Nations Industrial Development Organization (UNIDO), the number of National Clean Production Centers (NCPC) established since 1994 has reached 58 and this number is increasing day by day. NCPCs have carried out important works for the development of the concept of CP in their countries/regions with their capacity building works, handbooks they published, trainings they implemented, demonstration projects and other activities. In Turkey, the UNIDO Eco-efficiency (Clean Production) Program has been carried out by TDFT under the responsibility of UNIDO since 2008 as a sub-program within the scope of 'Developing Turkey's Capacity to Adapt to Climate Change'. One of the goals of this program is the establishment of a national Ecoefficiency (Clean Production) Center (The UNEP Working Group for Cleaner Production in the Food Industry, 2004).

The development of the institutional structuring in CP, the trainings given, and the increase in technical infrastructure and competence have also caused a rapid increase in the number of projects carried out on the subject all over the world. Many information sharing networks have been created in order to convey the CP activities implemented today to all stakeholders. These include UNEP/UNIDO National Network of Clean Production Centers (http://www.unep.fr/scp/cp/network/ncpc.htm), Western Network for Sustainability and Pollution Prevention (http://www.westp2net.org/), United

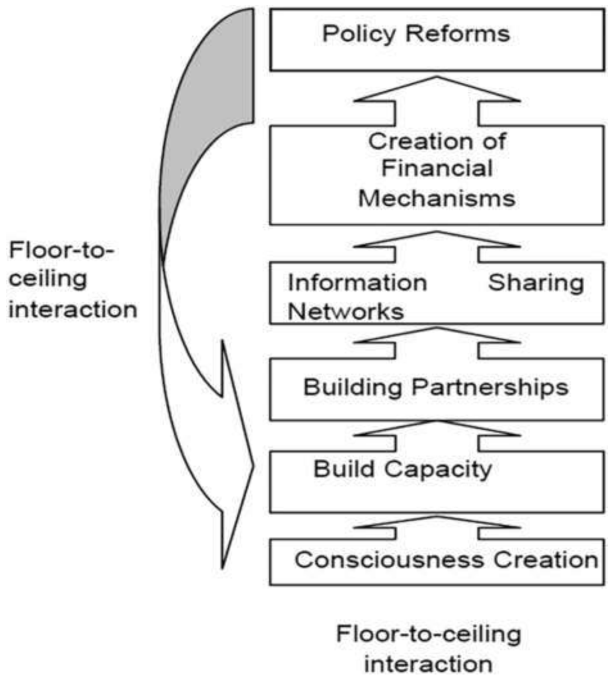

Figure 7.2 Typical process of development of CP concept in a country.
Source: UNEP (United Nations Environment Programme) (2002).

States Environmental Protection Agency (USEPA) Pollution Prevention Information Network (http://www.epa.gov/opptintr/ppic/) and others (http://www.cleanerproduction.com/Directory/networks.htm; http://www.serd.ait.ac.th/teenet/cleaner.htm). All these have made a serious knowledge on the subject available to the stakeholders.

At this point, different concepts, which are mentioned together with the concept of CP and overlap with the CP approach, have also been developed. One of the most interesting of these approaches is industrial symbiosis (industrial ecology). Industrial symbiosis represents that two or more economic processes that are physically close to each other and normally work independently of each other, establishing long-term partnerships to improve both environmental performance and competitiveness. In this respect, with the environmental management systems that can be designed for industrial zones similar to the Organized Industrial Zone (OIZ), by-products, residues, or wastes produced by one enterprise can be used as raw materials for another enterprise. In this way, it not only prevents environmental problems of industrial origin, but also provides economic returns.Consequently, after the pollution has occurred, the end-of-pipe approach, which consists of treating or eliminating with various equipment or technologies, has been replaced by methods that will prevent the generation of wastes from production processes with simple precautions to be taken. These methods were followed by approaches such as reducing the material content of the products, replacing the raw materials used for production processes with less harmful and less toxic ones to the environment, and reducing the water and energy needs required in the production and usage processes. This subject has become popular over time and it has become popular in terms of waste reduction, recycling, reuse, creation of products, and services with a more sensitive design on environmental damage, and so on. The number of researches and developed technologies on these issues has increased and has come to be known as 'Pollution Prevention' and, in parallel, 'Clean Production.'Clean production: it encompasses the continuous and regular implementation of a preventive and integrated environmental strategy applied to production processes, products, and services. CP also ensures that natural resources are

Figure 7.3 Typical CP methodology.

used more efficiently and that the risks of waste and pollution occurring from this process are likely to harm human health are reduced. In addition, it tries to eliminate environmental problems with the applications and technologies developed at the source of the production process, not with the end-of-pipe method at the end of the production process. Typical CP methodology is given in Figure 7.3.

7.1.3 CP to sustainable production

The rapid increase in studies conducted in different disciplines on the concept of 'sustainability' has also increased the use of different concepts and terminologies. CP is an interdisciplinary subject by its nature, so the concept and terminology developed by a particular discipline is used in different contexts by different disciplines. The emergence of a rapidly increasing number of concepts due to geographical and cultural differences causes various difficulties in understanding the studies carried out in the field of CP and in collaborating the concept and terminology. Although the concept of CP is still being used by many related organizations in the industry, sustainable production concept is also spreading rapidly in recent years.

The concept of CP has evolved into the concept of sustainable production in the last 5 years globally (Narayanaswamy & Stone, 2007). The concept of CP is still used by many related institutions (http://www.unido.org/index.php?id=o4460). Although it continues to be used, the concept of 'sustainable production' is rapidly spreading (http://sustainableproduction.org/). Sustainability can be defined that development of it has started recently (Clark, 2007; Luskin & Del Matto, 2007), an economically viable by preserving energy and natural resources with processes and systems and provide constructive and social benefits for all stakeholders in a safe and healthy environment for employees, consumers, and society in the production process.

The concept of CP has been used for the last 20 years. Different audiences have used the same concept in different contexts, in earlier studies, 'integrated pollution prevention and control,' 'life cycle analysis,' 'supply chain management,' and 'industrial ecology were used. The concepts of CP came to be used under the concept of 'umbrella.' However, these concepts are included in the subgroups of the concept of sustainable production, not CP, in the article of Glavic and Lukman (2007).

7.1.4 CP and eco-efficiency

Eco-efficiency is based on the principle of using less natural resources and energy and producing less waste for the same amount of production, with the use of highly efficient production technologies or managerial tools. The concept of eco-efficiency addresses not only environmental pollution and depletion of natural resources, but also many different areas such as 'protection of natural resources,' 'industrial efficiency,' and 'economic development.' In short, eco-efficiency can be defined as providing both environmental and economic benefits in parallel by increasing efficiency in production. In the eco-efficiency approach, 'end-of-pipe (pollution control) practices,' which refer to the disposal and treatment of waste after it is formed, is replaced by the selection of materials (raw materials and auxiliary materials), product design, procurement, production, transportation, and so on of environmental effects. It leaves it to an attitude that covers the processes and enables it to be projected in a wide framework. Eco-efficiency has goals and methods that conceptually overlap with the CP approach. Both concepts aim to minimize 'natural resource and energy consumption', 'use of toxic and dangerous chemicals,' and 'waste, wastewater and emission generation' in the production process (TDFT (Turkey Technology Development Foundation), 2011).

7.1.5 CP and industrial ecology (symbiosis)

Environmental management systems and CP practices not only increase the environmental performance of industrial organizations, but also positively affect their economic performance and corporate prestige. Although these practices are extremely important and effective, environmental performance can be improved to a certain extent since they remain within the company's boundaries. Providing additional gains in terms of environmental performances requires being able to go beyond company boundaries and often inter-firm cooperation. Industrial ecology is the presentation of a new conceptual framework for understanding the effects of industrial systems on the environment. This new framework sets out the ultimate goal of sustainable development, strategies to reduce, and then implement the environmental impacts of products and processes.

Industrial ecology is an approach used for the design of industrial products and processes by evaluating activities in terms of product competition and environmental interactions, in other words, the physical, chemical, and biological interactions between the industry and the environment. Because environmental problems are systematic, industrial applications require a systematic approach between human activities and environmental–ecological processes. A systematic approach involves identifying and solving problems, which makes it easier (Das, 2005; Davarcıoğlu, 2017).

At this point, different concepts, which are mentioned together with the concept of CP and overlap with the CP approach and have many aspects, have also been developed. One of the most interesting of these approaches is 'industrial symbiosis (industrial ecology)'. This approach has been implemented in many countries today. Industrial symbiosis, which first came to the fore in 1989, is based on the analogy between industry, natural life, and ecological systems and it is based on each other both economically and in terms of using each other's products and wastes (matter and energy) and it symbolizes the whole network of industrial processes. Industrial symbiosis represents that two or more economic processes, normally work independently of each other and preferably physically close to each other, coming together and establishing long-term partnerships that will increase both environmental performance and competitiveness, and working in solidarity. In this respect, with environmental management systems that can be designed for industrial zones similar to the organized industrial zone (OIZ), by-products, residues, or wastes produced by one enterprise can be used as raw materials for another enterprise. In this way, it not only prevents industrial environmental problems, but also provides economic returns. Especially implementation and investment projects in the field of waste evaluation (product from waste, energy from waste, etc.) continue at an increasing pace in many countries (Özbay, 2005).

7.2 CP BENEFITS/GAINS

CP approach is an approach that has the ability to provide permanent solutions to environmental pollution problems, which are on the agenda, by preventing pollution before it occurs. The number of organizations that are developing in this context is increasing day by day in the world. CP practices have many benefits for facilities (TDFT (Turkey Technology Development Foundation), 2011).

