Ravindra Soni
Deep Chandra Suyal
Lourdes Morales-Oyervides
Jaspal Singh Chauhan *Editors*

Current Status of Fresh Water Microbiology

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Preface

Freshwater ecosystems are dynamic natural resources, providing sources of potable water, food, animal habitats, and recreation. Perspectives of microbial dynamics in freshwater bodies provide a comprehensive and systematic analysis of microbial ecology in these ecosystems. These microbes are at the hub of biogeochemical cycles (carbon, nitrogen, phosphorus, potassium, and other elements). Moreover, they are an integral part of the aquatic food web and control the quality of freshwater bodies. Unfortunately, our freshwater microbial diversity is under threat due to several factors including overexploitation of species, increasing population, pollution, climate change, construction of dams, and introduction of exotic species. More specifically, pollution in freshwater reservoirs is a serious concern nowadays which affects the environment as well as humans. Therefore, multidirectional efforts viz. basic and advanced research, rapid and error-free data analysis, smart data compilation, and their worldwide sharing are urgently needed to identify, characterize, and conserve freshwater microbial diversity.

Nowadays, scientific knowledge is expanding very quickly due to the voluminous research, utilization of next-generation analytical tools, and higher data generation, which need to be compiled and shared effectively among the beneficiaries. From this perspective, this book is a perfect documentation of primary and secondary data-based information on the latest research findings, case studies, experiences, and innovations. This book deals with the various aspects of freshwater microbiology including diverse habitats, associated microorganisms, their ecological interactions, and applications. Moreover, it also discusses the issue of pollution in freshwater bodies and puts forward available strategies for its eco-friendly solution.

We acknowledge the suggestions and encouragement made by colleagues and well-wishers. Moreover, we are grateful to all the authors who have contributed to this book. Suggestions for the improvement of the book will be highly appreciated and incorporated in the subsequent editions.

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Bhopal, Madhya Pradesh, India — Deep Chandra Suyal
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Ravindra Soni is an assistant professor at the *Department of Agricultural Microbiology,* I.G.K.V., Raipur, CG, India. He has more than 13 years of experience in teaching and research. He is well experienced in the field of agricultural microbiology, microbial ecology, molecular biology and biotechnology, especially with reference to higher altitudes. He has published more than 100 research articles and chapters in well-reputed international journals and books. He also edited ten books with international publishers.

Deep Chandra Suyal is Assistant Professor at the Department of Science, Vidyadayini Institute of Science, Management and Technology, Bhopal, Madhya Pradesh, India. He has been actively engaged in research for 12 years and has experience in the fields of agricultural microbiology, molecular biology and biotechnology, and microbial ecology. He is currently investigating the genomics and proteomics of cold-adapted microorganisms. He has published several research articles, book chapters, and edited books with reputed international journals and publishers.

Lourdes Morales-Oyervides obtained her PhD in Engineering from University College Cork, Ireland (2015). Later on (2015–2016), she collaborated as a postdoctoral researcher at the Dairy Processing Technology Centre (Limerick, Ireland). Currently, she is a Professor at the Autonomous University of Coahuila (México) and she is recognized as a member of the National System of Researchers (SNI) from CONACyT (México) since 2016. Her research interests are focused on the design, optimization, and scale-up of sustainable bioprocesses to produce food additives, enzymes, biopolymers, and other added-value compounds.

Jaspal Singh Chauhan is a highly accomplished environmental scientist, renowned for his expertise in the field of environmental science and his significant contributions to aquatic ecology, water pollution, plastic pollution, and climate change. He earned his Doctorate in Environmental Science from GB Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India, in 2009. He currently serves as an Assistant Professor and Head of the Department of Himalayan Aquatic Biodiversity at Hemvati Nandan Garhwal University, a prestigious central university in Uttarakhand. Dr. Chauhan possesses an impressive 15 years of teaching and research experience in various aspects of the environment. He has authored approximately 25 research articles, which have been published in reputed national and international journals and books. His dedication to sharing his findings and expertise has led him to actively participate in numerous international and national seminars and conferences, where he has presented his research work to fellow experts in the field. In addition to his academic accomplishments, Dr. Chauhan is deeply committed to raising awareness about environmental protection among the general public. He has played a pivotal role in organizing several impactful programs, such as Save Ganga, Save Water Springs, Save Himalaya, and Plastic-Free Day. Recognizing his outstanding contributions to the field of environmental science, Dr. Chauhan was honored with the prestigious "Environmentalist of the Year" Award in 2021 by the National Environmental Science Academy.

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Freshwater Microbiology: Recent Updates and Prospects

1

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Abstract

Along with bacteria, other microorganisms are also key components of microbiota structure of freshwater, and those include archaea, microbial eukaryotes, and viruses that together build the diverse community of microorganisms. All those microorganisms play key ecological functions and can have important impacts on animal, plant, and human health. The traditional culture-based microbiology methods have been mostly replaced, or complemented, by molecular methods, due to their accuracy, fast response, and capability to detect microorganisms that are quite difficult to grow in culture media. This chapter addresses some of the current tendencies on microbial structure and its analysis. Those methods included the "omic-based" DNA studies that allow better and more comprehensive characterization of bacterial communities, leading to metagenomic studies that help to elucidate the population structure of microbiomes in aquatic environments. Strategies for detecting water-borne pathogens are focused on detecting bacteria, protozoa, yeast, and

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virus in different water sources, and these methods can also be complemented by molecular tools such as molecular marker (RAPD, Microsatellites, RFLPs, and AFLPs). Those methods also allow to detect pathogenic bacteria, fungus, and viruses of public health interest, such as the enterovirus and, more recently, the coronavirus. Water bodies can also be important reservoirs of antibiotic-resistant bacteria, and the group of ESKAPE bacteria represents an important study model for antibiotic resistance and its distribution. The acronym "ESKAPE" is associated with this group due to their ability to escape antimicrobial activity and develop high levels of resistance to multiple antibiotics (multidrug resistance). Water bodies play a central role on the selective pressure and is the most important cause of the dissemination and extension of resistance and has contributed to the genetic diversification of resistance genes also affecting ecological microbial structures.

Keywords

Microbiology · Molecular markers · Metagenomics · ESKAPE · Multi-drug resistance · Microbial ecology

1.1 Introduction

The biological role of freshwater microorganisms in the carbon cycle and its association with the uptake and emission of greenhouse gases is well documented as well as the impact that disturbance of microbial communities as a consequence of anthropogenic activities have on the climate and global change (Regnier et al. 2013; Premke et al. 2022).

Bacteria are prokaryote, unicellular, taxonomically diverse, and ubiquitous microorganisms. They are the most abundant organisms on earth and their habitats include virtually every ecosystem on the planet, even extreme aquatic environments such as hot springs and ice layers in the Arctic and Antarctic regions, under high pressures or extreme acidic environments (Takai et al. 2008; Dalmasso et al. 2016; Merino et al. 2019). The adaptation capabilities of bacteria to adverse environments explain their abundance within the different ecosystems on the earth; it is estimated that the average number of bacterial cells per gram of soil is around 40 million, whereas 1 million diverse bacterial cells can be found in 1 mL of environmental freshwater and it is even higher in estuaries and open ocean waters (Whitman et al. 1998; Anas et al. 2021).

In aquatic environments, the cross-feeding and metabolite interchange represent an important class of interactions, and thus, photosynthetically fixed dissolved carbon could produce chemotactic responses (Seymour et al. 2010). Early experimental evidence suggested the existence of strong positive and negative interactions among the photoautotroph and heterotroph microorganisms (Cole 1982). Besides bacteria, there are various microorganisms in fresh water, such as fungi, algae, and viruses, although they are not microorganisms. Their importance will be addressed in this chapter. The traditional culture-based microbiology methods have been mostly replaced, or complemented, by molecular methods, due to their accuracy and fast response. Here, some of the current tendencies on microbial structure and its analysis will be addressed.

1.2 Microbiome and DNA-Based Omic Studies

As we move forward to a better understanding at microorganic system level, complexity increases as the number of microorganisms interacting do. For this reason, it is important to develop pipelines to accurately characterize the diversity of bacteria and other microorganisms in specific niches and elucidate their role within the community and the ecosystem. Water is an essential natural resource for all living organisms, and the pollution consequence of human activities has a great impact ranging from environmental effects (sometime negative), animal and human health impact to important economic losses in food production. The causes of contamination of rivers, lakes, and ponds are diverse. Still, contamination with untreated or poorly treated sewage and residual waters containing heavy metals and other xenobiotics from industry, agriculture, and livestock production are among the main drivers that serve as disturbers of native bacteria communities. In the first case, sewage serves as an important source of enteric bacteria and viruses, whereas industrial residual waters modify the physical and chemical properties of the water itself, and these changes impact the bacterial population structure affecting the carbon flux between freshwater bodies and the environment among other important aspects (Schreiber et al. 2015).

Like other ecosystems, in freshwater bodies, bacteria and other microorganisms live in a complex multispecies structure. Begon et al. (1986) defined the microbial communities as the set of microorganisms coexisting in the same space and time. However, the characterization of bacterial communities was limited to microorganisms grown in culture media, and their study was focused mainly on morphological and biochemical profiles. In 1977, Woese and Fox proposed the 16S ribosomal RNA genes as a potential marker to establish phylogenetic relationships between bacteria, and it was only after the development of Sanger sequencing technology that the 16S ribosomal RNA genes analyses was used as the benchmark to fine-tune the classification system of microorganisms.

Later in 1998, Handelsman et al. proposed the term *metagenomics* in their research, which involved the isolation of total DNA from soil bacteria without previous culture on artificial media, and their cloning using BAC vectors in order to transform competent *E. coli* cells and then sequence the genomes and express genes detected, allowing a deep understanding of the bacterial population structures. The arrival of high-throughput next-generation sequencing (NGS) techniques in 2005 with the pyrosequencing method from Roche was a milestone in the microbial communities' characterization because of its relative low cost and reliable methodology to rapid sequencing of short fragments of DNA. However, this platform is discontinued today by more efficient sequencing strategies such as synthesis sequencing, initially proposed from ion torrent, followed and enhanced then by Illumina and PacBio, all collectively called next-generation sequencing (NGS), that now represents the most accurate and used technique to characterize the metagenome of environmental samples at a different resolution of taxonomic levels; these sequencing technologies allow the rapid detection of perturbations in populations of native bacteria in freshwater deposits under different conditions that can be associated to ecological phenomena (Escobar-Zepeda et al. 2015). In the past few years, through NGS techniques, researchers worldwide have been able to elucidate the population structure of microbiomes in aquatic environments under different and extreme conditions (Table 1.1).

To this day, metagenomic characterization is mostly descriptive and without a careful approach design it does not provide insights into genes of interest or metabolic integration pathways in the analyzed environments. However, a comprehensive approach integrating every member of the microbial community transcriptome (metatranscriptomics) studies and the total set of proteins (metaproteomics) could improve the insight into the role played by every microbial member at an ecosystem level establishing accurate relationships between taxonomy and functionality profiles within microorganisms.

1.3 Current Strategies for Detection of Water-Borne Pathogens

1.3.1 Incidence of Bacterial Pathogens in Water

Water bodies, such as rivers, lakes, irrigation channels, damps, and hospital and industrial wastewaters, harbor a wide variety of microorganisms. The microorganisms commonly found in wastewater are enteric anaerobic bacteria, some yeasts and fungi, and some protists especially those resistant to harsh environments. Regarding the bacteria present in water bodies, it has been estimated that 90% of the genera and species of bacteria characterized belong to 4 phyla: Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes. Some of the bacteria can be defined as pathogens such as *Escherichia coli* (Rice and Johnson 2000), *Pseudomonas* spp. (Pirnay et al. 2005), and *Stenotrophomonas maltophilia* (Brooke 2012). These bacteria, along with the emergence of antibiotic-resistant bacteria and its wide distribution in freshwater, is a concerning matter that will be addressed further in this chapter with the ESKAPE group.