7.2.1 Economic gains

On the one hand, CP practices integrated into processes will reduce the cost of the industry as it increases efficiency and reduces the use of energy, natural resources, and raw materials. On the other, since it minimizes the wastes to be generated, disposal costs are also avoided. Less energy, natural resource, and raw material consumption is a beneficial practice for the industry as well as the environment.

7.2.2 Compliance with regulations

Industries that adopt the CP approach are more likely to not only comply with today's environmental regulations, but also meet the requirements of regulatory rules that are expected to become more stringent in the future.

7.2.3 Compliance with legal sanctions

Implementations to be realized within the scope of CP will prevent legal sanctions that may occur due to incompatibilities that may arise against the demands of the regulations and environmental accidents that may take place.

7.2.4 Motivation of employees

Employees are the biggest factor in the success of an organization. Employees of an organization that adopts and implements CP strategies will be motivated by the satisfaction of working with an organization that respects society and the environment, and they continue to work in a more motivated way by embracing CP values.

7.2.5 Environmental benefits

As the studies carried out within the scope of CP destroy the wastes discharged to the environment at their source before they occur, they prevent the formation of waste water, emissions, and other waste types originating from processes. Another environmental benefit is that these applications minimize or eliminate the use of energy, raw materials, natural resources, and harmful substances.

7.2.6 Increasing institution and product image

An organization's care about the damage it causes to the environment due to the processes taking place within its body and taking actions for compensation in this regard ensures that it receives the support of the society and employees. In this way, it increases the chance of increasing its market share and being superior to its competitors.

7.2.7 Reducing possible risks against occupational health and safety

As a result of the practices carried out, working conditions are improved and developed, preventing possible occupational accidents, while also preventing workers from being exposed to pollution and dangerous substances.

7.3 OBSTACLES IN CP PRACTICES

Although CP technologies, whose application area has expanded in recent years, have many benefits for businesses, many different difficulties may be encountered during the implementation phase (Demirer & Mirata, 1999).

7.3.1 Economic challenges

Although a significant portion of pollution prevention practices can be implemented with no cost and/or very low-cost measures. However, the inability of organizations to find financing for these less costly or higher cost measures to be implemented in the future is one of the most important obstacles for CP practices. To overcome this obstacle, the benefits of CP technology applications should be well understood by the organizations.

7.3.2 Barriers to implementation and management

(a) **Management indifference:** The viability, success, and sustainability of each new approach to be implemented in an organization is possible if the senior management embraces the project and tries to embrace it by providing various trainings and necessary equipment to the employees.
(b) **Financing:** It is possible to have maintenance, repair, renewal, and equipment changes and additions over time in order to implement and ensure the continuity of the applications. Financial investments are required for all these situations. At this point, a budget should be allocated for CP practices in order to avoid problems.
(c) **Product quality:** The technology to be applied may cause changes in the raw materials and/or processes during the production stages, and the fear that this may lead to changes in product quality, decrease in market share, and bad reputation is an obstacle to the application of CP technologies.
(d) **Employee resistance:** Changes to be experienced in processes may lead to changes in the working routines of employees, and naturally, employees who have difficulty getting used to this situation may have difficulties during implementation. Adequate awareness and equipment will help overcome this obstacle.
(e) **Continuity:** Maintenance, repair, renewal, or add-on needs that may occur in plans and projects from the implementation phase of the applications should be foreseen in the plan and project stages, the necessary capital and awareness in this regard are necessary for the sustainability and longevity of the application.

7.4 COMPONENTS, TOOLS, AND METHODS OF CLEAN (SUSTAINABLE) PRODUCTION

7.4.1 Clean (sustainable) production components

Applications that can be realized within the scope of clean (sustainable) production can be classified under three main headings (Regional Activity Centre for Cleaner Production (CP/RAC), 2000):

- Waste reduction and resource consumption at source reduction
- Reuse and/or recycling
- Product modifications

Focusing on the source of pollution and reducing resource consumption is the basic principle of clean (sustainable) production (Figure 7.4), and in this context, the components of CP are summarized in Figure 7.5.

7.4.1.1 Reduction of waste at source

Administrative precautions constitute one of the simplest methods of clean (sustainable) production and do not bring any investment costs and can be implemented immediately after the determination of the opportunities. Keeping the water valves closed, not running the equipment in vain, optimizing the dosage of chemicals, and so on through prevention of water, energy, and other resource losses are among the examples that can be given by means of administrative methods. These methods require a particular focus on the management and training of employees. Better process control covers by checking optimum process operating conditions in terms of sources consumption, production, and waste generation whether it is suitable for the level includes proper arrangement. It covers parameters such as temperature, time, pressure, pH, process speed, and keeping them as close to optimum levels as possible. Better process control requires more advanced monitoring and management than administrative precautions. It covers parameters such as temperature, time, pressure, pH, process

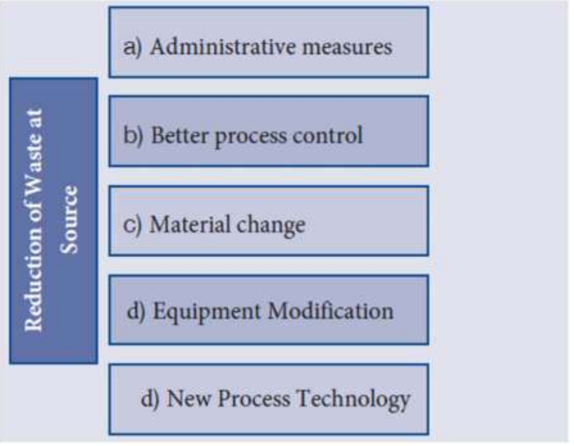

Figure 7.4 Practices for reducing wastes at source.
Source: Vietnam Cleaner Production Center (VNCPC) (2000).

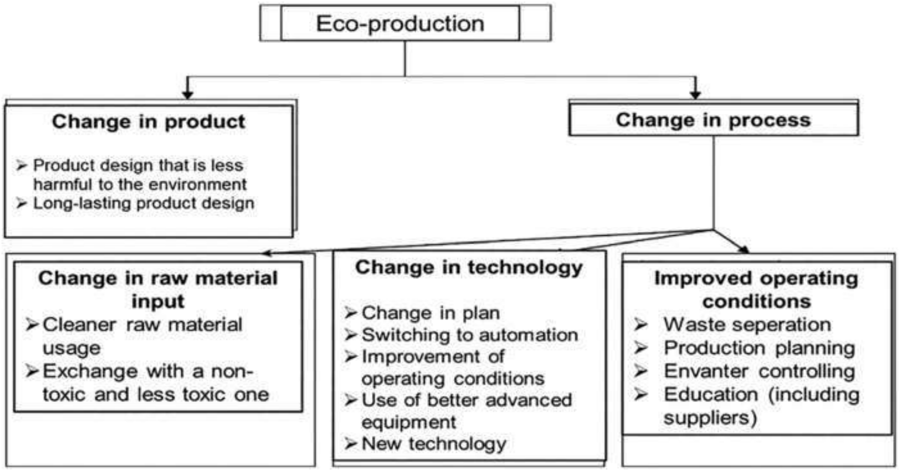

Figure 7.5 Components of eco-production.

speed and keeping them as close to optimum levels as possible. Better process control requires more advanced monitoring and management than administrative precautions. Material change, production efficiency from cost and quality, a higher quality will increase without compromising use of the material. Generally, there is a direct relationship between the quality of the materials and the quality and quantity of the products. In addition, material replacement includes replacing existing materials with those with better environmental properties. For example, the replacement of a paint containing hazardous chemicals with an environmentally friendly alternative, also ensures that the treatment requirements and costs are eliminated or reduced. Equipment modification is the improvement of existing equipment to create less waste and provide more efficient production processes. Adjusting engine speeds, reactors, tanks, optimization of their volumes, isolation of hot and cold surfaces, are some examples of equipment modification. Process change involves the use of modern and more efficient techniques and technologies. This application should be considered carefully as it requires a higher initial investment cost than other clean (sustainable) production applications. On the contrary, potential gains and increases in quality ensure that the investment returns in a short time, making it easier for the company to switch to more up-to-date and contemporary production processes. Such applications often provide to improve product and production quality (Vietnam Cleaner Production Center (VNCPC), 2000).

7.4.1.2 Reuse/recycle

Waste that cannot be avoided can be recycled within the company or as a by-product. On-site recycling or reuse covers collection of wastes and their reuse in the same or different parts of production. The reuse of washing water originating from one process in another cleaning process is an example that can be given to this application. Produced by-products covers waste sold to consumers or other companies as input after collection (if necessary after a certain processing). For example, waste yeast from the brewery can be used as animal feed, fish production and as a food additive (Vietnam Cleaner Production Center (VNCPC), 2000).

7.4.1.3 Product modification

Product specifications to reduce pollution from products replacement is among the main principles of clean (sustainable) production. Changing the product requires rethinking the product and its requirements. Improved product design allows for substantial reductions in material consumption and use of hazardous materials. Lightening the products, reducing the wall thickness, designs that make the product more recyclable, and so on can be given as an example of this approach. Packaging change is one of the important applications in this regard. The main point of view here is to minimize the amount of packaging material while guaranteeing the protection of the product. One of the most common examples in this field is the use of recycled cardboard instead of plastic foam to protect fragile materials (Vietnam Cleaner Production Center (VNCPC), 2000).

7.4.2 Clean (sustainable) production tools and methods

To implement the CP approach, different methods that can be applied to products and processes have been studied. As a result of these studies, many tools have been put forward to identify CP opportunities, and the tools that should be used change according to the application area. The choice of these tools depends on the problem in the business and the work to be done. One or more of the tools can be used depending on the nature of the study and the problem (Özbay, 2005).

The main tools and methods that make up the elements of CP practices are given in Figure 7.6.