1.3.2 Incidence of Yeast and Filamentous Fungi Pathogens in Water

The presence of these microorganisms in water has important ecological roles in organic matter transformation, and some have an additional impact, because these microorganisms can be pathogenic or display pathogenic properties and well as

Table 1.1 Metagenomic analyses from selected freshwater vessels under diverse physicochemical conditions

Water body	Location	Water samples	Metagenome composition	Conditions	Sequencing platform
Lake Baikal[a]	Siberia, Russia	2, from 5 and 20 m depth	Verrucomicrobia Actinobacteria Proteobacteria Acidobacteria Bacteroidetes Pseudomonadota	High oxygen levels.	Illumina HiSeq
Fetsui reservoir[b]	Taiwan	Samples taken from 5 m depth	Bacteroidetes Actinobacteria Cyanobacteria Flavobacteria Sphingobacteria Alphaproteobacteria	Evidence showed that typhoons disrupt the thermal stratification of the water column and change dissolved oxygen level by delivering oxygen-rich water to the reservoir.	454 Roche
Lake Tanganyka[c]	DRC, Tanzania, Burundi, Zambia	Tanzanian samples from surface to 1100 m depth	Bacteroidetes Ignavibacteria Betaproteobacteria Gammaproteobacteria Alphaproteobacteria Verrucomicrobia Planctomycetes Chloroflexi Actinobacteria [c]Tanganyka bacteria Cyanobacteria	Anoxic water, stratified. Metagenomes were compared from rich oxygen surface to dark, nonoxygen, nutrient-rich depth.	Illumina HiSeq
Lake Tai[d]	China	10 cm depth	Proteobacteria Firmicutes Actinobacteria Bacteroides Acidobacteria	Water body heavily contaminated with population residual water.	Illumina HiSeq
El chichon[e]	Mexico	Water column and sediment samples	Found at sediment 50 °C: Actinobacteria Proteobacteria Acidobacteria Found at sediment 92 °C	Crater lake of an active volcano, formed in 1982. Geothermal activity	Illumina NextSeq

(continued)

Table 1.1 (continued)

Water body	Location	Water samples	Metagenome composition	Conditions	Sequencing platform
			Firmicutes (*Alcylobacillus* and *Sulfobacillus*) Proteobacteria (*Bradtrhizobium*, *Methylobacterium*, *Sediminibacterium*)	including acidification, presence of sulfur compounds and metals, extreme water temperature.	
Lake Untersee[f]	Antarctica	80 m depth	Proteobacteria Bacteroidetes	High concentration of dissolved oxygen. High pH 9.8–12.1.	Illumina HiSeq
Lonar Lake[g]	India	Surface water samples	Proteobacteria Firmicutes Bacteroidetes	Hyperalkaline and hypersaline basin.	Illumina HiSeq
Lake Michigan[h]	U.S.A.	Samples from river, river mouth, near-shore, and offshore, at 45 cm depth	Proteobacteria Actinobacteria Bacteroidetes	Bacterial contamination originated from the Grand Calumet River.	Illumina

[a] Cabello-Yeves et al. (2017)
[b] Tseng et al. (2013)
[c] Tran et al. (2021)
[d] Chen et al. (2019)
[e] Peña-Ocaña et al. (2022)
[f] Koo et al. (2018)
[g] Chakraborty et al. (2020)
[h] Nakatsu et al. (2019)

antifungal resistance (Monapathi et al. 2020). Some species of yeast that have been identified in water bodies include *Candida*, *Clavispora*, *Cyberlindnera*, *Cryptococcus*, *Hanseniaspora,* and *Yarrowia.* Yeast detected in cities water distribution systems and tap water include *Candida*, *Clavispora*, *Cryptococcus*, *Debaromyces*, *Meyerozyma*, and *Pichia*. Yeast isolates identified in sewage treatment plants are *Candida*, *Cryptococcus*, *Debaryomyces*, and *Wickerhamonyces* (Monapathi et al. 2020). Yeasts isolated from different water samples recognized as pathogenic include *Candida* spp. *Cyberlindnera*, and *Cryptococcus*. Fungal infections caused by *Candida albicans* and other *Candida* spp. are important complications in immunosuppressed patients, since frequently, candidiasis in mucosal tissues (oral, gastrointestinal, and vaginal) represent an early sign of immune system malfunction

(Panizo and Reviákina 2001). *Cryptococcus neoformans* is an encapsulated yeast, and the cause of opportunistic infections, such as meningoencephalitis in immunocompromised patients (Pini et al. 2017).

Among filamentous fungi, some of the complications caused by some virulent species belonging to *Aspergillus, Fusarium,* and *Alternaria* may range from allergic to invasive syndromes. Immunocompetence facilitates clearance by initiating innate and adaptive host responses despite constant spore inhalation (Chotirmall et al. 2014). Regarding *Aspergillus* section Flavi includes 22 species, some of them with potentially pathogenic such as *Aspergillus felis*, *A. fischeri*, *A. fumigatiaffinis*, *A. fumisynnematus*, *A. hiratsukae, A. laciniosus*, *A. lentulus*, *A. novofumigatus*, *A. parafelis*, *A. pseudofelis*, *A. pseudoviridinutans*, *A. spinosus*, *A. thermomutatus*, and *A. udagawae*. The pathogenic species can display a wide range of differences in the frequency of production of toxins or toxic compounds (Tamayo-Ordóñez et al. 2021). Even though fewer pathogenic yeasts and molds have been reported in different water species compared to bacterial genera, their importance stands out, since their contact with humans with a compromised immune system could lead to serious complications and even cause death.

1.3.3 Identifying Virus in Water Samples

In raw wastewater, viruses may be ubiquitous and persistent and even after treatment, some viruses continue being distributed in treated wastewater that later arrive at water bodies. Adenovirus (HAdV), rotavirus (RoV), hepatitis A virus (HAV), and other enteric viruses, such as noroviruses (NoV), coxsackievirus, echovirus, reovirus, and astrovirus, are commonly detected in wastewater as well as some superficial water (rivers and irrigation channels) and are the principal human viral pathogens transmissible via water exposition (Corpuz et al. 2020). More dangerous viruses such as poliovirus can also be present in wastewaters. Enterovirus causing waterborne diseases such as diarrhea in children and adults are often associated with viral disease outbreaks, leading to several clinical manifestations (nausea, vomiting, and fever) and other gastrointestinal diseases, and some enteric viruses have also been related to respiratory, central nervous system (poliovirus), and other diseases (Corpuz et al. 2020).

Recently, a novel Coronavirus disease 2019 (COVID-19), which began in December 2019, that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Guarner 2020) was reported. Since this virus can invade the gastrointestinal mucosa, it can be released in feces, and the presence of nucleic acid of SARS-CoV-2 has been reported in raw wastewater (Randazzo et al. 2020), sewage samples collected from hospitals (Wang et al. 2020b), and wastewater sample after secondary treatment (Randazzo et al. 2020). However, infection caused by exposition to water is not been documented, and its detection functions as an early warning system to indirectly detect SARS-Cov-2 infections in the population.

1.4 Molecular Markers for Genotyping and Identification of Bacteria in Water Bodies

1.4.1 Intraspecific and Interspecific Variability and Genotyping

Molecular markers are DNA fragments sequences associated with a physiological trait, and are used to identify a particular DNA sequence related to a function or a trait of interest (Langridge and Chalmers 2004). Different types of markers are distinguished by their ability to detect polymorphisms at single or multiple loci, or sequence patterns and can be of dominant or codominant type. Below we describe some of the molecular markers that have been most used to determine intra- and interspecific variability in bacterial genera isolated from different bodies of water.

RAPDs RAPDs (randomly amplified DNA polymorphisms) are markers that randomly amplify segments of DNA in a wide variety of species. RAPDs are based on the statistical probability of complementary sites to the oligonucleotide of ten base pairs (bp) throughout the genome. The polymorphism of the bands between individuals is due to changes in the sequence of nucleotides at oligonucleotide coupling sites and by inserting or deleting fragments at these sites. These markers are dominant; they cannot discern the dominant homozygous or heterozygous for a particular segment, so allelic frequencies must be estimated indirectly, assuming Hardy Weinberg equilibrium. Among some of the applications of these molecular markers in bacteria, it has successfully demonstrated different RAPD profiles in multidrug-resistant coliform bacteria isolated from sewage samples of Ghaziabad city, India (Raj 2012). Since RAPD-derived markers include a genome-wide analysis, they can be a very good source of physiological markers that can be obtained even if little is known about the sequence of the microorganism being studied. Their application could be considered one of the first "genomic-wide" analysis performed before the massive sequencing technology was available; however, several problems with reproducibility limited its applications out of basic research.

Microsatellites Simple repeat sequences (SSRs) or ISBPs (Insertion Site-Based Polymorphisms) are DNA sequences made up of 1–4 base pairs (mononucleotides (TT)n, dinucleotides (AT)n, or tetranucleotides (AAGG)n). These loci are found both in coding and noncoding regions of DNA, formed by breakage events that generate polymorphisms with values greater than 90%. Challagundla et al. (2018) analyzed 598 genome sequences of *Staphylococcus aureus*; this study showed that depending on the geographical area of the isolate, certain mutations in the CC5-MRSA marker are present allowing a deeply analysis of the epidemiology of these strains. A particular important application of simple repeat sequences was on *M. tuberculosis* research, because this allowed the design of a classification system of *M. tuberculosis* strains, based on a VNTR (variable number of tandem repeats) that was so specific to *M. tuberculosis* that later these were called mycobacterial interspersed repetitive units or MIRUs, allowing a great advance on mycobacterial isolates classifications, both from clinical and environmental sources. Dangerous strains such as the Beijing lineage were spotted with these markers and later with complementary genomic analyses on Beijing strains obtained from different clinical samples, waters, and substrates: this allowed us to know its biogeographic structure and evolutionary history of the Beijing lineage worldwide through the SNPs analysis of 4987 isolates from 99 countries (Merker et al. 2015).

RFLPs This method allows the detection of specific DNA differences recognized by restriction enzymes. Each of the endonucleases (of bacterial origin) recognizes and cuts only a specific sequence in DNA, as long as they are not protected (methylated). Therefore, any DNA that is not methylated can be recognized and cut into fragments of defined length; any mutation within those sites could change the pattern of the fragment and allow an RFLP to be detected when comparing two or more genomes. Originally, RFLP were important tools to detect and related mutations and molecular marker to key physiological functions and to design the initial molecular diagnosis strategies, since sequencing was required only to confirm the design but not to apply the technique and had very good reproducibility results. RFLPs are still currently applied to understand bacterial community dynamics in wastewater treatment systems. Wang et al. (2010) investigated how bacterial communities change in treatment plants over a year using specific PCR followed by terminal restriction fragment length polymorphism (T-RFLP) of the 16S rRNA gene. The T-RFLP results indicated lack of stability in the bacterial community structures in 2 full-scale wastewater treatment systems, with 15 days average change rates observed in the 2 systems. On the other hand, through digestion of the 16S rRNA gene with the endonuclease *Mse* I, it was shown that distinctive patterns for *Acrobacter* species are observed, indicating variation in the population structure of the genera (Figueras et al. 2012).

AFLPs AFLPs (amplified fragment length polymorphisms) is a technique that combines the digestion of two restriction enzymes, generally MseI and EcoRI, within a sequence, involving a selective amplification of restriction fragment lengths from a genome using PCR, and followed by gel electrophoresis to separate the amplified fragments and obtain a banding pattern, which can be used to identify genetic differences between individuals or populations, and can also be used for genetic mapping and phylogenetic analysis (Tamayo-Ordoñez et al. 2012).

This technique has been used to determine the biodiversity of the bacterium *Pseudomonas aeruginosa* in an aquatic environment in a study including 100 isolates of *P. aeruginosa*, where the *oprL* gene was analyzed, and a DNA-based fingerprint (AFLP) indicated a positive relationship between pollution and prevalence of *P. aeruginosa*. In the Woluwe River, *P. aeruginosa* community was almost as diverse as the global *P. aeruginosa* population. These findings illustrate the significance of river water as a stable reservoir and source for the distribution of potentially pathogenic *P. aeruginosa* strains (Pirnay et al. 2005).

In another recent study, antimicrobial tolerance to oxytetracycline and taxonomic diversity relationship in the culturable oxytetracycline-resistant (Otr) isolates of heterotrophic bacteria in two Belgian aquatic sites receiving wastewater was investigated. Profiles for ampicillin and kanamicin tolerance were detected from the taxonomic differences in the Otr bacteria detected at genera and subgenera level. In addition, *Enterobacter* sp., *Stenotrophomonas maltophilia*, and *A. veronii* resulted as potential indicator organisms to help to assess microbial tolerance in various compartments of the aquatic environment (Huys et al. 2001).

1.4.2 Identification of Species Through Sequencing of Molecular Markers

To date, depending on the purpose of each investigation, different genetic regions can be used to identify species from various samples. Among the most explored genes used for species identification are the 16S rRNA, 23S rRNA, *rpo*B, *gyr*B, *dna*K, *dsr*AB, *amo*A, *amo*B, *mip*, *hor*A, *hit*A, *rec*A, *ica*, *frc*, *oxc*, 16S–23S rDNA ISR, and IS256.

Amplification and sequencing of 16S (V3–V4 of 16S rRNA) is commonly applied to study microbial communities with third-generation sequencers (MinION from Oxford Nanopore Technologies) and this made it possible to analyze the full length of the 16S rRNA gene, and this allows a deeper identification even to species level identification and at relative low cost. The analysis of nine indigenous bacteria that can be related to food poisoning and act as opportunistic infections was carried out with due diligence. *Enterococcus faecalis* and *Enterococcus hirae* were identified at the species level with an accuracy of 96.4–97.5%. Also, using these technologies, it is possible to evaluate the antibiotic sensitivities of multiple bacteria simultaneously. Kawai et al. (2022), using these technologies, allowed rapid evaluation of antibiotic activity spectrum at the species level containing a wide variety of bacteria, such as biofilm bacteria and gut microbiota. Even though the sequencing of these genes is a powerful tool to identify bacteria, it has some limitations when it comes to related bacterial complexes that can be difficult to distinguish.

Since the sequencing and analysis of 16S rDNA ribosomal regions is the most used molecular marker, we decided to find out the abundance of bacteria identified in bodies of water and of these, which accessions have been reported in the Gen Bank from NCBI. It was possible to identify 103 sequences of 16S rDNA regions of bacteria present in different bodies of water (Fig. 1.1). After analysis, a total of 58 bacterial genera were identified.

Conclusively, we want to emphasize that the use of molecular markers allows to identify and genotype a wide variety of microorganisms from different water samples, highlighting the importance of molecular markers in the clinical and environmental areas.