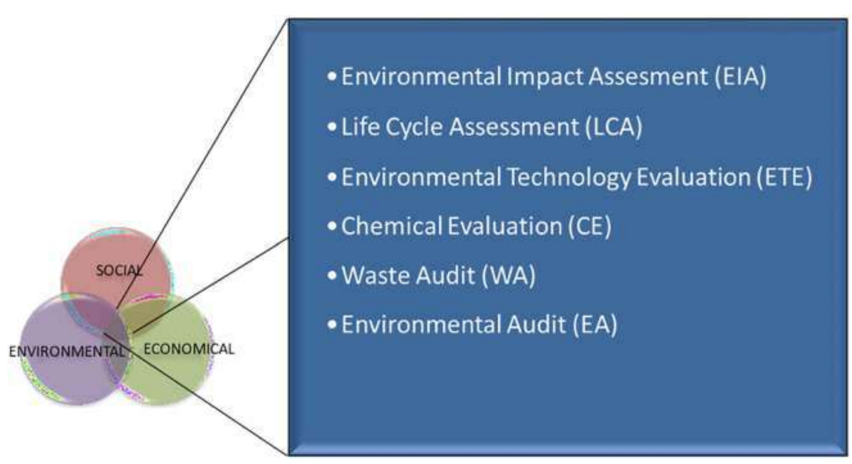

Figure 7.6 Main tools and methods of CP.

7.4.2.1 Environmental impact assessment

Environmental impact assessment (EIA) is a process that determines the significant impacts of a particular project or development on the environment (REC, 2011). EIA refers to the studies to be carried out in determining the positive and negative effects of the planned projects on the environment, in determining and evaluating the measures to be taken in order to prevent or minimize the negative effects in a way that does not harm the environment, the selected location and technology alternatives, and the monitoring and control of the implementation of the projects. In this context, EIA, which is used as a tool in CP practices, provides significant benefits within the framework of the following purposes:

- Identification of adverse environmental impacts expected to occur.
- Adding the necessary measures to the project in order to prevent these negative environmental effects.
- Determination of the project acceptability by the public in addition to the economic aspect also environmental.
- Determination of additional studies and monitoring mechanisms regarding the significant environmental impacts of the project.
- Ensuring public participation in decision-making processes.
- Helping all groups related to the realization of the project and its environmental impacts to understand their roles, responsibilities and relations with each other in this project.
- Ensuring that the process proposed in the project is BAT.

7.4.2.2 Life-cycle assessment

Life-cycle assessment (LCA) is a tool used to prevent or reduce these effects by analyzing the environmental impacts that occur in the process from the production of products and services to their use and disposal. The resources used throughout the entire life cycle of a product or service, as well as the environmental impact of emissions to air, water, and soil, and improvement opportunities can be systematically evaluated by means of LCA. The life-cycle stages cover the extraction and processing of raw materials, production, storage, and distribution of products, use, recycling, and disposal (cradle-to-cradle). LCA is mainly used in the development of environmental labeling criteria, raw materials of products, production processes, equipment, and so on. It is used to prevent/reduce environmental impacts through replacement and/or redesign. LCA studies are the activities in which the activity in question is monitored as 'cradle to grave,' 'cradle to door,' 'cradle to cradle,' and 'door to door' depending on the stage of a life cycle of all the stages, all inputs and intermediate and end products conducted for a defined time and place. LCA study is not a decision mechanism it has the quality of helping the decisions to be made. LCA is mainly used in the development of environmental labeling criteria, raw materials of products, production processes, equipment, and so on. It is used to prevent/reduce environmental impacts by changing and/or redesigning. Also useful for determining at what stage of a product's lifecycle a more detailed waste control exercise is required. The LCA approach is an important tool used in decision making in CP applications. However, the determination of the study limits, the quality and reliability of the collected information, and the approaches used in the analysis of this information, can give very different results depending on the differences in the viewpoints of the people and institutions conducting the study. Therefore, it would be beneficial to use it together with other tools (Özbay, 2005).

7.4.2.3 Environmental technology evaluation

Environmental technology evaluation (ETE) examines the effects of specific technology on human health and natural systems and resources. ETS is part of the technology assessment, which can be defined as the determination of the effects of a technology on human health and natural systems and resources. ETE includes (Özbay, 2005) environmental impact assessments of various facilities and projects, qualitative and quantitative determination of discharges resulting from the use of various technologies, determining the risks of various technologies on human health and environmental values using qualitative and quantitative methods.

7.4.2.4 Chemical evaluation

Chemical evaluation (CE) includes the analysis of the toxic effects and amounts of the chemicals used in the production stages by using various information sources and databases. Pursuant to CP, it is necessary to follow the approach of using chemicals that are the least harmful to human health and the environment in production (REC, 2011). For example, Materials Safety Data Sheets and the International Program on Chemical Safety are widely used information sources to determine the hazards of a chemical on human health and environmental quality. By using these resources, it is possible to choose the least harmful one for human health and environmental values from more than one chemical that can be used for the same job. CE is part of the risk assessment.

7.4.2.5 Waste audit

Requiring a balance of materials for each process and processes, waste audit (WA) includes identifying all inputs required for an industry, plant or process and all wastes generated from them. In waste inspection studies, all wastes generated during the production stages, the sources, quality and quantity of these wastes and the possibilities of reducing them are determined. Waste and emissions audit are terms used synonymously with waste and emissions prevention assessment and waste reduction audit. Waste inspection determines the following for CP: (REC, 2011);

- Input/output (mass balance) inventories of processes,
- Source, quality, and quantity of wastes generated,
- Efficiency and weak points of the current process,
- Waste minimization targets.

As a result of waste inspection, losses are reduced and/or prevented, thereby increasing process efficiency. A waste control application

- Defines the source, amount, and types of wastes produced.
- Gathers information on key processes used, raw materials, products, water use and waste generation.
- Identifies deficiencies and weak management points in the process.
- Determines the targets required for CP.
- Allows the development of inexpensive waste management plans.
- Helps to increase process efficiency by increasing the level of knowledge of the personnel working in the workplace about the processes used.

7.4.2.6 Environmental audit

The purpose of environmental audit (EA) is to determine the amount and character of waste streams originating from the production process or services and to make decisions about what should be done to reduce pollution. It is the most frequently used and most important application tool of CP. Since environmental audit is a very effective tool, there are also types developed for different purposes such as waste audit (WA), energy audit, and risk audit. Environmental auditing is an important tool for both business and government as its compliance with environmental policy and standards is evaluated. This tool of CP can enable industries to get rid of penal sanctions by detecting the measures to be taken regarding the environment in a timely manner (Özbay, 2005).

7.5 CP PRACTICES APPLIED IN DIFFERENT INDUSTRIES

7.5.1 Textile production facility: CP practices

7.5.1.1 In Korea (Asia Pacific Economic cooperation (APEC), 2006)

The main production divisions of the textile industry are fiber production (natural, artificial, synthetic, nonwoven), yarn production, fabric production (weaving, knitting), finishing (bleaching, dyeing, printing, finishing), ready-made garments. Pollution parameters in production include toxic chemicals discharged to air, water, and soil, as well as noise, energy consumption, consumption of natural resources, and waste. Textile finishing and dyeing companies are the ones that cause the most environmental pollution. In addition to the consumption of natural resources, noise, energy consumption and fiber dust parameters, which are also mentioned in textile finishing enterprises, yarn and weaving enterprises, toxic chemicals are used. Toxic chemicals pollute the soil with solid waste, environmental water with waste water, and air with flue gas.

In the study conducted by the Asia-Pacific Economic Cooperation (APEC) on CP case studies for selected industries in Asian and Pacific countries in 2006, two textile industries operating in Korea were examined. A group of experts assessed Wowcom Corp., a textile dyeing company in Korea, for process assessment, where possible CP technologies were identified. The general goal of the Clean Production Program was to achieve environment-friendly dyeing process with higher energy efficiency for the global competitiveness of these companies.

7.5.1.1.1 Evaluating dyeing processes

The problems identified before CP practices are listed below:

- The dyeing liquor is not measured quantitatively during the continuous process that generates waste.
- Since there is no inverter in large pumps, there is unnecessary energy consumption.
- Insulation of pipes is insufficient.
- Since the ventilation system is insufficient in the area where the tentering machine is located, it is operated continuously and unnecessary energy is consumed.
- Irregularities in the dyeing process due to the formation of unsuitable dye combinations cause a decrease in efficiency.
- Insufficient ventilation and lighting near the jigger machine causes a decrease in work efficiency and product quality.

Implemented application: CP options determined for these identified problems are listed as follows:

- For the proper management of chemicals, pumps capable of quantitative measurement have been placed in areas where chemicals are used, and an investment of $2900 has been made for this (*reduced wasted water by 6%, reduced energy consumption by 9%, reduced material usage by 5%, payback period: 4 months*).
- To reduce unnecessary energy consumption in the jet dyeing machine, an inverter application was made to control the main motor and an investment of approximately $26 000 was made in this regard (*reduced energy consumption, reduced air emissions, payback period: 34 months*).
- To prevent unnecessary energy consumption due to the lack of insulation in the pipes, pipe insulation was made and an investment of $2500 was made for this (*reduced energy consumption, reduced air emissions, payback period: 0, 6 months*).
- A new system was installed to reduce ventilation runtime when the tentering machine is not in use, with an investment of $4200 (*reduced energy consumption, reduced air pollution, payback period: 4 months*).
- In the dyeing process, dyeing recipes were optimized for each color, and an investment of $1700 was made for this (*reduced energy and chemical consumption, payback period: 1 month*).

- In the section where the jigger machine is located, arrangements were made on the ceiling to allow more sunlight to enter and to increase the lighting in the environment, and an investment of $2700 was made for this (*increased in labor productivity and improved safety of the work place, payback period: immediately*).

Benefits: As a result of all these improvements, with an investment of $40 000, an annual economic benefit of approximately $103 400 was provided to this industry, with a payback period of minimum 0.6 months (*insulation of pipes*) and maximum 34 months (*invertor usage*).

7.5.1.1.2 Evaluating waste minimization and cost reduction

The problems identified before CP practices are listed below:

- The cooling waters in the industry are not recycled.
- Energy loss occurs due to the high-speed operation of the fans when the tentering machine is in the standing position.
- Due to the lack of insulation in the steam distribution pipes within the facility, heat loss and thus energy loss occur.
- Even when the anti-felting machine is in the standby position, steam flow occurs and therefore unnecessary energy consumption occurs.
- Since dyes with low compatibility are mixed in dyeing prescriptions, the color quality decreases, which leads to a decrease in product quality.
- Uncontrolled liquor flow management in the dyeing process causes a decrease in product quality.
- Leakage occurs due to aging of pipes throughout the facility.

Implemented application: CP options determined for these identified problems are listed as follows:

- An investment of approximately $5800 was made for the reuse of the cooling water for the wool washing process before the dyeing process in the facility (*reduced wastewater generation, payback period: 5 months*).
- An investment of approximately $500 was made in order to prevent unnecessary energy losses, the tentering machine is equipped with a sensor that will allow the modification of operating conditions (*reduced gas emission, reduced energy consumption, payback period: immediate*).
- An insulation unit has been installed throughout the facility to prevent heat and energy losses, with an investment of approximately $2700 was made (*reduced energy consumption, payback period: 3 months*).
- An automatic valve system is installed to stop the steam supply when the anti-felting machine is on standby with an investment of approximately $1250 was made (*reduced energy consumption, payback period: 4 months*).
- For highly compatible dyestuff mixtures to be applied in dyeing recipes, the adsorption behavior of each dyestuff was determined by examining without any investment was made (*higher product quality, higher reproducibility of color, payback period: immediate*).
- A flow detector was installed to control the liquor flow in the dyeing process with an investment of approximately $10 000 was made (*reduced wastewater generation, payback period: 4 months*).
- Aged pipes throughout the facility were replaced with new ones with an investment of approximately $15 000 was made (*reduced wastewater generation, payback period: 5,5 months*).

Benefits: As a result of all these improvements, with an investment of $32 250, an annual economic benefit of approximately $135 200 was provided to this industry, with a payback period of minimum 3 months (*insulation unit*) and maximum 5,5 months (*pipe replacement*).

7.5.1.2 In Peru (Asia Pacific Economic cooperation (APEC), 2006)

In this study, a Peruvian textile company that deals with fiber spinning, knitting, fabric dyeing, and finishing was investigated. During CP studies, energy was focused on as the most important CP opportunities. These issues included thermal energy generation, energy recovery of hot gases released from the chimneys of boilers, inefficiencies in the combustion process in boilers, the presence of leaks in switches and steam pipelines, and high power consumption due to compressed air leakage in distribution lines.

Implemented application: CP options determined for these identified problems can be listed as follows:

- Administrative practices such as optimization of the combustion process, continuous control of fuel quality and keeping the boiler clean were carried out by making an investment of $1000 in order to eliminate the inefficiency in energy-generating boiler systems (*reduced fuel consumption by 28 800 gal/yr. Reduced gas emission of effect conservatory (GEI) and polluting gases of the atmosphere (GCA), payback period: 3 months*).
- With an investment of $11 000, the steam traps were reviewed to reduce heat losses in the steam distribution line and during condensation, eliminating the identified losses and optimizing the use of electricity to prevent compressed air losses during dry cleaning (*increased boiler efficiency by 16.5, 7.8, and 33.5%. Reduced CO_2 emissions by 850 tons/yr, payback period: 5 months*).

Benefits: As a result of all these improvements, with an investment of $12 000, an annual economic benefit of approximately $27 400 was provided to this industry, with a payback period of minimum 3 months (*optimization and maintenance*) and maximum 5 months (*isolated steam pipelines*).

7.5.1.3 In Turkey (TDFT (Turkey Technology Development Foundation), 2011; Alkaya et al., 2011)

In this study, a facility with a fabric production capacity of 15 00 000 meters/month, which produces fabrics (polyester, cotton, and lycra-based fabrics) for textile dyeing and finishing, women's outerwear group, was examined within the scope of Unido Eco-Efficiency Program.

Before the CP application, it was determined that a total of 300 000 m^3 of water was consumed annually depending on the production amount in the facility and 80–85% of the total water use of the facility was realized in the dyeing and finishing processes. In addition, it has been determined that the facility meets its energy needs with natural gas and electricity and has an annual consumption of approximately 1 300 000 m^3 natural gas and 4 250 000 kWh electricity. It has been determined that an old technology water-softening equipment used in the water-softening processes that form the heart of the facility is used, this equipment is operated manually and the amount of water consumed during the regeneration of the device is 15 m^3/rej and the amount of salt consumed is 450/kg/rej.

Implemented application: CP options determined in this facility making textile dyeing and finishing processes can be listed as follows:

- To better control the water consumption values for each process and to determine the optimum water consumption values in these processes, flow meters are installed at the process inlets and outlets.
- To prevent unnecessary water consumption, washing times were reduced and the overflow washing valves in the overflow washing used in finishing were closed.
- In the dyeing section of the facility, where a high amount of water is consumed, the inlet–outlet valves in the cooling water sections of the dyeing machines were renewed in order to reduce water losses and leakages.
- Dryer cooling water was collected and sent to the soft water pool for reuse.
- To determine the optimum flow rate of the water used in the fabric opening machine, a flow meter is mounted on the machine line.
- Reuse of fabric fluff burning machine cooling water was directed to the soft water pool.

- The old technology water softener is fully automatic and the new system, which also increases the quality of the water produced, has been replaced with a water-softening unit.
- A monitoring system has been set up to monitor the water savings on a monthly basis on the basis of the product produced.
- Heat exchangers have been installed at the entrance of the wastewater treatment plant of the enterprise to provide heat recovery from the wastewater.
- A system has been set up to provide heat recovery from the high-temperature flue gas released to the atmosphere from the RAM unit.

Benefits: As a result of all these improvements, with an investment of $34 659, an annual economic benefit of approximately $345 161 was provided to this industry, with a payback period of <2 months. While 111.7–129.4 L of water was consumed per kg of product produced before the project, water consumption was reduced to 50.9 L/kg of product with the project. Unnecessary consumption of approximately 162 000 m^3 of water per year is prevented and total water consumption of the facility was reduced by 54%. Moreover, as a result of the improvements in the water-softening system, 192 tons of salt (NaCl) was saved annually. Furthermore, with the heat recovery systems from waste water and flue gas, the energy used for hot water production was also saved and in this sense, 22% increase in efficiency was achieved and annual natural gas and electricity consumption was reduced by 4 780 000 kWh in total. Thus, the total CO_2 emission of the facility was reduced by 879.6 tons/year.

7.5.2 Rubber production facility CP practices in New Zealand (Asia Pacific Economic cooperation (APEC), 2006)

Rubber has been used for centuries, indispensable in our daily lives, from the shoes we wear to the wheels and windscreen wipers in our cars, from diving suits to hoses and soccer balls. The gasket that integrates the natural gas pipes with the stove, the bellows that protects the axle shafts in our cars, the gloves used in the operating room, the insulators that surround the conductive copper wire, the raincoats that protect us from the rain and the insulation materials used in house roofs are made of rubber. The reason why rubber is used in such a wide range is its properties such as flexibility, softness, durability, stickiness, and waterproofing. Compounds such as zinc oxide and lead oxide are used in the vulcanization process of rubber. These chemicals can poison living things by mixing with the soil, streams and water bodies in the form of liquid waste. The rate of waste material generated in the rubber processing process is approximately 25 times that of the rubber produced.

In the study conducted by the Asia-Pacific Economic Cooperation (APEC) on CP case studies for selected industries in Asian and Pacific countries in 2006, a rubber manufacturing company, implemented CP introduced by the Target Zero program, was examined. The objective of this program is to enhance efficiency in every aspect of the business from manpower right down to production. The problems identified before CP practices are listed below:

- The calender machine, which is an important part of production, is operated in an inefficient way in terms of waste generation, raw material use, and fuel use in the production of certain products.
- The plastic strips used in banding and wrapping the products are used once and thrown away.
- Leaks are detected in most of the faucets connected to the water mains throughout the facility.
- The extruder machine cooling waters drawn from the aquifer are discarded after using the water once.
- Blank cards attached to the cards used for tracking the production stages of rubber products throughout the facility cause unnecessary waste generation and resource consumption.
- Unnecessarily large amounts of paper are routinely used to print production line flow reports on site throughout the facility.
- Cardboard packaging is not recycled throughout the facility.

- Low efficiency monophosphor lamps are used throughout the facility in terms of energy efficiency.
- Steel strips, which are used as a component of the packaging in the palletized packaging of raw materials, are disposed of landfill and are not recycled.

Implemented application: CP options determined for the above identified problems are listed as follows:

- A special attachment was fitted to the calender machine to reduce waste generation, manual machining, and edge misalignment efficient operation of the machine with an investment of approximately \$20 000 was made (*reduced waste rubber and usage of raw materials and fuel, increased machine productivity, payback period: <1 month*).
- The plastic strips used in banding and wrapping the products were recycled without any investment being made (*reduced raw material consumption, payback period: immediate*).
- By applying a regular maintenance program throughout the facility, unnecessary water consumption was prevented and savings were achieved with an investment of approximately \$1000 was made (*reduced water consumption, Payback period: 4 months*).
- The amount of wastewater was reduced by feeding the cooling water used in the extruder mill back to the groundwater with an investment of approximately \$1500 (*reduced wastewater generation, payback period: 2 years*).
- Blank cards attached to the cards used for tracking the production stages of rubber products throughout the facility were eliminated without any investment was made (*reduced cardboard wastes, reduced resource consumption, payback period: immediately*).
- Unnecessary paper usage and resource consumption have been reduced by rationalizing the report printing format throughout the facility without making any investment (*reduced paper usage, reduced landfill disposal, payback period: immediately*).
- Cardboard packages are segregated within the facility to ensure recycling without making any investment (*reduced landfill disposal, reduced raw material usage, payback period: immediately*).
- Monophosphor lamps with low energy efficiency throughout the facility were gradually replaced with triphosphor lamps without making any investment (*reduced amount of mercury disposed, reduced energy consumption, payback period: immediately*).
- Steel strips, which are used as a component of the packaging in the palletized packaging of raw materials are recycled without making any investment (*reduced resource requirement, reduced landfill disposal, payback period: immediately*).