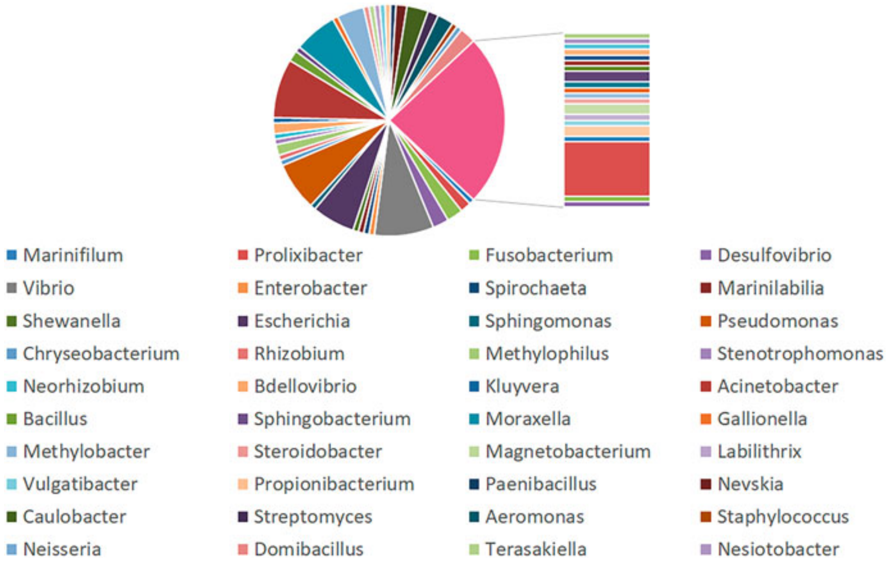

Fig. 1.1 Representation of the bacterial species identified in water bodies. The bacterial species were identified by searching in the sequences reported in the NCBI

1.4.3 Methods of Detection of Viruses in Waters

Viruses are important components of aquatic systems, since they are heavy regulators of bacteria populations, and some of them can be related to plants, animal, and human diseases, so their detection is crucial as indicated in numerous studies, including different water matrices such as surface water, treated wastewater, and irrigation water (Wang et al. 2020a; Ji et al. 2020; Rusiñol et al. 2020). Several methods to directly and indirectly detect and quantify viruses found in wastewater include epifluorescence microscopy, transmission electronic microscopy, pulsed-field gel electrophoresis, immunofluorescence assay, flow cytometry, traditional cell-culture, and molecular detection (Corpuz et al. 2020). Among the molecular techniques, PCR and especially real-time PCR are currently used.

PCR is an in vitro enzymatic reaction that amplifies or generates millions of copies of a specific DNA sequence during several repeated cycles in which the target sequence is faithfully copied. This technique uses thermostable DNA polymerase to replicate DNA strands, for which alternate high and low temperature cycles are used to separate the newly formed DNA strands after each replication phase. Among the components used in this in vitro reaction are amplification template (DNA or complementary DNA, or RNA in the case of viruses), oligonucleotides, DNA polymerase (Taq polymerase), polymerase cofactor ($MgCl_2$), PCR buffer, deoxyribonucleotide triphosphates, water, and equipment (thermocycler). Since viruses have DNA or RNA genomes, PCR is an important tool for detecting them in water samples. For the detection of RNA genome viruses, it is necessary to carry out an RT-PCR (Reverse Transcription-Polymerase Chain Reaction) that is a derived technique with a step where a RNA complementary DNA is generated that can be used directly in the PCR (Kadri 2020). An important derivative technique for virus study is the Real-time PCR or quantitative PCR that is a method that allows us to monitor the evolution (simultaneous amplification and quantification) of the PCR while it is being carried out. Cycle-by-cycle analysis of the changes in the accumulation of the PCR product, detected by a change in the fluorescent signal generated in the three steps of the PCR, is done with this technique and allows two types of determinations: the quantification absolute and quantitative comparison between samples. The quantification absolute determines the exact number of DNA or RNA molecules present in each sample. Applying this quantification method requires generating a standard curve and using the comparative Ct method. The other type of determination is the quantitative comparison of target nucleic acid, expressed in orders of magnitude concerning a calibrator.

Variants have been derived from both techniques, such as Multiplex PCR and ICC-PCR (integrated-cell culture PCR) (Corpuz et al. 2020). The application of multiplex PCR allows the simultaneous detection of multiple viruses present in a single sample using more than one set of primers in one reaction. Multiplex qPCR also enables the detection of different specific viruses using more than one fluorescent reporter. In the ICC-PCR method, the samples in which the viruses may be present are used to infect cell culture media and incubated for several days. Then the samples of cell culture are subjected to freezing to cause lysis of the cells. If viable viruses were present in the original sample and invaded the culture cells, those are from their host cells when lysis occurs. After this, PCR-based amplification is applied to the lysate (Corpuz et al. 2020), so the ICC-PCR is an effective enrichment method for virus detection.

1.4.3.1 Detection of Coronaviruses in Samples of Waters by Molecular Methods

There are extensive reviews in the literature describing the persistence of coronavirus in different types of wastewaters. The integrity of the coronavirus particle in water depends upon the wastewater characteristics, presence of suspended solids, organic matter, and temperature (Mandal et al. 2020). The methods of early detection of the virus on waters help to detect future immediate outbreaks, allow to implement measures to minimize the infections and deaths caused by COVID-19, and new molecular techniques are currently being developed that allow the effective detection of other viruses relevant for public health such as adenovirus and poliovirus,

1.5 ESKAPE Bacteria as a Model of Antibiotic-Resistant Bacteria Distribution in Water

1.5.1 Antibiotics and Drug Resistance

The use of antibiotic-producing microbes to prevent disease goes to back more than 2000 years ago from the use of moldy bread to treat open wounds (Hutchings et al. 2019). Still, it was not until the twentieth century that the microbiologist Alexander Fleming discovered the origin of Penicillin in 1928, a substance produced by mold (*Penicillum notatum*), that was lethal to several bacteria giving rise to the birth of the antibiotic era (Patel 2016), but also to the next milestone in history "antibiotic resistance" statement made by Alexander Fleming during his Nobel Prize acceptance speech (1945) (Podolsky 2018) predicting the extent and severity of antibiotic resistance over the next 10 years, statement that then became a reality, with hundreds of cases of resistance recorded throughout the world (Rosales Magallanes 2018).

Currently, antimicrobial resistance is one of the greatest threats to global health (Maillard et al. 2020). The widespread and inappropriate use of antibiotics has led to an increased prevalence of multidrug-resistant (MDR) bacteria in the environment. MDR infections led to 700,000 deaths globally in 2016 and are expected to rise to10 million by 2050 (Brooks et al. 2018). The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeroginosa,* and *Enterobacter* species) (Tigabu and Getaneh 2021) are a group of common opportunistic pathogens associated mainly with nosocomial infections (Santaniello et al. 2020), the acronym "ESKAPE" is associated to this group due to their ability to escape the antimicrobial activity and development of resistance to multiple antibiotics (multidrug-resistance) (Founou et al. 2018). The World Health Organization (WHO) listed in the critical priority list carbapenem-resistant *A. baumannii, P. aeruginosa, K. pneumoniae*, and *Enterobacter* spp., whereas vancomycin-resistant *E. faecium* (VRE) and methicillin/vancomycin-resistant *S. aureus* (MRSA or VRSA) in the list of high priority group (Mulani et al. 2019). The presence of the ESKAPE pathogens in the environment is likely due to contamination via sewage spills, hospital waste that has been discarded incorrectly; consequently, several antibiotic-resistant microorganisms and antibiotic-resistance genes (ARGs) have been detected in water bodies (Denissen et al. 2022). The water sources represent a reservoir of various chemical and microbiological pollutants such as chemical, heavy metal, organic pollutants, residues of pharmaceuticals (Voigt et al. 2020), including horizontal gene transfer of resistance genes, the misuse or overuse of antibiotics, and the contamination through livestock slurry and plant wastewater (Daniel et al. 2017) are factors that contribute to genetic selection pressure for the development of MDR bacterial infections in the community (Wang et al. 2018).

1.5.2 *Enterococcus*

Enterococci is a Gram-positive bacteria that commonly resides in the intestinal tracts of humans and animals but is also found in water, soil, and plants due to their high tolerance to different conditions (Cho et al. 2020), they can survive in a variety of environments, such as soil, water, food, plants, and animals (Castillo-Rojas et al. 2013), and they can survive in a wide range of temperatures (10–45 °C), pH (4.4–9.6), and in a hypersalty media with 6.5% NaCl (Ben Braïek and Smaoui 2019). Nowadays, *Enterococcus* is the leading cause of nosocomial infections, responsible for causing endocarditis, urinary tract infections, and bloodstream infections (Raza et al. 2018). There are more than 50 species of enterococci where *E. faecium* and *E. faecalis* are the most ecology and epidemiological relevant (Ramos et al. 2020). Furthermore, enterococci have intrinsic antimicrobial resistance to aminoglycosides, and cephalosporins antibiotic agents and can adapt to acquire resistance to antimicrobials such as β-lactams and vancomycin from the environment (Lee et al. 2019), by mutation and/or acquisition of genes through the plasmids and transposons (Grassotti et al. 2018). Vancomycin is an antibiotic of last resort used to treat MRSA. The vancomycin-resistance among clinical isolates is a growing health problem that involves the exchange of *van* genes between strains; where *vanA* and *vanB* are the most prevalent genotypes in *E. faecium* in clinical isolates (Melese et al. 2020), where the *vanA* gene encodes an enzyme that modifies the vancomycin binding site through the substitution of D-ala-D-ala by D-ala-D-lactate (Liu et al. 2021). Water ecosystems, in particular wastewater treatment plants, are considered one of the main hotspots of the evolution and spread of antibiotic-resistant bacteria (ARB) and ARGs into the natural environment that leads easily reach communities via aquatic environments (Ekwanzala et al. 2020). Enterococci and antimicrobials are commonly excreted in urine and feces, and most of this waste is treated in wastewater treatment plants where *E. faecalis* and *E. faecium* are dominant species identified before being discharged into surface water mainly during the dry season (Sanderson et al. 2020). *E. faecalis* and *E. faecium* are also used as an indicator of water quality criteria by fecal contamination in freshwater in the United States of America at geometric means of 33 and 35 CFU/100 mL (Wen et al. 2020) due to they are more resistant to stress than *E. coli* and other fecal coliform bacteria (Chidamba and Korsten 2018). Nevertheless, the distribution of resistant bacteria from the environment to humans may occur through contaminated food, manure, and contaminated surface water used for irrigation (Taučer-Kapteijn et al. 2016).

1.5.3 *Staphylococcus*

Staphylococcus aureus is a commensal opportunistic Gram-positive bacterium in humans (Pasachova et al. 2019) and are able to survive at a wide range of temperatures, dryness, dehydration, and low water activity (Silva et al. 2020). The genus *Staphylococcus* comprises more than 50 species, including coagulase-positive (*S. aureus*) and coagulase-negative staphylococci (*E. epidermidis*) (Kosecka-Strojek et al. 2019). *S. aureus* is one of the main pathogens in hospital and community infections associated with skin infections, pneumonia, and nosocomial bacteremia and represents public problem health (Cheung et al. 2021). The staphylococci became antibiotic resistant through genetic mutations, reducing outer membrane proteins, and acquiring resistance genes through HGT (Guo et al. 2020). The methicillin resistance in *S. aureus* (MRSA) is a significant global health concern determined by the expression of *mecA* gene, which encodes a penicillin-binding protein (PBP2a) with low affinity for β-lactam agents (Watkins et al. 2019). MRSA strains show a multidrug-resistant pattern with resistance to fluoroquinolones, macrolides, aminoglycosides, clindamycin, penicillins, cephalosporins, and carbapenem, making β-lactams ineffective (Gajdács 2019). MRSA is known as the major cause of hospital-/community-acquired infections, and morbidity infections are elevated worldwide. On the other hand, vancomycin has been one of the first-line drugs to treat MRSA infections; nowadays the intermediate and complete vancomycin-resistant *S. aureus* (VRSA) is a severe public health concern (Cong et al. 2020).

Environmental factors such as pH, temperature, nutrient content, salinity, and dissolved oxygen play important roles in biofilm development, influencing their persistence in water, especially in piping due to inefficient water treatment (Silva et al. 2022). Despite this, wastewater treatment reduces the prevalence of *S. aureus*, thus reducing its presence in surface water, a fact attributable to the fact that the effluents are diluted in the river water (Zieliński et al. 2020). Although Staphylococci are less frequent in surface water than *E. coli, Enterococcus,* and other ESKAPE pathogens, they are indicators of antimicrobial resistance in the environment mainly by the presence of *mecC* gen associated with *S. aureus* (Silva et al. 2021).

1.5.4 *Klebsiella pneumoniae*

Klebsiella spp. are Gram-negative and nonmotile bacteria resident in the environment (surface water, sewage, soil, and plants), and on mucosal surfaces such as humans and animals (Bengoechea and Sa Pessoa 2019). In humans, *K. pneumoniae* is a commensal and opportunistic pathogen found in gastrointestinal and respiratory tracts and skin of healthy people, and is responsible for causing community-acquired and nosocomial infections (urinary, respiratory tract infections, and infections of wounds and soft tissue) (Herridge et al. 2020). In contrast to other nosocomial pathogens, *K. pneumoniae* clones are divided into two phenotypic groups characterized by multidrug resistance and hypervirulence (Gonzalez-Ferrer et al. 2021). *K. pneumoniae* shows high resistance to a broad spectrum of antibiotics such as ESBL, carbapenem, quinolones, polymyxin, tigecycline, fluoroquinolones, and aminoglycosides. Recently, the WHO recognized extended-spectrum β-lactam (ESBL) producing and carbapenem-resistant *K. pneumoniae* as a critical public health threat (Wyres et al. 2020).

β-lactam antibiotics are the first-line option for the treatment of infections, but ESBL enzymes hydrolyze β-lactams (penicillins, monobactams, carbapenems, and cephalosporins) (Pillai 2022); on the other hand, the carbapenems are β-lactam antibiotics of last resort with the broadest spectrum of activity, that, in less than a decade after their use, become a global public health crisis (Cherak et al. 2021), mainly because infections caused by the ESBL/carbapenemase-producing *Enterobacteriaceae* are associated with increased mortality, length stay, and health-care costs (De angelis et al. 2020).