Benefits: As a result of all these improvements, with an investment of \$22 500, an annual economic benefit of approximately \$ 297 010 was provided to this industry, with a payback period of minimum <1 month (*fitted a special attachment to the calender machine*) and maximum 2 years (*recovered the extruder machine cooling water*).

7.5.3 Fertilizer manufacturer facility CP practices in New Zealand (Asia Pacific Economic cooperation (APEC), 2006)

Fertilizer industry is the branch of the chemical industry that produces phosphorus, nitrogen, potassium, and other plant nutrients required for plants in agriculture, either simply or in combination. Products in the fertilizer industry; nitrogen fertilizers, phosphate fertilizers, potash fertilizers, mixed fertilizers, and compound fertilizers are divided into five groups. Various processes are applied by using different raw materials according to the type of fertilizer to be produced in the fertilizer industry. Some products can be used as a raw material for another product. In the industry, some of the raw materials are imported, and some of them are produced in-house as intermediate products. Intermediate products used in the fertilizer industry are sulfuric acid, phosphoric acid, and nitric acid.

The Ravensdown Fertiliser Co-op site at Hornby in Christchurch is one of the three sites in the Ravensdown group that manufactures phosphate fertilizers for New Zealand's agricultural industry, has been a participant in the Target Zero Program. The Sustainable Management Fund of the Ministry for the Environment, Meridian Energy Ltd (formerly ECNZ), and the local authority and electricity company in each area sponsored the program. The objective of this program is to enhance efficiency in every aspect of the business from manpower right down to production. The problems identified before CP practices are listed below:

- The lamps that illuminate the roads inside the facility are left on during the day. Since electricity is generated on site, electricity is traditionally considered a free good and consumes unnecessary resources.
- The drums are not recycled due to the large amount of lithium grease residues under the drums used in production.
- The cooling water supplied by drilling in the acid plant is treated after use and then discharged to the ground. In addition, the drilling water used in acid-plant plate heat exchangers is stored in an open pond, which forms algae before use and requires chemical treatment.

Implemented application: CP options determined for the above identified problems are listed as follows:

- A $279 investment was made for the timing system, especially for the automatic on and off of road lighting systems throughout the facility (*reduced electricity consumption and increased bulb life, payback period: 9 months*).
- For an investment of $65, grease suction is made efficient by adding a 10 mm thick metal plate to the suction pump at the bottom of the drums (the extra weight allows more grease to be sucked in by the pump) (*increased amount of grease removed by 9.45 kg/drum, reduced grease waste from 22% to 4.5%, allowed recycling of drums, payback period: 3 weeks*).
- Existing bore water ponds were used for the acid plant wastewaters, make-up water for the wet scrubber of the facility was supplied from these ponds and also four new closed-hole water tanks were installed to prevent algal growth. All these were done with an investment of $185 344 (*compliance to regional council regulations, reduced bore water consumption and chemical use, payback period: several years*).

Benefits: As a result of all these improvements, with an investment of $185 344, an annual economic benefit of approximately $12 983 was provided to this industry, with a payback period of minimum 3 weeks (*by adding a 10 mm thick metal plate to the suction pump*) and maximum several years (*acid plant cooling water reuse*).

7.5.4 Leather processing facility CP practices in Croatia (Greco Initiative & Regional Activity Centre for Cleaner Production (CP/RAC), 2008)

Chemicals such as sulfites, acids, alkalis, and chromium are used in leather production. If these chemicals are not properly managed or removed, they cause adverse effects for the environment. Organic substances from raw leather materials such as leather or hide cutting, sawdust, scraps, hair, hair protein and keratin, dissolved in wastewater, lead to a decrease in oxygen in receiving environments such as lakes and rivers. If wastewater and solid wastes are not treated and/or managed well before discharge, they also harm flora and fauna.

Within the scope of the study, the following subjects were determined as the focal points of the CP activities carried out in the facility:

- Determination of actual volumes and pollution parameters of tannery waste.
- Choosing the most appropriate methods to reduce water consumption and pollution.
- Developing the most suitable waste and sludge treatment alternatives.

- Developing a financing program for the implementation of an environmentally friendly water management program by reducing operating and maintenance costs.
- It is aimed to establish standards, regulations, and pricing guides for discharge and sludge disposal.

Implemented application: The clean (sustainable) production possibilities determined for the facility within the framework of these focal points are listed below:

- Salt forging: reduction of chloride concentration at the tannery outlet (total reduction in 0.3–0.4%, chemical reduction with 5% for washing and soaking, reduction in pollution parameters are 0.6%, 4%, 3%, and 25% for SS, BOD_5, COD, and chloride, respectively).
- Recovery of residues: reduction of organic pollution and sludge volume by reusing residues through reduction of sulfite and lime pollution (*total reduction in 1.4%, chemical reduction with 9–10% for liming, reduction in pollution parameter for sulfide is 9–10%)*. 16% of leather residues are converted into useful products (biogas, compost).
- **Hair removal:** reduction of organic pollution, sludge volume, raw material, and water consumption by reducing the amount of hair on the material before the waste is discharged into the sewer system (*chemical reduction with 14–15% for lime, 100% for NaHS (%72 Na_2S), 6–7% Na_2S (%62–67), reduction in pollution parameters are 6.7%, 41%, 25%, 25%, 18%, 35%, and 2% for TSS, SS, COD, BOD_5, sulfide, N-total and N-NH_4, respectively*). 47% of waste by weight has been reduced.
- **Chromium reduction:** reduction of chromium concentration in sludge and wastewater through the recovery of chromium from the tanning process (*chemical reduction cannot be provided but reduction in pollution parameters is determined as 98–99 for Cr*). The amount of chromium in the sludge has been reduced below the legal limits for regular storage.

Waste treatment and sludge treatment methods implemented are as follows:

- Sulfite removal
- Use of efficient flocculants and coagulants
- Sludge treatment, disposal, and reuse
- Laboratory analysis of discharge and sludge composition

Benefits: With the project, a model that can be applied by other tanneries in Croatia has been developed in order to reduce environmental pollution in leather production. A remarkable example has been set as a result of reducing environmental impacts and production costs through practices such as salt forging, hair reduction, and chrome recovery. As a result of the implementation of CP practices only in the hair removal process, BOD_5 in the wastewater was reduced to 25% with an investment on equipment and construction works of 49.384 € + annual operating costs of 14 523€. In addition, a total of 85 000€/year savings was achieved, with a chemical savings of €22 252/year and a savings of €53 526/year in sludge disposal costs. Total payback period was calculated as 1 year. On the contrary, chromium process with the chromium recovery system has an economic value gained with an annual investment cost at 218 352€, the operating cost is 70 253–80 077€ and a payback period of 5–7 years (when indirect savings costs for chromium recovery are included, for example, storage cost for sludge, reduction of discharge amount – payback period is calculated as 1–2 years). With this application, 111 512€ was gained by recovering 26 400 kg of chrome. However, here, basic sludge treatment methods in different sludge types are discussed in reducing the chromium content (below 1000 mg/L), and hazardous waste storage systems are not considered.

In addition, end-of-pipe measures were also included in the project and solid waste management strategy took place at the end of the project and uncontrolled waste, which was specified as 14 567 kg/year, was also disposed of.

7.5.5 Canned food production facility CP practices for water and energy saving in Egypt (Greco Initiative & Regional Activity Centre for Cleaner Production (CP/RAC), 2008)

In this study, two facilities were examined, one in İskenderiye and the other in Kaha, employing approximately 600–650 people. Two selected facilities, one private and one public, are one of the largest canned food producers in Egypt. The production line of both plants includes juice, jam, frozen vegetables, canned beans, and tomato paste. Canning facilities and a cooling unit are located on the premises of the facilities, and production is seasonal at both plants. Both facilities have high water and energy consumption and they discharge 780 680 and 520 000 m^3/year wastewater to the sewerage network, respectively.

As a result of the preliminary examinations carried out within the scope of CP practices in both facilities, it was determined as a priority to address the following issues:

- At the beginning of the energy-related problems in facilities, steam leaks in processes, steam lines, and traps (steam traps) take place.
- In addition to these, facilities experience heat losses due to poor steam line insulation, steam and water losses occur due to the can sterilization unit and the inability to manage condensate water well.
- As for water problems, a very high amount of water is wasted due to open cooling cycles and water leaks, some malfunctioning cooling towers, insufficient water recovery systems, water dripping from taps and hoses, water consumption for washing vegetables, washing equipment, and floors.

Implemented application: To prevent energy loss, the steam escape points on the steam lines are insulated. Steam and water losses occur due to not taking the necessary precautions in the can sterilization unit and the condensate water cycle, this situation is monitored by water meters placed in the company's 13 separate water-using processes, the establishment of a cooling tower for the bottled fruit juice line for the recycling and recovery of cooling water, the water loss. With the improvement of the storage system and the addition of hose caps that allow water to slow down, water usage has been reduced.

The following measures have been taken to conserve energy:

- Insulation of open steam pipes (investment cost for facility-1: 38 009€, payback period: 19 months, annual cost saving: 24 504€, facility-2: 38 009€, payback period: 10 months, annual cost saving: 22 307€).
- Replacing leaky steam traps (investment cost for facility-1: 4277€, payback period: 8 months, annual cost saving: 6182€, facility-2: 4430€, payback period: 5 months, annual cost saving: 9777€).
- Replacing leaky steam valves (investment cost for facility-1: 14 379€, payback period: 36 months, annual cost saving: 4790€, facility-2: 11 891€, payback period: 17 months, annual cost saving: 8400€).
- Establishment of pressure regulators in the sterilization system (investment cost for facility-1: 13 329€, payback period: 10 months, annual cost saving: 16 373€, facility-2: 13 822€, payback period: 4 months, annual cost saving: 47 231€).
- Installation of condensate water recovery system (investment cost for facility-1: 10 154€, payback period: 44 months, annual cost saving: 2772€, facility-2: 12 183€, payback period: 14 months, annual cost saving: 10 190€).
- Improved boiler efficiency (investment cost for facility-1: annual cost saving: 4734€, facility-2: annual cost saving: 10 603€).

The following measures have been taken for water saving:

- Use of hose nozzles (investment cost: 2587€, payback period: 7 months, annual cost saving: 2754€).
- Improvement of the water collection system (investment cost: 1499€, payback period: 5 months, annual cost saving: 7344€).
- Cooling tower investment (investment cost: 29 953€, payback period: 12 months, annual cost saving: 26 438€).

Benefits: As a result of the implementation of the above-mentioned options, 15 278 m^3/year steam savings were achieved in facility-1 and 18 125 m^3/year in facility-2. Fuel consumption was reduced by 40% in facility-1 and up to 34% in facility-2. As a result of all these improvements, with an investment of $80 148 for facility-1 and $61 288 for facility-2, an annual economic benefit of approximately $59 355 for facility-1 and $108 508 for facility-2 was provided to this industry, with a payback period of minimum 4 months and maximum 44 months.

7.5.6 Oil and soap facility CP practices in Egypt (Greco Initiative & Regional Activity Centre for Cleaner Production (CP/RAC), 2008)

The seed varieties processed in the facility, which processes an average of 68 000 tons of seeds per year, include sunflower, corn, soybean, and cotton seeds. The main by-products produced in the facility, which produces up to 24 000 tons of first-class edible oil per year, are approximately 40 000 tons of dry pulp (packaged in bags as animal feed) per year and up to 1800 tons of soapy substances annually.

The processing of oil at the plant is carried out in five stages:

(1) Storage after the seeds are separated from the broken seeds.
(2) Seed preparation, extraction of 50% of the crude oil content and obtaining the seed pulp containing 30% oil.
(3) Sending the seed cake to the solvent extraction section where a solvent–oil mixer is located and forming pulp (containing 2% oil), extracting the crude oil in a mixer with a three-stage evaporation system and removing the pulp from the solvent, and finally, the hexane used as a solvent is recovered in the system.
(4) Refining of oil neutralized with caustic soda (for soapy substances), washed, separated by centrifugation and separated from its odor, color, and aromatic flavoring substances.
(5) Bottling and packaging of the oil produced.

The following clean (sustainable) production opportunities have been determined as a priority within the scope of the project, whose facility is carried out through clean (sustainable) production inspection:

- Reducing steam losses due to damaged pipes and valves and lack of adequate insulation.
- Reuse of broken seeds and hulls from processes in the hull removal unit.
- Reducing fuel leaks and losses.
- Treatment and reuse of refining wastewater with high organic matter content.
- Reducing oil losses in chemical processes and storage/packaging areas in the refining unit.
- Reduction of oil losses in refining due to leakage.

Implemented application: Activities realized within the scope of CP practices:

(1) Simple productivity and good business practices:
   - Preventive maintenance program (maintenance and repair of steam lines and traps, repair of leaks, broken valves, damaged water and steam pipes, etc.) and reducing steam/hot water losses and process optimization have been put into practice (*investment cost: 4500€, payback period: 6 months, annual cost saving: 9000€, annual saving: 34 tons/year*).

- ◦ The oils in the packaging unit were collected and recycled. These oils were pumped into the collection tank and recovered at the refinery for processing and thus production increased (*investment cost: 750€, payback period: <1 month, annual cost saving: 10 500€, annual saving: 1392 tons/year*).

(2) Process change:

- ◦ Fine grains from the preparation unit are reused. A unique design has been implemented for the recycling of sunflower seed parts. At this stage, the seed pieces were changed in such a way that they were quickly directed to the sections with fresh seeds that provided high yields. Seed breaking capacity has been increased by reusing the seed pieces (*investment cost: 3000€, payback period: 1 month, annual cost saving: 36 000€, annual saving: 120 tons/year*).

(3) Changing chemicals:

- ◦ Caustic soda instead of liquid caustic soda solution was used during neutralization and thus caustic soda losses were reduced. As a result of the use of liquid caustic soda, a decrease of up to 47% in neutralization costs, an increase in the quality of soapy substances, corrosion reduction in the amount of caustic soda, reduction in caustic soda losses and improvement in working conditions have been observed (*investment cost: No investment, payback period: immediately, annual cost saving: 75 000€*).

(4) Water and energy saving:

- ◦ Development of steam network, rehabilitation of steam pipes, improvement of boiler combustion settings and improvement of boiler feedback water treatment, recovery of condensed steam, replacement of defective/broken valves, replacement/repair of steam traps and pipes, improvement of hot water and steam pipes measures such as isolation. As a result of these applications, a decrease in steam consumption, removal of a boiler from the production line (saving in the use of diesel oil), reduction in water consumption and maintenance costs have been achieved (*investment cost: 9000€, payback period: <1 month, annual cost saving: 165 888€, annual saving: 3600 tons/year reduction in steam consumption, 1728 tons/year for fuel consumption savings, and 28 800 tons/year reduction in water consumption and maintenance cost*).

(5) Recovery and reuse:

- ◦ Before the project, the shells were sold as animal feed. With the application, the shells were transferred to the preparation unit where they will be processed with the help of a screw conveyor, and then the processing started and the broken seeds and seed shells were recovered. As a result of the recovery of crushed seeds, the production of oil and pulp has been increased (*investment cost: 2700€, payback period: <1 month, annual cost saving: 138 975€, annual saving: increase in oil production, 78 tons/year for oil and 595 tons/year for pulp, respectively*).
- ◦ As a result of the application of the recovery of soapy substances, the pollution load of the wastewater has been reduced (*investment cost: 1500€, payback period: 4 months, annual cost saving: 4320€, annual saving: 29 tons/year*).

(6) Treatment of wastewater:

- ◦ Process water from refining has been treated and the remaining water has been used for irrigation near the factory. As a result of waste water separation, improvement was achieved in waste water disposal (*investment cost: no investment cost, payback period: immediately, annual cost saving: 5400€, annual saving: 13 464 m^3/year*).

Benefits: As a result of low-cost or no-cost applications, the plant has achieved significant benefits. Maintenance costs decreased by 10%, water consumption decreased to 46%, wastewater treatment requirement decreased by 66%, fuel consumption of the boiler decreased to 48% and annual oil, pulp, and soapy matter valued at 207 795€ recovery has been achieved. Compliance with legal discharge limits was also ensured with the implementations.

7.5.7 Beverage facility CP practices

7.5.7.1 In New Zealand (Asia Pacific Economic cooperation (APEC), 2006)

Investigated facility is a major manufacturer of fruit and vegetable juice concentrates and other processed apple and vegetable products in New Zealand. The facility manufactures clear and cloudy apple juice concentrates, as well as pear, kiwifruit, and carrot concentrates. The facility operates all year round with high levels of production in the winter months.

The main possibilities determined for the facility within the framework of below focal points are listed below:

- Fuel consumption between 125 000 and 187 500 L/h at the facility and a borehole water with 156 900 L/hour extraction permit was detected.
- It was determined that approximately 150 000 L/year fruit concentrate loss occurred in the facility and this lost stream was not returned to the main process.

Implemented application: The clean (sustainable) production possibilities determined for the facility within the framework of these focal points are listed below:

- To reduce the amount of water drawn and consumed from the drilling well in the facility and to ensure the limit values determined by the competent authorities in resource consumption, to recover the wastewater from the final cooling towers and evaporators of the fruit juices and the use of belt filters in pasteurization and heat exchanger plates in cooling towers (*investment cost: $67 287, payback period: 19 years, annual cost saving: $3536*).
- The recovery of lost sugar-rich product was maximized by using decanters capable of capturing all the retained material to prevent 23 000 L of sugar-rich product from entering the wastewater stream to trade waste and to achieve the predicted productivity increase for 150 000 L of additive product (*investment cost: $400 000, payback period: 1.8 years, annual cost saving: $226 036*).

Benefits: As a result of all these improvements, with an investment of $467 287, an annual economic benefit of approximately $226 036 was provided to this industry, with a payback period of minimum 1.8 years (*decanter usage*) and maximum 19 years (*re-used waste bore water*).

7.5.7.2 In Turkey (TDFT (Turkey Technology Development Foundation), 2011; Alkaya et al., 2011)

CP activities were carried out in a facility with 15 000 m² closed and 2000 m² open area, which is one of the leading producers of the non-alcoholic beverage (soft drink) sector in Turkey. The facility offers different products to the soft drink market such as fruit nectar, fruit drink, carbonated drink, and 100% fruit juice. In addition, there is a milk production line in its facilities.

The fruits coming under the Fruit Concentrate Production section of the facility are washed and after pre-treatment, are pasteurized and then are converted into fruit concentrate. Groundwater is used in the cooling process during the concentrate production phase. At this stage of production, where approximately 346 000 m³ of water is used annually, there is no recycling or reuse activity conducted.