The environment is an important reservoir for disseminating ARB, antibiotic-resistance genes, and mobile genetic elements that interact and spread to other parts or to human and animal hosts (Samreen et al. 2021). Carbapenemase and ESBL-producing *K. pneumoniae* have been reported in aquatic environments, such as rivers, hospital effluents, and posthospital wastewater (Furlan et al. 2020). Some human health concerns associated with antibiotic resistance in the environment are the alteration of the human microbiome toward the emergence of antibiotic resistance and the potential hazard of selective pressure to create an antibiotic resistance environment (Ben et al. 2019). Nevertheless, measurements of the amounts and types of hazards (ARBs and ARGs) associated with environmental sources (water, air, soil, and food) that are related to the acquisition of resistant infections in humans are essential to establish suitable mitigation strategies (Vikesland et al. 2017).

1.5.5 *Acinetobacter baumannii*

The genus *Acinetobacter* comprises a group of Gram-negative, nonfermenting, strictly aerobic, catalase-positive and oxidase-negative coccobacillus (Torres et al. 2010). *Acinetobacter* species are widely distributed in the environment, making it possible to recover *Acinetobacter* isolates in almost 100% of the soil and water samples, as these are considered their main ecological niches (Salazar de Vegasa and Nieves 2005). Among the 32 varieties of species of the genus *Acinetobacter*, the most representative species is *Acinetobacter baumanii*, whose, while being distributed in nature, prevalence is strongly associated with the clinical-hospital environment, where some strains can survive in wet or dry inanimate objects in the hospital environment for weeks or months; in addition, it is a microorganism that is part of the normal flora of human skin and is distinguished as the microorganism that is most frequently carried persistently on the skin by hospital personnel (Phillips 2014).

Thus, all these factors increase the chances that patients will be colonized, in addition to promoting contamination of medical equipment, thus playing a determining role in the spread of infection and prolonged hospital outbreaks. The risk factors for *Acinetobacter baumannii* infection will depend on the type of infection (intrahospital, extrahospital) and its drug resistance profile, the latter being the most important. Community-acquired *Acinetobacter baumannii* infections, unlike those acquired in the hospital environment, are reported less frequently, because other risk factors are involved, such as residence in a tropical developing country, alcoholism, smoking, or diseases such as diabetes mellitus and chronic lung disease, conditions that easily allow infection to result in bacteremia or pneumonia.

It is worth mentioning that despite being rarely detected, this type of infection is of greater concern once it is acquired in the community, since it is a more severe infection than nosocomial infection and, in most cases, it is fulminant, dying in 60% of patients (Antunes et al. 2014). The *A. baumannii* genome contains several genes grouped into resistance islands, whose structure, in addition to providing high intrinsic resistance to antimicrobials, facilitates the acquisition of resistance mechanisms from other species of bacteria present in its environment, such as *Pseudomonas* spp. through mobile genetic elements (plasmids, transposons and integrons) (Agodi et al. 2013; Fournier et al. 2006), thus giving rise to an *Acinetobacter* isolate with a multidrug-resistant (MDR) phenotype with the capacity to produce extended-spectrum beta-lactamases, which confers resistance to most of the known antimicrobials, including carbapenems and colistin (Towner 2009). Therapeutic difficulties, added to the great capacity that it must survive for a long time in the hospital environment, together with the potential to develop in an acidic pH and at low temperatures, which allows it to increase its ability to invade devitalized tissues, together with the development of a biofilm ("biofilm") on human surfaces and cells, constitute this microorganism as an emerging public health problem (Gaddy and Actis 2009).

1.5.6 *Pseudomonas aeruginosa*

Bacteria of the genus *Pseudomonas* are Gram-negative microorganisms that can be classified as facultative anaerobes and are characterized as being ubiquitous and preferring humid environments (Luján Roca 2014). Specifically, *Pseudomonas aeruginosa* is a bacterium that successfully colonizes an enormous diversity of niches, as a consequence of its great nutritional versatility, it can survive in extreme environmental conditions; however, naturally, we can find it widely distributed in nature, its reservoir is the moist soil, water, wastewater, vegetation, humans and animals, humans and animals being the main hosts (CDC 2016). *P. aeruginosa* is an opportunistic pathogen responsible for a wide range of infections, mainly nosocomial, which is why it is considered highly prevalent in infections associated with health-care infections (HAIs), since it has a high adaptation to repeated changes in the microenvironment, nutrient availability, resistance mechanisms, and capacities to form biofilms (Villanueva-Ramos et al. 2019).

In humans, the species most at risk from infection is *Pseudomonas aeruginosa*, but infections by other species such as *P. paucimobilis*, *P. putida*, *P. fluorescens*, or *P. acidovorans* may also occur (Dumaru et al. 2019). Invasive infections by *P. aeruginosa* occur mainly in patients with underlying diseases that cause immunosuppression and mostly occur in the hospital setting. Community-acquired infection may occasionally occur in previously healthy patients. These infections are usually localized at the skin level (folliculitis, cellulitis, or ecthyma gangrenosum) without associated bacteremia, or present as severe clinical forms (Brady 2004; De almeida et al. 2002;). In the case of community-acquired infections, such as sepsis due to *Pseudomonas aeruginosa* in previously healthy patients, it is an infrequent clinical entity that must be considered when ecthyma gangrenosum, neutropenia, and gastrointestinal manifestations are present.

However, there is a possibility that *P. aeruginosa* can colonize parts of the human body; however, the prevalence of this colonization in healthy people is low (Rossolini and Mantengoli 2005). In case of acquiring an infection caused by *P. aeruginosa*, the vast majority of these will be related to the hospital environment, constituting a severe clinical problem, since, in most cases, there is a compromise of the host's defenses (Lyczak et al. 2000). This is because once the infection is established, *P. aeruginosa* produces a series of toxic compounds that cause not only extensive tissue damage but also interfere with the functioning of the immune system. Among the proteins involved in *P. aeruginosa* infection, we find toxins, as well as hydrolytic enzymes that degrade the membranes and connective tissue of various organs. This situation is aggravated by the difficulty in treating *P. aeruginosa* infections, since this bacterium is intrinsically resistant to multiple classes of antibiotics that are not structurally related to each other (Strateva and Yordanov 2009) due to decreased permeability of its outer membrane, the constitutive expression of several efflux pumps, and the production of enzymes that inactivate antibiotics. In addition, it can acquire new resistance mechanisms via mutations (Mesaros et al. 2007). Furthermore, in aqueous environments, this bacterium adheres to surfaces, producing a kind of aggregate called a biofilm. The formation of these accumulations of bacteria and extracellular material represents a health problem, since it contaminates devices implanted inside the body, such as intrauterine devices, catheters, or heart valves. Biofilms also represent a problem in the production process of various industries as they cause clogging and corrosion of connections and filters (Villanueva-Ramos et al. 2019). It is estimated that 65% of bacterial infections are due to the formation of biofilm and its mechanisms of tolerance to antibiotics, which represents a problem in the health field; the appearance of biofilms is strongly associated with recurrent chronic and delayed wound healing (Romeo 2020). When growing in a biofilm state, *Pseudomonas aeruginosa* increases tolerance to antibiotics up to a thousand times more than when it grows in its free form (Torres et al. 2010) and is mainly associated with the following mechanisms: quorum sensing (QS) signal, porins, efflux pumps, gene expression, membrane vesicles, extracellular DNA, and enzymes (Bolívar-Vargas et al. 2021). *P. aeruginosa* appears as an exceptional bacterium; the wide variety of virulence factors, the breadth of infections it causes, and its multiple mechanisms of resistance to antibiotics make it stand out among the pathogenic microorganisms for humans.

1.5.7 *Enterobacter* spp.

The *Enterobacter* genus is a member of the ESKAPE group, which contains the main resistant bacterial pathogens (Rice 2010). It consists of fermentative, facultative anaerobic, Gram-negative bacterial species belonging to the Enterobacteriaceae family. This genus is associated with a variety of environmental habitats, their presence in the intestinal tract as natural commensals of animal and human intestinal microbiota resulting in their wide distribution in soil, plants, water, and wastewater (Singh et al. 2018). Among this genus of bacteria, only certain subspecies/species have been associated with hospital-acquired infections and outbreaks. In humans, multiple *Enterobacter* species are known to act as opportunistic pathogens (disease-causing organisms): species, such as *E. cloacae*, *E. aerogenes*, *E. gergoviae*, and *E. agglomerans*, are associated with hospital-acquired infections and outbreaks (Akbari et al. 2016). These bacteria cause a variety of conditions, including eye and skin infections, meningitis, bacteremia (bacterial infection blood), pneumonia, and urinary tract infections.

The *Enterobacter aerogenes*, *E. cloacae*, and *E. hormaechei* species represent the most frequently described isolated species in clinical infections, especially in immunocompromised patients hospitalized in an intensive care unit (ICU), due to the adaptability of these species to antimicrobial agents and their behavior as opportunistic pathogens. These pathogens are frequently associated with a multidrug resistance (MDR) phenotype, mainly due to their adaptation to the hospital setting and their ability to readily acquire numerous mobile genetic elements containing resistance and virulence genes. These species have intrinsic resistance to ampicillin, amoxicillin, first-generation cephalosporins, and cefoxitin due to the expression of a constitutive AmpC β-lactamase. In addition, the production of extended-spectrum β-lactamases has been reported in these bacteria, making their treatment difficult (Davin-Regli et al. 2016). Antibiotic resistance, the regulation of resistance genes, and the expression of extended-spectrum beta-lactamases and carbapenemases significantly reduce therapeutic options, creating a particularly worrisome scenario with this microorganism as the protagonist of a global crisis of multiresistance to antibiotics.

Recent studies show that carbapenem-resistant enterobacteriaceae (CRE) can contaminate aquatic environments such as marine surface waters, rivers, estuaries, and contaminated drinking water (Mahon et al. 2019). CRE can reach aquatic bodies as a consequence of organic contamination from multiple sources (Mathys et al. 2019), including hospital effluents, wastewater treatment plants (PTARs), discharges from livestock and agricultural farms, water seepage, and others. Once mixed with the aquatic body, these effluents can introduce not only resistant microorganisms but also high doses of antibiotics, likely triggering the spread of resistance (Aydin et al. 2019). However, evaluating the link between the clinical and aquatic epidemiology of CRE is often challenging. While some studies have shown that clinical strains can be found in aquatic bodies, others have yet to prove such a link (Piedra-Carrasco et al. 2017).

1.5.8 Current Situation of Resistance

During the last 20 years, an increase in resistance has been observed in the 6 bacteria of the ESKAPE group; this represents a new crisis and a global public health problem (WHO 2015). Specifically, in Latin America, more than 50% of infections acquired in intensive care units (ICU) are caused by ESKAPE bacteria, with a growing tendency toward extreme drug resistance (resistance to all families of antimicrobials except 2 or 1 of them) and pan drug resistance (resistance to all families of antimicrobials) (WHO 2015).

Selective pressure is the most important cause of the dissemination and extension of resistance; in the last 70 years, the indiscriminate use of antibiotics had contributed to the genetic diversification of resistance genes, as can be seen in the current number of TEM beta-lactamases, where to date, there are at least 187 described, when in 1982 before third-generation cephalosporins were introduced into the clinic, only TEM-1 and TEM-2 were known (Corvec et al. 2013).

Antimicrobial resistance is favored by the inappropriate use of antimicrobials in human medicine, veterinary medicine, agriculture, and aquaculture. Insufficient prevention and control measures for infections associated with hospital care, incomplete treatment by patients, and lack of hygiene and sanitation, are the other factors that complicate global efforts to contain it (Bolívar-Vargas et al. 2021). The prevalence of resistance has not only affected the efforts made to hold it but has also complicated the interpretation of the phenotypic profile and addressing the appropriate treatment, since the association of different resistance mechanisms for the same is increasingly frequent.

Furthermore, the prevalence of resistance has not only increased in infection-causing bacteria. Intestinal colonization of healthy people by ESBL-producing Enterobacteriaceae has reached pandemic levels worldwide in just a few years, and it is estimated that there are 1753 million colonized people worldwide (Phillips 2014; Woerther et al. 2013). Other factors that contribute to the extension and dissemination of resistance are a consequence of the current situation in the world, where the hyperconnection among borders, for example, the food and animal trade, tourism, health and business trips, emigration, and refugees, among other events, allows resistant strains to reach anywhere (Rogers et al. 2011). In addition, wild animals can also act as a reservoir and a potential source of dissemination. An example of this is the presence of bacteria resistant to antibiotics in migratory birds, a situation that could undoubtedly favor the spread of resistance over long distances (Simoes et al. 2010). In all of the above examples, water bodies do play an important role as reservoirs and distribution nodes of antibiotic-resistant bacteria, especially those of the ESKAPE group, which, besides commonly being multidrug resistant, have an enhanced ability to transfer antibiotic-resistance genes to related bacteria present in the water bodies.

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2 The Chemical Composition of the Water in the Rivers, Lakes, and Wetlands of Uttarakhand

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Abstract

Ions like calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), and potassium (K^{+}) predominate among the chemical components of the water in Uttarakhand's rivers, lakes, and wetlands. These waters may also contain the following significant ions: bicarbonate (HCO_3^{-}), sulfate (SO_4^{2-}), chloride (Cl^{-}), and nitrate (NO_3^{-}). Depending on the type of water body and the area, different ions have different concentrations. Other significant chemical components of the water in these areas include suspended particles, organic matter, and dissolved organic carbon. The concentrations of nutrients such as phosphorus (P) and nitrogen (N) vary depending on the geographical area and season. The water also contains trace amounts of elements like iron (Fe), manganese (Mn), and zinc (Zn). The water quality of the rivers, lakes, and wetlands in Uttarakhand is impacted by the presence of certain chemical components, which may have an effect on the health of aquatic life, human activities, and the environment. These water bodies need to be protected; therefore, it is important to keep an eye on their chemical makeup and take precautions to keep them healthy.