In the soft drink production industry, fruit concentrate is combined with water and other additives to turn it into a soft drink. As in the concentrate production, groundwater is used for cooling purposes during the beverage production phase and approximately 173 000 m³ of water is consumed annually at this stage. In this production line, which causes one of the most intense water consumption of the facility, any recycling or reuse of water is not performed.

Table 7.2 Annual water consumption and savings in the facility.

Process	Before CP Applications (m^3/year)	After CP Applications (m^3/year)	Annual Water Consumption Savings (%)
Fruit concentrate production			
Fruit washing	11 500	11 500	–
Cooling	519 000	18 000	96
Cleaning	72 000	36 000	50
Juice production			
Cooling	173 000	28 000	84
Cleaning	36 000	36 000	–
Carbonated drink Production	55 000	55 000	–
Process operations (steam production, pasteurization, etc. ancillary processes)	14 000	14 000	–
Other (domestic use, in the product, etc.)	180 000	180 000	–
Total	851 500	378 500	56

Source: TDFT, Turkey Technology Development Foundation (2011).

The wastewater produced by the facility, which consumes a large amount of water, is sent to the central wastewater treatment plant of the organized industrial zone (OIZ) to be treated. Especially in the summer months, due to the intensification of activities in fruit processing, water consumption, and the amount of wastewater sent to the treatment plant increases. This situation forces the existing capacity of the OSB treatment system. On the contrary, intensive water consumption is an important cost factor for the facility. For these reasons, it was necessary to take precautions in the cooling processes, which are primarily responsible for water consumption in the facility, and water saving was taken into account as the focus of CP.

Implemented application: Two separate systems were implemented for the recovery and reuse of cooling waters used in fruit concentrate and soft drink production lines. Instead of existing open cooling systems (once-through cooling), two similar closed-loop cooling systems of different capacities have been installed, consisting of cooling tower, stainless-steel water pump, stainless-steel pipes/connections, inverter and control panel units. The water consumption determined before and after the CP practices in the facility is given in Table 7.2.

7.5.8 Dairy production facility CP technology practices (Kotan & Bakan, 2007)

In this facility, which produces dairy products, the milk purchased daily from the surrounding villages is first pasteurized. The pasteurization process is carried out by heating the milk in metal tanks to a certain temperature for a while with water vapor and suddenly decreasing it to a lower temperature. The facility decided to prevent heat losses by improving the insulation in the steam line to the pasteurization tank.

Implemented application: The company is a production facility that starts the process by pasteurizing freshly brought milk on a daily basis. This pasteurization process is carried out by heating the milk with water vapor in metal tanks and then freezing it with the help of a sudden reduction in temperature when the desired temperature is reached. The heat loss experienced during heating with this steam is prevented by insulation systems.

Benefits: With the insulation renewals carried out, fuel savings of 40% were achieved. In this way, the amount of pomace burned in a year has been reduced by 87 tons. This reduction in the amount of burned pomace also reduced air pollutant emissions such as NO_x, CO, CO_2, and organic substances formed in the burning process at the same rate. As a result of all these improvements, an annual economic benefit of approximately $4000 was provided to this industry with a payback period of immediately with an investment of $100.

7.5.9 Sugar production facility clean (sustainable) production practices (Greco Initiative & Regional Activity Centre for Cleaner Production (CP/RAC), 2008)

7.5.9.1 Facility-1 in Fes, Morocco

Sugar factories operate seasonally, intensive water consumption in production is estimated to be 80 000 m^3/day. In sugar factories, water is used for processes such as washing, conveying of beets, cooling, diffusion of sugar during extraction, as well as for washing floors, tanks, and machines. Washing and transport waters constitute 50% of organic pollution and 90% of suspended solids in wastewater. 200 000 tons of solid waste is emitted annually around the factory. Due to the intense energy production in the factory, 120 000 tons of CO_2 is released into the atmosphere annually. The factory was established at the end of the 1960s. The economic aspect of consumption or wastewater discharge was not considered. By the 1980s, the company became more sensitive to environmental problems and especially to the issue of water saving. As a matter of fact, the drought that occurred in Morocco during this period caused a decrease in the supply of water difficulties and increased production costs. Faced with this problem, the facility determined the focus of CP as water saving and reducing pollution after environmental inspection.

Implemented application: The following CP practices have been implemented within the facility:

- By adding a sugar beet cleaning process to the beet loading and unloading line, some of the soil in the untreated sugar beet was separated at the initial stage, thus reducing both the amount of waste and the amount of soil going to the filter and sludge pool.
- Necessary equipment changes were made in order to separate the organic substances that affect the quality from the hydraulic transport water.
- Recycling of beet transport water at the level of washing, removing stones and washing roots has been achieved, thus reducing the overflowing water during this process.
- The water that feeds the processes inside the facility is filtered, the quality of the water is increased by lime and aluminum sulfate treatment, and its recycling is ensured.
- The recovery of hot water overflowing from the processes inside the facility and its cooling by spraying (pulverized) was ensured.
- Cooling water was recycled in the gas scrubbing section, the liquid cycle section with CO_2 pumps, and the small cooling tower section.
- A plate heat exchanger system was installed to heat the printing water starting from the hot water, thus making it possible to use the energy of the hot water. With this application, steam consumption was reduced and heat recovery was ensured by passing all hot water through the heat exchanger.

Benefits: As a result of the technological improvements realized, the pollution load of the process wastewater and the amount of water usage in the processes have decreased. In this facility with a capacity of 3000 tons/day, 60% water savings were achieved with the investments made with an investment of $204 000 with a payback period of 26 months, while wastewater generation was reduced at the same time. Thus, it also contributed to reducing the production costs of the facility and reducing its environmental impacts.

7.5.9.2 Facility-2 in Slovenia

Sugar production and processing factories include processes that intensively consume water and energy, as well as solid wastes and emission of CO_2 wastes. In this study, water reduction possibilities as a CP focus on a sugar factory in Slovenia were evaluated (Kotan & Bakan, 2007; Zver, 2005).

Benefits: With the improvements made, the use of water has been reduced to approximately 54 250 m³/h (69% of total water consumption) and in parallel, a decrease in waste discharge has been achieved, while the discharge standards have been met. As a result of all these improvements, an annual economic benefit of approximately 51 144€/day was provided to this industry with a payback period of 5 days with an investment of 2500€.

7.5.10 Metal coating and painting facility in CP practices

7.5.10.1 Facility-1 (MPM Publications, 2007)

The focus of CP in the investigated facility was the rinsing and filtrate areas of the cyanide zinc coating process.

Implemented application: The rinsing process was conducted by reducing the amount of leachate formation and other chemicals used during the rinsing and leaching of the cyanide zinc coating. This was done by reducing the amount of chromium 6 used in the current state of the rinsing baths and rinsing tanks.

Benefits: As a result of the improvements made in the rinsing process, 65% reduction in water savings per product and up to 60% reduction in the use of some chemicals has been achieved. The decrease in the use of chemicals provided a reduction in the pollution load in the process wastewater. These improvements, with an investment of 7316 TL an annual economic benefit of approximately 18 875 was provided to this industry, with a payback period 4,7 months. It was reported that an annual reduction of 3000 TL was achieved in water costs.

7.5.10.2 Facility-2 (Demirer, 2009)

Implemented application: In the facility, the surface preparation process before painting was carried out by the workers using thinner for certain parts. With the applications carried out within the scope of the project, a more environmentally friendly production system was started, which includes degreasing, rinsing, oxylan chemical and drying steps instead of cleaning and cadmium coating processes. Labor force and transportation costs were also saved with applications where significant savings were achieved in the use of chemicals such as thinner, cadmium oxide (CdO) and sodium cyanide (NaCN).

Benefits: As a result of the improvements made in facility-2 are given in Table 7.3.

Table 7.3 Improvements in metal processing industry (Facility-2) with CP.

Facility Sector	Metal Processing Industry: Metal Coating and Painting
Chemical consumption subject to the project	Thinner: 9000 kg/year cadmium oxide (CdO): 1230 kg/year sodium cyanide (NaCN): 5219 kg/year
Chemical savings	Thinner: 7650 kg/year (85%) cadmium oxide (CdO): 1230 kg/year (100%) sodium cyanide (NaCN): 5219 kg/year (100%)
Project budget	UNIDO contribution: $25 000, Facility contribution: $4500
Total investment cost	$29 500
Thinner savings	14 645 $/year
Elimination of service procurement	15 329 $/year
Labor savings	17 947 $/year
Payback period	7 months

7.6 CASE STUDY ON CP PRACTICES AT PULP AND PAPER PRODUCTION FACILITY IN TURKEY

The paper industry is the industry that produces cellulose and paper products such as paper and cardboard from plants such as wood, jute, and hemp. In recent years, the production of paper products from used paper has gained great importance. This industry covers the preparation of raw material, its conversion into cellulose, bleaching, and conversion into paper products.

In this study, CP practices realized in real scale in the processes of the facility examined were evaluated.

7.6.1 Processes in the facility

This study was carried out in all kinds of sanitary and cleaning paper production facilities with a paper production capacity of 385 837.26 tons/year. The facility consists of two main processes, the paper production section and the converting units.

Paper preparation unit; cellulose, which is a raw material and supplied from abroad, is loaded into the machine in bales in the feeding band section. According to the production plan and to meet the determined semi-product standards, the type and amount of strength, softener, and chemicals are added to the process according to their requirements. By systematically controlling the entire process, the paper is produced as a buffer in the wrapper part of the machine. Paper bobbins hung on the rubber bumper or axial bumper are directed to narrow dubbing or wide dubbing machines to be sized and wound according to their combination. The bobbins coming out of the dubbing machines are stretched several times and labeled and directed to the warehouse. In the warehouse, the coils are shipped to the coil customer according to the order, or they are forwarded to the converting department to be converted into products. The bobbins coming to the converting department are sent to the toilet paper/towel, napkin, and tissue machines in accordance with the production plan. The general flow chart of paper products production is given in Figure 7.7.