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Keywords

Water body · Chemical components · Uttarakhand · Water · Wetland

2.1 Introduction

Water is an inorganic chemical substance that is clear, odorless, tasteless, and almost colorless; it is the primary component of the hydrosphere of the Earth and the fluids of all known forms of life; its chemical formula is H_2O and acts as a solvent. Despite the fact that it does not contain any calories or organic nutrients, it is necessary for all kinds of life that have ever been discovered. In accordance with its chemical formula, H_2O, each of its molecules is composed of two hydrogen atoms and one oxygen atom that are covalently bonded to one another. The angle between connected hydrogen and oxygen atoms is 104.45° (Brini et al. 2017). The term "water" refers to the aqueous phase of the molecule H_2O when it is at a standard pressure and temperature.

There are many different natural states that water may be found in. It results in the production of aerosols that resemble fog as well as precipitation in the form of rain. Clouds are formed by ice and water droplets that are suspended in the atmosphere. When the crystalline ice is broken up into smaller pieces, it has the ability to fall to the ground as snow. The gaseous state of water is referred to as steam or water vapors. The majority of the surface of the Earth is covered by water, the bulk of which may be found in the world's oceans and seas (Chaplin 2019). The groundwater (1.7%), glaciers and ice caps of Antarctica and Greenland (1.7%), clouds (made up of ice and liquid water suspended in air), and precipitation (0.001%) of the atmosphere all contain small quantities of water. Precipitation also contains a minimal quantity of water (Ho 1972).

Water is a very important component of the world economy. More than seventy percent of the fresh water that humans consume is put to agricultural use (Baroni et al. 2007). Water is the most vital resource necessary for the continuation of life. The pollution of water makes it unsuitable for human consumption, and the causes of this contamination include both natural processes (such as rock weathering and erosion) and human activities (such as urbanization, agriculture, industry, and population growth), among others (Oluyemi et al. 2010). Water is crucial to the continued existence of human beings. It was estimated by the WHO that 36% of Indians living in urban areas and 65% of Indians living in rural areas did not have access to safe drinking water (Kılıç 2021).

People place the most importance on the presence of naturally occurring water in the physical world that surrounds them. It is one of the most incredible, but inexplicable, combinations that can ever exist on our earth. Because of a wide range of characteristics that are unique to it, it stands out above all other substances (e.g., anomalously high values for the temperature of melting, boiling, and evaporation, and heavy dissolving capacity) (Nilsson and Pettersson 2015).

The hydrological cycle, which is a connection between the hydrosphere and the atmosphere, lithosphere, and biosphere, is responsible for the creation of the chemical composition of water on Earth. Water is a universal solvent that interacts with every component of its natural environment and is influenced by both natural and man-made factors. Water is enriched by a wide range of different substances in gaseous, solid, and liquid states that produce a huge variety of natural water types from the perspective of their chemical composition. Water is a universal solvent that interacts with every component of its natural environment and is influenced by both natural and man-made factors (Quattrini et al. 2016).

The boundaries of Uttarakhand are denoted by the presence of the Himalayas to the north, the Shivalik Hills to the south, the Ganges to the east, and the Yamuna to the west. The climate there is classified as moderate. The lowest it gets in the winter is approximately 5 °C, while the highest it gets in the summer is around 36 °C (Kumar et al. 2010).

The weathering of the rocks and improper disposal of sewage waste are the key factors that contribute to water source pollution in Uttarakhand. The mountains in this region are sloped, making these factors more likely to occur. Problems with water quality are being experienced by Uttarakhand's water sector as a consequence of turbidity and bacterial contamination of the available drinking water sources in the state. The mountainous section of the state is home to a sizeable portion of the state's population, and it is estimated that around 90% of the rural population gets the water they need for everyday life from natural water sources (Jain et al. 2010).

Fecal contamination in water is still the pollutant that most seriously affects human health. This includes the major water-borne diseases such as diarrhea, cholera, typhoid, and schistosomiasis. This is especially true in the hills of Uttarakhand and other similar states like Himachal, Jammu and Kashmir, and the North East states (Joshi et al. 2009).

2.2 The Composition of Natural Waters from a Chemical Standpoint

The dissolved inorganic components and the dissolved organic components make up the two main parts of natural water. The main inorganic elements of natural water are calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), oxygen (O_2), hydrogen (H_2), carbon dioxide (CO_2), nitrogen (N_2), and sulphur (S). Together, these elements create a complex mixture of ions and molecules that constitutes the majority of natural water (Rittmann and McCarty 2001).

Proteins, carbohydrates, lipids, and other organic compounds make up the majority of natural water's organic constituents. Depending on the source of the water and the surrounding environment, these organic components can vary significantly. For instance, the organic components of groundwater or seawater may be different from those of river water. In addition, biological processes like photosynthesis and respiration can have a big impact on how natural water is made (Schwarzenbach et al. 2003).

Natural water's chemical makeup can vary significantly depending on its source, the surrounding environment, and the extent of chemical and biological processes. Each component's concentration can also differ significantly, with some components present in only trace amounts and others possibly in high concentrations (Ašperger 2004).

2.3 Ions Found in Nature

Conventionally, the ions, complex ions, undissociated chemicals, and colloids (in the dissolved form) that make up natural fluids are classified into macrocomponents and microcomponents. These components include: ions, complex ions, dissolved colloids, and undissociated chemicals. The macrocomponents are what make up the so-called principal ions, which define the chemical type of water and make up the majority of its natural minerals. The percentage of natural minerals in highly mineralized water can reach up to 99%, while the percentage of natural minerals in fresh water only reaches 95%. In a solution of water, hydrogen ions, nitrogen molecules, phosphorus molecules, and silicon molecules all take up positions in the center.

The quantity of different chemical elements present in the crust of the Earth and the ease with which their compounds may be dissolved in water are the two primary factors that determine the relative amounts of each mineral.

An abundance of cations such as Ca^{2+}, Na^{+}, Mg^{2+}, and K^{+} as well as anions such as Cl^{-}, SO_4^{2}, HCO_3^{-}, and CO_3^{2-} are found in natural water.

Ions of chloride (Cl^{-}): This ion has a substantial capacity for migration, because sodium, magnesium, and calcium chloride compounds are very soluble in water, and chloride ions have the same property. Because of natural processes such as leaching from rocks (e.g., nephelines), minerals (such as gallite, sylvite, carnallite, and bischofite), and saline deposits, they are able to live in water. In addition to being present in the precipitation of the atmosphere, it is currently mostly associated with pollution from urban and industrial sources. Chloride ions are present in varying concentrations throughout all types of water, ranging from a few parts per million to hundreds per kilogram (in brines).

2.3.1 Ions of Sulfate (SO_4^{2-})

These are present in all surface waters, and the presence of calcium ions, with which they mix to generate the compound $CaSO_4$, which is relatively soluble, restricts their concentration. Sulfate ions may be found in all surface waters. The sedimentary rocks that are the primary contributors of sulfate to water are gypsum and anhydride, two examples of which are described below. Sulfates contribute to the enrichment of water through a variety of processes, including the oxidation of sulfide, which is abundant in the crust of the Earth, as well as the oxidation of hydrogen sulfide, which is produced during volcanic eruptions and is found in the precipitation of the atmosphere. The concentration of sulfur in water bodies may be affected by human economic activity, processes involving the breakdown and oxidation of sulfur, as well as compounds of vegetative and organic origin.

2.3.2 Ions of Carbonate and Hydrocarbonate (HCO_3^- and CO_3^{2-})

Ions of carbonate and hydrocarbonate. It is found in waters that have formed naturally in a dynamic equilibrium with carbonic acid in particular quantitative ratios and, together, the two substances produce a carbonate system of chemical equilibrium that is related to the pH of the water. When the pH of the water in a system ranges from 7 to 8.5, hydrocarbonate will predominate as the predominant ion. When the pH is lower than 5, the number of hydrocarbonate ions present is almost nonexistent. When the pH is higher than 8, the majority of ions present are carbonate ions. Various types of carbonate rocks, such as limestones, dolomites, and magnesites, are the primary contributors of HCO_3^- and CO_3^{2-}, and the decomposition of these compounds requires the presence of carbon dioxide.

The predominance of hydrocarbonate ions is seen most often in waters with considerable mineralization but sometimes occurs in fluids with low mineralization. There is some buildup of hydrocarbonate ions, but just a little. Because calcium ions are present, these ions interact with the salt HCO_3^- to generate calcium carbonate. Surface freshwaters typically have an HCO_3^- concentration that is lower than or equal to 250/mg (with the exception of soda alkaline waters in which HCO_3^- and CO_3^{2-} content can reach grams and even dozens of grams per kilogram). The sodium ion, denoted by the symbol Na^+, has a comparatively strong tendency to migrate. This may be attributed to the high solubility of all of sodium's salts. When there is very little mineralization in the water, the concentration of Na^+ is third. As the mineralization of a fluid grows, so does the concentration of sodium, and once the mineralization reaches a certain g/kg, sodium becomes the predominate ion in the fluid. The presence of chlorine ions brings a considerable portion of the sodium ions into equilibrium, which results in the formation of a stable mobile combination that moves fast through a solution.

The presence of salt deposits (rock salt), products derived from the weathering of limestone, and the removal of sodium from the absorption complex of rocks and soils by calcium and magnesium are all potential sources of sodium in rivers.

2.3.3 Potassium Ions (K^+)

Potassium is pretty similar to sodium in terms of the quantity present in the Earth's crust and the solubility of its elements. Potassium is also fairly comparable to sodium in terms of the stability of its ions. Because of its restricted potential for migration, it is only found in surface waters in very small quantities. This is the case because it plays an active role in several biological processes, such as the absorption of nutrients by living plants and other microbes.

2.3.4 Ions of Calcium (Ca^{2+})

The principal sources of calcium are carbonate rocks (limestones, dolomites), which are broken down by the carbonic acid found in water. Other sources of calcium include algae and shellfish. The reaction, on the other hand, begins to proceed in the opposite direction when the availability of carbon dioxide (with which it is in equilibrium) is low, which is then followed by the precipitation of $CaCO_3$ crystals. Gypsum, which is often found in sedimentary rocks, provides an additional source of Ca^+ in the streams that are found naturally. Calcium ions dominate the cation composition of fluids that have a relatively modest mineral content.

2.3.5 Magnesium Ions (Mg^{2+})

There is a greater concentration of calcium than magnesium in the crust of the Earth. It is possible for it to make its way into surface water as a result of the chemical weathering and disintegration of rocks such as dolomites, marls, and others. Magnesium ions are present in all naturally occurring fluids, although they very seldom ever steal the spotlight. River waters may include anything from one milligram per liter to tens of milligrams per liter of this substance. The lower biological activity of magnesium as compared to calcium, as well as the higher solubility of magnesium sulfate and magnesium hydrocarbonate in comparison to the corresponding calcium compounds, both induce an increase in the Mg^{2+} content in water. With a rise in the mineral content of the water, the proportion of magnesium to calcium begins to move toward a greater abundance of the latter.

1. **Ammonia:** Ammonia is naturally present in rivers, lakes, and wetlands due to the decomposition of organic material.
2. **Nitrate:** Nitrate is naturally present in rivers, lakes, and wetlands due to the decomposition of organic material.
3. **Phosphate:** Phosphate is naturally present in rivers, lakes, and wetlands due to the decomposition of organic material.
4. **Chloride:** Chloride is naturally present in rivers, lakes, and wetlands due to the runoff of salt from the mountain soils.
5. **Sulfate:** Sulfate is naturally present in rivers, lakes, and wetlands due to the decomposition of organic material and the runoff of sulfur-containing minerals from the mountain soils.
6. **Magnesium:** Magnesium is naturally present in rivers, lakes, and wetlands due to the dissolution of soluble salts from the mountain soils.
7. **Calcium:** Calcium is naturally present in rivers, lakes, and wetlands due to the dissolution of soluble salts from the mountain soils.
8. **Iron:** Iron is naturally present in rivers, lakes, and wetlands due to the runoff of iron-containing minerals from the mountain soils.
9. **Manganese:** Manganese is naturally present in rivers, lakes, and wetlands due to the runoff of manganese-containing minerals from the mountain soils.

10. **Zinc:** Zinc is naturally present in rivers, lakes, and wetlands due to the runoff of zinc-containing minerals from the mountain soils.
11. **Copper:** Copper is naturally present in rivers, lakes, and wetlands due to the runoff of copper-containing minerals from the mountain soils.
12. **Lead:** Lead is naturally present in rivers, lakes, and wetlands due to the runoff of lead-containing minerals from the mountain soils.
13. **Arsenic:** Arsenic is naturally present in rivers, lakes, and wetlands due to the runoff of arsenic-containing minerals from the mountain soils.
14. **Mercury:** Mercury is naturally present in rivers, lakes, and wetlands due to the runoff of mercury-containing minerals from the mountain soils.
15. **Cadmium:** Cadmium is naturally present in rivers, lakes, and wetlands due to the runoff of cadmium-containing minerals from the mountain soils.
16. **Beryllium:** Beryllium is naturally present in rivers, lakes, and wetlands due to the runoff of beryllium-containing minerals from the mountain soils.

2.3.6 Ions of Hydrogen

Despite their comparatively modest absolute number in contrast to that of other ions, hydrogen ions play a particular function in the composition of natural waters. This is because hydrogen ions have a positive charge. Hydrogen ions are always present in water, because they are formed during the electrolytic dissociation of water.

The formula for hydrogen ions is

$$H_2O \rightarrow H^+ + OH^-$$

The concentration of hydrogen ions in a water solution can be calculated using the "ionic product" of water, which is denoted by the formula

$$K_w = [H^+] \cdot [OH^-]$$

Therefore, $K_w = 10^{-14}$ is a constant value that is always equal to the product of the concentrations (in g molecules) of hydrogen ions and hydroxyl at a temperature of 22 °C. This is because $K_w = 10^{-14}$ is a product of the concentrations of hydrogen ions and hydroxyl ions concentration. Since the concentrations of hydrogen and hydroxyl ions are so negligible, it is customary to express them in the form of their logarithms with the sign switched around:

$$p^H = -\log\ [H^+]; p^{OH} = -\log\ [OH^-]$$

When describing water processes, the concentration of hydrogen ions is often utilized. When pH equals 7, the interaction between water and other substances is considered neutral. However, if pH is more than 7 or less than 7, the reaction might go in either an acidic or alkaline direction. The amount of hydrogen ions that are included within natural fluids is determined by the dissociation and hydrolysis of the combinations that are already there (Brezonik and Arnold 2012).

2.4 Gases Dissolved

Depending on the location and the surrounding environment, the percentage composition of dissolved gases in water with a focus on the biological oxygen demand (BOD) can change. In general, nitrogen, oxygen, carbon dioxide, and argon are the most prevalent dissolved gases in water. Since oxygen is necessary for aquatic organisms' respiration and metabolism, it is the most significant of these for BOD. Nitrogen, carbon dioxide, and argon make up the remaining portion of the total dissolved gas in water, which is typically between 10 and 14% oxygen. Due to higher levels of pollutants and organic matter in the water, polluted waters can have significantly lower oxygen percentages. The total amount of dissolved gas in these situations can be between 0 and 7%, with oxygen making up roughly 0–3% of the total. A significant indicator of water quality is the proportion of oxygen in the water because it can show how polluted the water is. It is also one of the key elements in determining a water body's BOD level. Overall, depending on the environment and the level of water pollution, the oxygen content of water with a focus on the BOD can range from 0 to 14% (Kumar 2020; Theodore et al. 2022).

2.5 Biological Substances

Biogenous substances are those whose origins are tied to aquatic species' critical functions, which determine whether they can survive in bodies of water. These include silicon, nitrogen, phosphorus, and iron compounds.

2.5.1 Silicon (Si)

Natural waters always include silicon, which is one of the ionogenic elements. Due to the limited solubility of silicate minerals and certain species' consumption of them, their quantity in natural waters is minimal compared to the overall salt composition (in land surface waters up to 10–20 mg/L). In the form of the meta- and ortho-silicic acids H_2SiO_3 and H_4SiO_4, as well as in colloidal form of the $x\ SiO_2 \times yH_2O$ type, it may be found in fluids in a completely dissolved condition.

2.5.2 Nitrogen (N)

It is found in natural waters as both organic molecules and a variety of inorganic ions, including ammonium NH_4^+, nitrite NO_2^-, and nitrate NO_3^- (in the amino acids and proteins of organisms, and the products of their vital activity and decomposition). These may be found in water as dissolved molecules, colloidal particles, and suspended substances.

2.5.3 Phosphorus (P)

Phosphorus is an inorganic and organic chemical that exists in water as dissolved, suspended, and colloidal entities. Being an anionogenic element, phosphorus generates the neutral phosphoric acid H_3PO_4 that dissociates into many derivative forms, including H_2PO_4, HPO_4^{2-} and PO_4^{3-}, the relationship between which is influenced by the pH of water.

2.6 High-Quality Water

Water that satisfies drinking water standards set by the Environmental Protection Agency (EPA) and other organizations and is free of contaminants is considered to be of high quality. In general, there shouldn't be a lot of bacteria, viruses, heavy metals, or other chemicals in the water. It should also look good and have a pleasing flavor and aroma.

The Environmental Protection Agency (EPA) establishes guidelines for contaminants in public drinking water systems, including bacteria, lead, arsenic, and other pollutants. Depending on the type of water system, the water source, and the population served, these standards may change. For instance, the EPA mandates that public water systems test for lead, copper, and other contaminants and disinfect water using chlorine.

Some states and localities may have their own standards or requirements for the quality of drinking water in addition to the EPA guidelines. Water filters and other items that can enhance the quality of a person's water are available for purchase by both homeowners and businesses. Many communities also have water treatment facilities that can clean up contaminants and raise the standard of the water. No matter where your water comes from, it's crucial to test it frequently to make sure it satisfies the requirements for safe drinking water. For more information if you are worried about the quality of your water, get in touch with the EPA or your local health department (EPA 2020).

2.7 River's Chemical Makeup

The chemicals in the rivers in Uttarakhand depend on where the river starts, how fast it flows, and what is around it. Most rivers in Uttarakhand have different amounts of calcium, magnesium, sodium, chloride, and sulfate ions. Ions like nitrate, phosphate, potassium, bicarbonate, iron, manganese, and copper are also often found in the rivers of Uttarakhand. Some rivers in Uttarakhand may also be affected by the runoff from factories and farms, which can add chemicals like nitrates and phosphates to the water (Seth et al. 2016; Sharma et al. 2022).

The way the rivers in Uttarakhand are made up chemically also changes with altitude. For example, rivers at higher elevations tend to have more calcium, magnesium, and bicarbonate ions, while rivers at lower elevations tend to have more sodium, chloride, and sulfate ions. The chemicals in the rivers of Uttarakhand are important for the environment because they affect the rivers' health and the health of the ecosystems around them. It can also affect the health of people, since rivers are often used to get drinking water (Ruhela et al. 2018).

2.8 Various Rivers of Uttarakhand with Their Ionic Composition

The rivers of Uttarakhand with their chemical composition are as

2.8.1 Bhagirathi River

The ions calcium, magnesium, sodium, potassium, sulfate, chloride, nitrate, and bicarbonate are found in the Bhagirathi River in Uttarakhand. By preserving the pH balance, these ions help the river remain suitable for aquatic life. As some of these ions serve as nitrogen sources for the growth of algae and other types of aquatic plants, they can also aid in reducing the eutrophication of the river (Arora et al. 2017).

High concentrations of some of these ions, including nitrate and chloride, can, however, be harmful to the river. An imbalance in the river's ecology can result from an increase in these ions, which can also promote the growth of algae and other aquatic plants. This may result in lower oxygen levels, which may have an impact on aquatic life's health. Additionally affecting the quality of drinking water and rendering it dangerous for ingestion are high quantities of nitrate and chloride (Khan et al. 2022).

2.8.2 Bhilanagna River

In general, calcium and bicarbonate predominate in the chemical makeup of the Bhilangana River in Uttarakhand, India, with minor levels of magnesium, sodium, potassium, and chloride. The amounts of dissolved organic carbon and nitrate are typically low. Aquatic life can benefit from the high calcium and bicarbonate concentrations. While bicarbonate helps to buffer the water and maintain an ideal pH for aquatic life, calcium is crucial for the skeletal development of fish and other aquatic organisms (Agarwal et al. 2018).

High calcium and bicarbonate concentrations, however, can also be harmful to aquatic life. An increase in the river's hardness brought on by too much calcium can have a detrimental effect on the health of the fish. High bicarbonate concentrations can also cause carbonates to build up on the river bottom, lowering the amount of oxygen in the water. The variety of aquatic life may suffer as a result (Amoatey and Baawain 2019).

A decrease in water clarity brought on by high quantities of dissolved organic carbon can also restrict the amount of aquatic animals' habitat. Nitrate concentrations can also be an issue since they raise the possibility of eutrophication, which can cause the water to lose oxygen (Annayat et al. 2022).

2.8.3 Alaknanda River

The Alaknanda River, which originates in the Himalayas of Uttarakhand, India, is a significant tributary of the Ganges River. Calcium, magnesium, sodium, potassium, bicarbonate, sulphate, chloride, nitrate, and silicate are the main dissolved inorganic components of the Alaknanda River. Ca^{2+} (2.68 mg/L), Mg^{2+} (1.48 mg/L), Na^{+} (4.22 mg/L), K^{+} (0.19 mg/L), HCO_3^{-} (81.41 mg/L), SO_4^{2-} (2.66 mg/L), Cl^{-} (2.19 mg/L), NO_3^{-} (8.96 mg/L), and SiO_2 (4.09 mg/L) are the elements with the highest average values in the Alaknanda River.

These inorganic substances in the Alaknanda River have both beneficial and detrimental effects on the ecology. When calcium and magnesium are present, for instance, the pH of the water is balanced and fish and other aquatic creatures are protected against environmental stresses like temperature and oxygen level variations. For aquatic plants and animals, sodium and potassium are crucial minerals for growth and reproduction. Bicarbonate, sulfate, and chloride work together to control the acidity of the water and to shield aquatic life from harmful metals. The growth of phytoplankton, the main producers in aquatic habitats, depends on nitrate and silicate.

High levels of these substances, however, can contaminate and eutrophize the water. High amounts of nutrients like nitrates and phosphates can cause excessive development of aquatic plants and algae, which lowers the amount of oxygen available to other aquatic creatures, a process known as eutrophication. Fish and other aquatic species may perish as a result of this. High levels of nitrate, chloride, and sulfate can also contaminate water, rendering it unfit for consumption or other uses. In order to safeguard the aquatic ecology of the Alaknanda River, it is crucial to maintain a healthy balance of these components (Kumar et al. 2016).

2.8.4 Mandakini River

The Mandakini River in Uttarakhand has significant amounts of the following chemical elements: calcium, magnesium, sodium, potassium, chloride, sulfate, nitrate, and phosphate. These Mandakini River elements may have a significant impact on the environment (Goswami and Singh 2018).

The presence of calcium and magnesium in the river can support aquatic life, because these ions are necessary for the growth of aquatic plants and animals. Major ions in the water like sodium and potassium can make the water more electrically conductive, which can have a big impact on the aquatic ecosystem. Pollutants that can damage the ecosystem include chloride, sulfate, nitrate, and phosphate. These contaminants have the potential to significantly affect aquatic life by causing eutrophication and oxygen depletion at high concentrations (Sain et al. 2023).

2.8.5 Ganga River

The Ganga River, commonly known as the Ganges, originates in Devprayag and flows through Biyasi Rishikesh before ending in Haridwar in the Uttarakhand region. Gangotri Glacier, Satopanth Glacier, Khatling Glacier, as well as the water melting from the snow-covered peaks of Nanda Devi, Trishul, Kedarnath, Nanda Kot, and Kamet, are the sources of the Ganga River. In Uttarakhand, the Ganga River is distinguished by a variety of chemical elements. Calcium, magnesium, sodium, potassium, chloride, sulfate, nitrate, dissolved organic carbon, and total dissolved solids are important constituents. These substances have an impact on the water's quality, which is crucial for supporting aquatic life. The majority of the dissolved solids in the Ganga River—between 15 and 25%—come from calcium. Aquatic species benefit from it, because it keeps the pH balance of water. Magnesium makes up between 10 and 15% of all dissolved solids and is the second most prevalent element. It is crucial for preserving the proper nutritional balance in water and aids in controlling the water's ionic composition. Another crucial element and crucial nutrient for aquatic life is sodium. It adds between 5 and 10% of the total dissolved solids. Potassium is found in smaller concentrations and makes up about 1–3% of all dissolved solids. For keeping the osmotic balance in water, it is crucial. Another crucial element is chloride, which accounts for 1–5% of all dissolved solids. It benefits aquatic life and aids in maintaining the ionic balance of water. Sulfate is less prevalent and makes up 0.5–2% of the total dissolved solids. It benefits aquatic life and aids in keeping the pH balance of the water. The Ganga River has a significant amount of nitrate, which makes up 0.5–2% of the total dissolved solids. Due to its role in preserving the water's nutritional balance, it is crucial for the survival of aquatic life (Mukherjee et al. 2021).

The Ganga River contains higher concentrations of dissolved organic carbon, which makes up around 5–10% of the total dissolved solids. It benefits aquatic life and aids in keeping the pH balance of the water. Total dissolved solids are a key contaminant indicator that helps determine how much contamination is present in the water. The total dissolved solids in the Ganga River range from 200 to 400 mg/L. These substances have an impact on the Ganga River's water quality, which is crucial for supporting aquatic life. Reducing the amount of pollutants and other impurities in the water is crucial for maintaining the health of the Ganga River (Kumar et al. 2015; Ahmad and Chaurasia 2019).

2.8.6 Ramganga

The Ramganga River in Uttarakhand has a complicated chemical makeup that is heavily influenced by the local geology and human activity. Bicarbonates, sulfates, chlorides, nitrates, and phosphates are some of the major ions found in water. In addition, the water contains trace amounts of iron, magnesium, calcium, zinc, copper, cadmium, lead, and arsenic (Paul 2017; Khan et al. 2015).

These chemicals have mostly had a negative impact on the Ramganga River. High levels of nitrates, phosphates, and heavy metals, for instance, can lead to eutrophication, which increases the growth of aquatic vegetation, lowers oxygen levels, and increases the frequency of hazardous algae blooms. This may lead to a decline in water quality and an increase in aquatic species mortality. In addition, exposure to heavy metals poses major health concerns for people, including neurological diseases, cancer, and renal damage. High levels of nitrates and phosphates can also result in a rise in algae, which can lower the water's oxygen content and result in a decline in fish populations (Tirth et al. 2022).