Cellulose bales are separated according to their types in the dough preparation section. Depending on the customer's demand, a mixture of long and short fibers is adjusted in accordance with the type and quality of the product to be produced. Cellulose bales are brought to the pulpers, which act as a special blade mixer, in order to open them and turn them into pulp in an aqueous medium. It is broken down with the help of white water system and chemicals that circulate in pulpers and save water. They are brought to a size that can pass through the holes under the pulpers. There are two pulp stock warehouses, long and short fibers, to be used in the production facility. It is passed through a coarse clean that cleans the foreign materials in the dough coming to the process from the dough stock tank. They are brought to a size that can pass through the holes under the pulpers. Thus, a more homogeneous and individual fibered suspension will be obtained. The homogenized fibers pass to the grinder, and the milled pulps coming out of the machine are taken to the mixing tank.

Converting unit: The coils kept in the warehouse are sent to the converting department in line with the order specifications. Coils, which are in various sizes in dubbing machines come to the converting department, and are directed to toilet paper/towel, napkin, or tissue machines in accordance with the production plan.

7.6.2 CP practices in the facility

The aim of the environmental engineers, environmental consultants and auxiliary technical personnel in the paper production facility is to develop environmentally friendly products and processes by using proactive methods, to minimize environmental impacts, and to continue production by minimizing environmental and energy losses in compliance with legal requirements.

7.6.2.1 Selection of production techniques that pollute the environment less

As a result of the use of the advantage air cap (AAC) system in the production process, the gap between the 'Hood' and 'Yankee Drying Cylinder' machines, which affect natural gas consumption,

Figure 7.7 Pulp and paper manufacturing production processes.

can be reduced from 20 to 15 mm in order to save on natural gas consumption. Since the investigated facility is a pulp and paper production facility, the cellulose used as raw material is supplied from companies obtained from sustainable forests in accordance with the Forest Stewardship Council-Forest Management Council (FSC) and Program for the Endorsement of Forest Certification – Forest certification approval program (PEFC) standards. This is documented by the ECOLabel certificate, which states that the processes taking place in the production and use stages of the products cause less harm to the environment. There is an ECOLabel certificate stating that the processes that take place in the production and usage stages of the products are less harmful to the environment. There is a system called multiplayer in the paper production machines in the facility. There are long fibre and short fibre cellulose lines in the paper machines currently used in the facility. It is advantageous over a single type line. There are four paper production machines at the facility. The fourth machine in the facility uses completely pure cellulose, the pure cellulose used is supplied from forests grown for industrial purposes. Using pure cellulose requires less energy and water use than using recycled cellulose. Production at the facility continues with the product production plans created. While making the production plan, product productions of the same quality are planned one after the other. This reduces water and energy consumption during production.

7.6.2.2 Correct selection of the treatment system and reuse of waste water in paper processing

There is a wastewater treatment plant where the industrial wastewater from the factory and the domestic wastewater generated by the employees are treated. The pulp is squeezed and filtered between the sieve and the felt in the pulp and paper production machines. This filtered water returns to the White Water System. The so-called white water (filtered from the fibers) returns to the dough production process in the system. Thus, the need for raw water required for the production of pulp is reduced.

7.6.2.3 Reducing the amount of chemical substance used

Chemicals with Ecolabel Certificate are preferred in the processes. Ecolabel Certificate is an eco-label certification system that enables products to be distinguished as environmentally friendly and sustainable. To minimize the waste of chemicals used, chemicals are fed to the process with an automation system. Thus, unnecessary chemical use is prevented.

7.6.2.4 Evaluation of waste heat and energy saving

Equipment such as machinery, pipes and valves are insulated to prevent energy losses. Uninsulated valves are a constant source of energy consumption. This energy loss has been tried to be prevented by applying jackets to the valves. The jacket application is an isolation system installed on the valves and provides energy savings. An economizer was installed in the boiler in order to benefit from the waste flue gas heat of the natural gas steam boiler. 102° of feed water coming from the deaerator passes through the economizer and is fed to the boiler at 150°. With this application, waste heat transfer was made and natural gas usage amounts were saved.

In the paper production line, after the drying process is completed with the yankee cylinder, the saturated steam leaving its heat in the cylinder leaves the cylinder as condensate (condensed steam) and low-temperature steam. The condensate separated in the condensate tanks is fed back to the steam boiler. The rotten steam is mixed with fresh steam in the thermocompressor and fed back to the cylinder. Thanks to this heat recovery, it is ensured that all of the steam heat is used and turned into condensate. The method that enables us to save heating energy is: in the steam boiler fed by using coal, the air at atmospheric temperature coming from the primary and secondary fans with the air exchanger called requperator is heated up to 200° and pressed in the boiler. In the fourth paper production machine in the facility, the air inlet–outlet flow rates, temperature values, humidity values required for drying the paper are controlled by the air balance control (ABC) system, and the system is always operated at optimum values. In this way, 30–40 kWh of energy is saved for the electricity consumed per ton of paper. While processes are carried out using fresh steam in conventional paper production systems, the exhaust air from the process will be used thanks to the ReDry System implemented. Thanks to this system, 0.4 tons of steam will be saved per ton of paper and the waste heat is reused in the process and is not given back to the atmosphere. With the waste heat recovery system, the energy of the exhaust air is utilized, while the exhaust air is passed through a washer, reducing the emission values given to the environment and the fibers escaping from the process in this air are recovered. This system is environmentally friendly in terms of emission values and energy-friendly in terms of energy recovery. With this system, approximately 5.5 MWh of energy is recovered. Another environmentally friendly technology applied is the IQ Fiber Measurement system, which does not contain a radioactive source like conventional systems and measures the weight and moisture profile without carbon emission.

7.6.2.5 Wastes

The company has a Zero Waste Certificate at the basic level; within the scope of the Zero Waste Regulation applied in Turkey, all wastes generated as a result of the process are collected separately and sent to waste disposal facilities.

7.6.2.6 Emissions

The amount of dust emission generated in the machine is reduced with the help of Advantage Run System technology in the paper production machine. It is pulverized with the help of vacuuming technology and recycled to the system in order to reduce the volatile organic carbon (VOC) and dust emissions expected to occur in the drying section. In this way, emission reduction takes place. The advantage wet dust removal system in the facility is a wet-based system. A wet dust collector is integrated in the pulper under the machine, thus providing a clean, safe, and environmentally friendly tissue paper production environment. The system prevents dust from spreading by flying. Increases work environment safety and eliminates the risk of fire and clogging.

7.7 DISCUSSION AND CONCLUSION

As a result of eco-efficiency practices, the environmental issue for the industry is no longer just 'environmental legislation pressures' and 'additional costs for environmental protection,' it has evolved into a concept that includes opportunities for minimizing production and environmental costs by increasing efficiency in production and providing both environmental and economic benefits. The development and implementation of CP strategies for an organization can be beneficial in many ways.

One of the main goals of CP is to increase process efficiency. Increasing process efficiency for a facility will affect important cost items such as reduction in energy, natural resources and raw materials, and increase the profitability of the facilities. Increasing the efficiency of the process will also lead to results such as minimizing the use of energy, natural resources and raw materials, and increasing the efficiency and profitability of a facility directly due to the decrease in product or service costs. Technology applications used within the scope of CP, all production processes, machinery, raw materials, auxiliary chemicals and dyestuffs, water and energy consumption, all kinds of waste production, occupational and worker health as a result of examining and evaluating the negative effects of the facility on the environment, while minimizing the negative impact of the facility. At the same time, it will ensure quality production at the facility and the sustainability of environmentally friendly production by providing great financial gains.

CP strategies, due to their proactive nature, will ensure that the facility fulfills its legal obligations regarding environmental issues and that any incompatibilities are eliminated. Facilities that develop and implement clean technology strategies not only will gain the advantage of being in compliance with current regulations, they will also be prepared to comply with environmental regulations and international/national standards and regulations, which will be under even greater pressure in the future. Moreover, the resulting solid/liquid/gas wastes will be reduced at their source, if possible, and compliance with the relevant environmental legislation will be facilitated. The industries, on the one hand, while they contribute to economics by producing more and also providing development, on the other, they have to find a solution to reduce the wastes resulting in their production. In other words, they have to realize development with a sustainable approach in harmony with the environment.

In this chapter, it is seen that the industries (mostly small manufacture enterprises – SMEs) examined in the literature and the paper industry, which is examined as a case study, mostly focused on energy recovery and waste water recovery as CP practices. Therefore, management skills and institutional competencies of especially SMEs should be developed and studies should be carried out to increase the ability to comply with relevant national and international standards. 'Know-how' practices including a responsible environmental management should be developed. In other words, efficiency should be increased by applying new techniques and work programs to existing ones without applying a new technology or process, and by reviewing the policies of production, processes, and organizations. CP technology practices such as the manufacturing technology or manufacturing process, changing inputs, changing the final product, using non-product materials formed during production at the production site and in the process, or improving the existing ones, should be developed on a sectoral basis. The approach in the industry in the new millennium is not only to have an obligation to create a healthy society, but also to develop sustainable production systems that minimize environmental negatives. Minimizing environmental problems is perhaps the most important social responsibility of industries. In order to fulfill these responsibilities, industries should show sensitive approaches to environmental issues in decisions regarding production management and evaluate the production function and environmental issues together.

The future is focused on CP and consumption processes. While industries investing in this field with a sustainable society understanding, should consider not only the solution of short-term problems, but also to assess the contributions in medium- and/or long term to the environment and the society in general regarding these investments.

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Author's contributions
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