2.8.7 The Yamana River

Depending on the season, the Yamuna River in Uttarakhand has a different chemical makeup. High concentrations of calcium, magnesium, chloride, nitrate, sulfate, and potassium are typically present in the water. In addition, the water has very little dissolved oxygen and is mildly acidic (Sharma and Kansal 2011).

The ecology, especially aquatic life, may suffer as a result of the Yamuna River's chemical makeup. Eutrophication, which can result in algal blooms, lower oxygen levels, and other environmental changes, is brought on by high levels of nitrates and sulfates. High calcium and magnesium levels can produce calcium carbonate deposits as well, which can clog pipes and cause a scarcity of water. In addition, aquatic life may become hazardous at high chloride levels (Sharma et al. 2020).

2.8.8 The Kali River

A significant tributary of the Ganges is the Kali River in Uttarakhand. For the people of Uttarakhand, this river is crucial, since it supplies water for drinking, agriculture, and other uses. A wide variety of aquatic life can be found in the river.

The main ions that make up the chemical makeup of the Kali River include calcium, magnesium, sodium, potassium, sulfate, chloride, nitrate, and bicarbonate ions. Trace levels of other elements like iron, copper, zinc, and manganese are also present in the river (Trivedi 2010).

These substances are found in the Kali River, and they have both beneficial and harmful effects on the ecology. On the plus side, these components supply vital nutrients and minerals to the river's aquatic life. Additionally, they support the growth of advantageous bacteria and maintain the pH balance, which help to clean and maintain the health of the water. On the downside, certain of these elements, like iron and manganese, can contaminate water at high concentrations, which is bad for human health. In addition, sulfate and chloride levels that are too high can make the water corrosive, harming pumps, pipes, and other infrastructure (Ghosh and McBean 1998).

2.9 Wetlands' Chemical Composition

Depending on the type of wetland and its location, wetlands in Uttarakhand have different chemical compositions. Wetlands typically include high levels of organic matter, nitrogen, phosphorus, and sulfur as well as trace elements including iron, manganese, copper, and zinc. Decomposing plants, fungus, bacteria, and other microbes make up the organic matter. The trace elements may have an effect on the well-being of the wetland environment, whereas the nitrogen, phosphorus, and sulfur are vital nutrients for aquatic life. Some wetlands may have significant amounts of particular chemical substances including arsenic, mercury, and lead. In order to maintain the ecosystem's health, it is crucial to keep an eye on the chemical makeup of wetlands (Mitsch and Gosselink 2000; Sharma et al. 2018).

2.9.1 Availability of Wetland's Geotagging Data and Class of Wetlands

The Uttarakhand State Council for Science and Technology (UCOST), on the other hand, has started mapping the state's wetlands, and its findings should be made public soon. The goal of this project is to create a 1:50,000 scale map of the state's wetlands, and then categorize them according to the criteria established by the Ramsar Convention. In-depth data about the state of Uttarakhand's wetlands are available in the atlas form. Details about the wetland's location, size, type, and other attributes are included. There are many different kinds of wetlands listed in the atlas, such as marshes, ponds, lakes, reservoirs, oxbows, swamps, floodplains, and rivers (Bachheti et al. 2023).

The Uttarakhand Forest Department also keeps a database of the state's wetlands called the State Wetland Database. Details about the wetland's location, size, and composition are included. Marshes, ponds, lakes, reservoirs, oxbows, swamps, floodplains, and rivers are only some of the wetland types represented in the database.

There is a wealth of data about the state's wetlands available from the Uttarakhand State Biodiversity Board as well. The document details the wetland's precise location, as well as its kind, size, and other attributes. Marshes, ponds, lakes, reservoirs, oxbows, swamps, floodplains, and riverine wetlands are all represented in this catalogue. The wetlands of Uttarakhand are also depicted on the UK Land Cover Map. Details on the wetland's location, kind, extent, and other attributes are included. This map depicts a variety of wetland ecosystems, such as marshes, ponds, lakes, reservoirs, oxbows, swamps, floodplains, and rivers (Patakamuri 2013).

2.9.2 Wetland Coding and Classification System

Ramsar classification and the Indian Wetland Classification System serve as the foundation for Uttarakhand, India's wetland coding and classification system (IWCS). The system is made to recognize and classify wetlands based on their purpose and significance to the environment. In Uttarakhand, there are four primary categories of wetlands:

1. Natural Wetlands: These include lakes, ponds, marshes, swamps, estuaries, mangrove forests, coral reefs, lagoons, and wetlands created artificially by the construction of dams, reservoirs, and canals.
2. Human-made Wetlands: These comprise built-in wetland structures including sewage treatment plants, agricultural fields, industrial locations, and other man-made bodies of water utilized for aquaculture or irrigation.
3. Transitional Wetlands: Also known as "intermittent wetland systems," they are regions that provide as a transition between wetland and dryland ecosystems.
4. Wetlands that are particularly crucial to the preservation of biodiversity and ecosystem services are known as ecologically important wetlands.

Based on their size, hydrological properties, and other characteristics, these four categories are further subdivided (Bassi et al. 2014; Sharma and Kumar 2017).

2.10 Lake's Chemical Makeup

There are more than 500 glacial lakes in Uttarakhand. The geological and hydrological characteristics of these lakes' catchment areas determine their chemical composition. In general, the lakes have high levels of silica, magnesium, sodium, potassium, and calcium ions. They are also abundant in nutrients including nitrogen, phosphate, and sulfur as well as organic materials. Additionally, the lakes have large concentrations of suspended materials, primarily clay particles, which can change the hue and transparency of the water. The lakes may also contain significant amounts of heavy metals, including lead, arsenic, mercury, and cadmium, which are harmful to aquatic life. The amount of glacial meltwater that the Himalayan glacial lakes get also has an impact on their chemical makeup. Due to higher levels of dissolved carbon dioxide, lake water may turn acidic in the presence of glacial meltwater. Aluminum, iron, and manganese are just a few of the trace elements that the meltwater may add to the lake's water. Pollution from human activities can potentially change the lakes' chemical composition. Agricultural runoff, industrial waste, sewage, and other contaminants can contaminate the lakes. These contaminants may raise the concentrations of nutrients, organic matter, and heavy metals in the lake, which may be hazardous to aquatic life (Kumar and Sharma 2019; Babuji et al. 2023).

2.11 Effect of Chemical Constituents Present in Water of Uttarakhand on Human Beings, Marine Biota, and Environment

2.11.1 Human Beings

Uttarakhand water is generally high in minerals, which can lead to a range of health benefits. Examples of these minerals include calcium, magnesium, potassium, sodium, and iron. These minerals can help improve blood circulation, reduce blood pressure, and can improve the overall metabolism of the body. However, it can also contain pollutants and contaminants, such as pesticides, heavy metals, and industrial chemicals, which can lead to health risks if consumed in large quantities.

2.11.2 Marine Biota

The high mineral content of water in Uttarakhand can help improve the health of marine biota by providing essential nutrients. However, it can also contain pollutants, such as pesticides, heavy metals, and industrial chemicals, which can lead to toxicity and other health risks if consumed in large quantities.

2.11.3 Environment

The presence of pollutants and contaminants in the water can lead to environmental damage if not managed properly. For example, high levels of heavy metals can lead to eutrophication, which can reduce the amount of oxygen in the water and damage aquatic ecosystems. Additionally, industrial chemicals can pollute the air, land, and water and can lead to soil and water contamination (Lin et al. 2022; PR 2020).

2.12 Conclusion

The chemical composition of the water in rivers, lakes, and wetlands in Uttarakhand varies greatly depending on the water's point of origin, the time of year, and the location in which it is located. In addition to main cations like calcium, magnesium, and potassium and trace metals, the water typically contains dissolved organic and inorganic compounds including nitrates, phosphates, sulfates, and chloride. Dissolved particles and suspended sediments in the water may also be a source of toxic metals. In addition, eutrophication can lead to a rise in nutrient and contaminant levels in the water. The ecological systems of the rivers, lakes, and wetlands of

Uttarakhand are vulnerable to the effects of these substances; thus, they must be carefully monitored and maintained.

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3 Microbial Diversity of Cold-Water Reservoirs and Their Prospective Applications

Vidhi Jain, Prashant Arya, Shivani Maheshkumar Yagnik, Vikram Hiren Raval, and Namita Ashish Singh

Abstract

Microorganisms including bacteria, archaea, viruses, fungi, and protists have evolved into every possible niche on the globe and now dominate the living biomass in marine environments. This chapter highlights the bacterial, fungal as well as viral diversity of microbes isolated from cold-water reservoir viz. lakes, ocean, glaciers, sea, etc. The next section focuses on the applications of microbes isolated from the cold-water reservoirs mainly in bioremediation, food industry, textile industry, and pharmaceuticals. Psychrophilic enzymes, that is, amylase, cellulase, and protease, are used in textile, detergent, cosmetics, and leather industry for different purposes. Primary and secondary metabolites produced by cold-water reservoir microbes play major role in bioremediation of hydrocarbons and development of novel drugs, which are very useful against antimicrobial resistance (AMR) pathogens. Current research on microbes that can survive in the cold and on their biomolecules has amply shown the enormous potential of psychrophiles.

Keywords

Cold-water reservoir · Diversity · Bioremediation · Metabolites · Probiotic · Drug

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3.1 Introduction

More than 70% of the earth's surface is covered by cold-water reservoirs, including seas, oceans, lakes, rivers, ponds, and glaciers. These reservoirs support majority of the planet's biomass and play a substantial role in the global cycles of matter and energy that are necessary for life to continue on earth (Hunter-Cevera et al. 2005). The biosphere is dominated by a cold environment known as the "cryosphere" (area >33 million km^2) (Boetius et al. 2015). Microbes in the sea are most likely the source of all life on earth. They serve as the foundation of life on earth representing the richest assets and molecular diversity in nature (Sean and Jack 2015). They are referred to as multicultural tiny creatures possessing a vast range of metabolism that are cosmopolitan in nature, that is, widely distributed in air, water, soil, the sea, mountains, hot springs, and also in the bodies of living plants and animals, including humans (Onen et al. 2020). Marine microorganisms enable the storage, transfer, and turnover of essential biological materials in addition to producing the organic matter and oxygen necessary to support life. Over 50% of the oxygen produced on earth is produced by marine phototrophic microorganisms, including cyanobacteria, diatoms, and pico- and nanophytoplankton.

Ecosystem biodiversity has become a topic of rigorous study; subsequently, a great deal of information has been gathered on the distribution of microorganisms around the world. In addition, there is growing interest in the role of marine microorganisms in biogeochemical processes, biotechnology, pollution, and health (Poli et al. 2017). The immense diversity of microorganisms also gives rise to a huge amount of genetic data, bioactive substances, and biomaterials that are largely untapped but could have significant societal benefits and applications, such as improving medical treatments, probiotics and bioremediation applications, the supply of energy, and the development of industrial products and processes (Glöckner et al. 2012). Since microbes constitute the basis of life, they are vital to the habitability and sustainability of our planet and represent a largely unexplored supply of novel bioactive substances and metabolic pathways that might be utilized for new biotechnological applications and products (York 2020).

3.2 Diversity of Microbes Isolated from Cold Water Reservoirs

Microbial diversity encompasses a vast collection of variability in all kinds of microorganisms like fungi, bacteria, and viruses in the natural world vital for the continuation and conservation of global genetic resources. The structure and dynamics of food webs, global biogeochemical cycles, and the remineralization of organic matter rely on marine microbial communities (Parvathi et al. 2020; Wei et al. 2020). Psychrophiles are microorganisms capable of growing at very low temperatures ranging from −12 to 20 °C, where the optimum temperature of 15 °C shows the best growth (Sensoy 2021).

Marine bacteria, fungi, and other microorganisms build up exceptional metabolic and physiological capabilities that allow them to endure in hostile habitats and to generate compounds that might not be formed by their earthly counterparts (Shaaban 2022). The microbial diversity of various cold-water reservoirs is listed in Table 3.1 and explained in following sections.

3.2.1 Microbial Diversity of Oceans and Seas

The abyssal plain referred to as the deep-sea is typically sited between 3000 and 6000 m deep in the global ocean. The deep sea is composed of an extensive diversity of ecosystems and is the biggest and most distant biome of the biosphere (Corinaldesi 2015). Light intensity declines with depth within this zone, and photosynthetic yield is halted due to the absence of light. The temperatures nearing freezing and lack of organic matter at the ocean floor are the primary factors of the deep sea that control benthic productivity and biomass (Jørgensen and Boetius 2007). Microbes are the crucial members of the biota present and are believed to play a significant function in mineralization of organic matter, which in turn impacts the global biogeochemical cycles and carbon sequestration capacity of oceans. The Archaea, which degrade detrital proteins by secreting enzymes like proteases and peptidases, are one of the predominant microbial life forms found in marine sediments (Schippers et al. 2012). Bacteria and Archaea, which represent around 90% of the total benthic biomass in deep-sea sediments, contribute the highest proportion of taxonomic richness and productivity (Barnes et al. 2021).

In the sediments of Atlantic ocean and the deeper depths of the Arctic and Pacific Oceans, *Proteobacteria* is found to be dominant (Schauer et al. 2009). In the Pacific Ocean, seasonal variations have been noted, with *Bacteroidetes* being more prevalent than *Proteobacteria* in the winter (Suh et al. 2014). Sixty-four clones collected from the top surface sediments were divided into *Firmicutes* and *Gamma-proteobacteria* in a study by Khandeparker et al. (2014). *Bacillus* was the most prevalent among the *Firmicutes*, followed by *Dolosigranulum*. The dominant *Gamma-proteobacteria* was *Pseudomonas*, *Shigella*, and occasionally *Escherichia*. *Gamma-proteobacteria*, represented by *Pseudomonas* and *Enterobacteriaceae*, predominated the 58 clones isolated from deeper sediments, followed by *Beta-proteobacteria*, represented by *Limnobacter* and *Burkholderiales*. Shao et al. (2015) isolated 51 bacteria from the deep seawater column above the South-West Indian Ridge that belonged to Alpha-proteobacteria within the genera *Alterierythrobacter*, *Citricella*, *Erythrobacter*, *Kaistia*, *Lutibacterium*, *Maricaulis*, *Martelella*, *Mesorhizobium*, *Novosphingobium*, *Pseudomonas*, *Phenylobacterium*, *Roseovarius*, *Rhodobacter*, *Salipiger*, *Stappia*, *Sphingopyxis*, *Sphingomonas*, *Tistrella*, and *Thalassospira*, Gamma-proteobacteria within the genera *Alkaligens*, *Alkanovorax*, *Halomonas*, *Idiomarina*, *Marinobacter*, *Pseudoidiomarina*, and *Pseudomonas*, *Muricauda* and *Salegentibacter* (*Bacteroides*), *Bacillus* (Firmicutes) and *Microbacterium* (Actinobacteria). A novel thermophilic sulfur-reducing bacterium, *Desulfurobacteium indicum*, was reported by Cao et al. (2017) from hydrothermal vent in the Indian Ocean. Wang et al. (2018) characterized the bacterial diversity from rare-earth-elements-rich sediment in the Indian Ocean and revealed occurrence

Table 3.1 Microbial diversity of cold-water reservoirs

Cold-water reservoir and its location	Diversity type	Abundant phyla/genera	References
Polythermal glacier, Svalbard, Norway	Bacterial	*Proteobacteria, Firmicutes, Actinobacteria*	Amato et al. (2007)
Lakes in Schirmacher Oasis, Antarctica		*Proteobacteria, Actinobacteria, Bacteroidetes, Fusobacteria, Verrucomicrobia,* and *Chlorobi*	Mojib et al. (2009)
Surface snow, glacial stream, and moraine lake in Yala glacier, Nepal		*Bacteroidetes* and *Betaproteobacteria, followed by Actinobacteria*	Liu et al. (2011)
Kafni glacier, India		*Proteobacteria, Bacteroidetes, Actinobacteria, Chloroflexi, Spirochaetae, Tenericutes, Verrucomicrobia*	Srinivas et al. (2011)
Continental Antarctica and Antarctic Peninsula		*Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes, Deinococcus-Thermus*	Peeters et al. (2012)
Austrian Alps		*Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, Nitrospira, Cyanobacteria*	Wilhelm et al. (2013)
Pacific Ocean	Fungal	*Ascomycota, Basidiomycota*	Xu et al. (2014)
Jiulong River Estuary, China	Viral	*Pelagibacter phage HTVC010P, Puniceispirillum phage HMO-2011, Thalassomonas phage BA3*	Cai et al. (2016)
Alpine glacial-fed lake, Western Tibet, Kalakuli	Bacterial	*Actinobacteria, Proteobacteria, Verrucomicrobia, Firmicutes, Planctomycetes*	Liu et al. (2017)
Tietê River, Brazil	Fungal	*Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota, Zygomycota, Neocallimastigomycota*	Ortiz-Vera et al. (2018)
East China Sea (ECS)		*Byssochlamys, Aspergillus*	Li et al. (2018)
Lake Baikal	Viral	*Myoviridae, Poxviridae, Mimiviridae, Siphoviridae, Phycodnaviridae,* and *Podoviridae*	Butina et al. (2019)
Revelva River and Lake Revvatnet, Southwestern Spitsbergen, Norway	Bacterial	*Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, Tenericutes, Cyanobacteria,* and *Acidobacteria*	Kosek et al. (2019)
Antarctic freshwater	Fungal	*Ascomycota, Basidiomycota, Mortierellomycota, Mucoromycota, Chytridiomycota, Oomycota*	Ogaki et al. (2019)
Lake Magadi, Kenya		*Aspergillus, Penicillium, Acremonium, Phoma, Cladosporium, Septoriella, Talaromyces, Zasmidium, Chaetomium, Aniptodera, Pyrenochaeta, Septoria*	Orwa et al. (2020)

(continued)

Table 3.1 (continued)

Cold-water reservoir and its location	Diversity type	Abundant phyla/genera	References
Gulf of Gabès, Southern Mediterranean Sea	Bacterial	*Firmicutes, Proteobacteria, Bacteroidetes, Chloroflexi*	Jeddi et al. (2022)
Lake area of Huaibei, China		*Pseudomonadota, Bacteroidales, Chloroflexales, Acidobacteriales,* and *Firmicutes*	Shen et al. (2022)

of 49 different phyla, among which the most abundant bacteria were *Proteobacteria*, with Gamma-proteobacteria being noted in all sections of the core; this was followed by *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Chloroflexi*. *Lactobacillus*, *Profundibacterium, Shigella, Escherichia, Pseudoalteromonas, Vibrio, Propionibacterium, Alteromonas, Enterobacter, Sphingomonas*, and *Staphylococcus* were the major genera isolated. Gawas et al. (2019) identified 43 heterotrophic bacteria from Central Indian Ocean Basin including members of phyla *Proteobacteria, Actinobacteria*, and *Firmicutes*. This was one of the earliest reports of *Oceanobacillus* (Firmicutes*)* and *Brachybacterium* (Actinobacteria). *Bacillus subtilis* strain (G7) was isolated by Gu et al. (2019) from deep-sea hydrothermal vent. From deep-sea sediments of the Indian Ocean, primarily from the Bay of Bengal and the Andaman Sea, Padmanaban et al. (2019) recovered 34 bacterial isolates associated with *Firmicutes*, *Proteobacteria*, and *Actinobacteria*, with *Firmicutes* and *Actinobacteria* being the major phyla. Bottom water samples collected from Northwest Indian Ocean reported presence of a novel planctomycete, *Gimesia benthica* (Wang et al. 2020). Qiu et al. (2021) reported a halotolerant *Halomonas sedimenti* from the deep sea sediment of the Southwest Indian Ocean. A novel hydrogen- and sulfur-oxidizing chemolithoautotroph, *Sulfovorum indicum* was reported by Xie et al. (2019) from deep sea hydrothermal plumes in the Northwestern Indian Ocean. Yaylacı (2021) isolated 13 strains of *Bacillus* species from cagewater aquaculture. In a study from different sites at varying depths of the South Indian Ocean, bacterial species belonging to *Proteobacteria*, *Firmicutes*, and *Chloroflexi* along with uncultured species of archaebacteria belonging to the phyla *Thaumarchaeota* and *Euryarchaeota* were identified (Zhu et al. 2022).

In all natural ecosystems, including fresh and marine waters from the ocean surface to the deep sea, eutrophic to ultra-oligotrophic lakes, lagoons, rivers, ground waters, melting water, and glacier ice, fungi are omnipresent microorganisms that are a part of the microbiota (Grum-Grzhimaylo et al. 2015; Mokhtarnejad et al. 2016). *Aspergillus sydowii*, in both nonsporulating and sporulating forms, was reported by Raghukumar et al. (2004) in the Chagos trench in the Indian Ocean. Damare et al. (2006) isolated 181 fungal cultures from 5000 m depth in the Central Indian Ocean Basin and found that most were terrestrial sporulating species belonging to *Aspergillus*, *Penicillium*, and *Cladosporium* genera. Various studies from deep-sea environments including hydrothermal vents and the Mariana Trench have reported fungal isolates (Nagahama et al. 2008). Singh et al. (2010) isolated 16 filamentous and 12 yeast cultures, belonging to *Ascomycota* and *Basidiomycota*, from sediments of the Central Indian Ocean Basin at depths of 5000 m. This was the first account of the isolation of filamentous fungi (*Capronia*, *Exophiala*, *Sagenomella*, and *Tilletiopsis*) from deep-sea sediments, demonstrating the unique diversity of fungi that inhabit the Indian Ocean basin. A report on fungal diversity in the Indian Ocean sediments published by Singh et al. (2011) revealed 32 fungal taxa including majorly *Ascomycota* and *Basidiomycota*. Within the *Ascomycota*, members of *Sordariomycetes*, *Dothideomycetes*, and *Saccharomycetes* and members belonging to *Tremellomycetes*, *Microbotryomycetes*, and *Ustilaginomycetes* were identified within the *Basidiomycota*. Zhang et al. (2014) conducted investigation on the Eastern Indian Ocean and found that filamentous fungi such as *Aspergillus*, *Penicillium*, *Simplicillium*, *Cladosporium*, and *Phoma* dominated the culturable percentage. *Ascomycota* and *Chytridiomycota* were found to be the two primary phyla of the fungal diversity in the coastal water and sediment samples from North Carolina, coupled with a significant number of novel sequences (Picard 2017). According to a study by Fotedar et al. (2018), basidiomycetous and ascomycetous yeasts were approximately equally prevalent in hypersaline Inland Sea in Qatar. The three main phyla found in the deep-sea sediments of the Indian Ocean are *Ascomycota*, *Basidiomycota*, and *Zygomycota* (Xu et al. 2019). In a recent study, the abyssal zones of the Indian Ocean yielded fungal phylotypes primarily from *Aspergillus*, *Penicillium*, *Ophiocordyceps*, and *Phoma* (Tang et al. 2020). The fungal diversity and composition of the Continental Solar Saltern in Añana Salt Valley was studied by Azpiazu-Muniozguren et al. (2021), and a total of 380 fungal genera were detected. *Saccharomyces* was highly prevalent in the saltern, although other halotolerant and halophilic fungi like *Wallemia*, *Cladosporium*, and *Trimmatostroma* were also present.

In cold seep sediments of the Gulf of Mexico, it was discovered that virus-to-prokaryote ratios and viral-like particle counts were substantially greater than in nearby sediments, indicating that these habitats may be hot spots for viruses (Kellogg 2010). Double-stranded DNA (dsDNA) viruses, particularly bacteriophages, make up the majority of virioplankton communities in the ocean, according to previous virome-based studies (Etiope et al. 2014). Novel viruses have also been identified in methane seep sediments, including a novel sister lineage to the *Microvirus* genus of *Enterobacteria* phage and a putative archaeal virus linked to an anaerobic methane-oxidizing clade (Bryson et al. 2015). In the Antarctic surface oceanic area, *Caudovirales* makes up to 72% of the total dsDNA virus community (Kennicutt 2017). Five surface and two bottom water samples from Prydz Bay were analyzed by Gong et al. (2018), and the findings showed that the majority of the DNA viruses were dsDNA viruses. The nucleocytoplasmic large DNA viruses (*Phycodnaviridae*, *Mimiviridae*, and *Pandoraviridae* viruses) were most prevalent in the bottom water, while *Caudovirales* (*Siphoviridae*, *Myoviridae,* and *Podoviridae*) phages were most prevalent in the surface seawater. *Microviridae*, *Inoviridae*, *Cellulophaga*, and *Flavobacterium* phages were found among the ssDNA viruses**.** Wu et al. (2020) reported that the majority of viruses they found in the surface water of the East China Sea had the characteristic head-and-tail form. These viruses belonged to *Siphoviridae*, *Myoviridae*, and *Podoviridae* of the

Caudovirales order. Deep sea sediments associated with cold seeps studied by Li et al. (2021) revealed the presence of *Caudovirales* order, specifically *Podoviradae*, *Myoviradae*, and *Siphoviradae*.

3.2.2 Microbial Diversity of Lakes, Rivers, and Ponds

The study of inland waterways, such as lakes, rivers, ponds, and reservoirs, is crucial for determining how human activity and climate change affect ecosystem architecture. Due to their abundance, diversity, and metabolic activity, these microprobes dominate aquatic environments and play a key role in numerous ecological processes (Savio et al. 2015). In freshwater lakes with available food, microorganisms adapt to the conditions and exist in every zone (horizontal and vertical). Microbes have a significant impact on the various biogeochemical (C, N, P, S, and other elements) cycling pathways and global energy fluxes of deep reservoirs and small lakes in freshwater environments (Savvichev et al. 2018; Liu et al. 2019b).

In comparison to Lake Urmia, which primarily contained *Proteobacteria*, *Firmicutes*, and *Actinobacteria*, the culturable microbial diversity of the Aran-Bidgol saltlake contained isolates from the genera *Halorubrum*, *Haloarcula*, *Salinibacter*, *Salicola*, and *Rhodovibrio* (Kashi et al. 2014). In their study of the bacterial diversity and abundance in pond water in Hubei Province, China, Qin et al. (2016) discovered that *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, and *Actinobacteria* dominated the microbial communities, while potential pathogens such as *Acinetobacter*, *Aeromonas*, and some probiotics like *Comamonadaceae* unclassified and *Bacillales* unclassified were also present. After examining the microbial diversity in the hypersaline Lake Meyghan in Iran, Naghoni et al. (2017) discovered that *Haloarchaea* predominated in the high salinity brines, while bacteria dominated the low salinity brines, with *Alteromonadales* (Gammaproteobacteria) being a particularly significant taxon. Touka et al. (2018) conducted an analysis of the Ancient European Lake, The Lake Pamvotis, and found that the bacterial community comprised primarily *Proteobacteria* (β-, γ-, δ- and α-Proteobacteria), followed by phylotypes belonging to *Cyanobacteria*, *Nitrospirae*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Spirochaetes*, *Planctomycetes*, *Actinobacteria*, *Gemmatimonadetes*, and most of the sequences of Archaea belonged to *Euryarchaeota*. *Bacillus shivajii* sp. nov. was discovered by Kumar et al. (2018) in a water sample from the Indian Salt Lake Sambhar. In their study of the microbial diversity in river water and sediment in Hong Kong, Deng et al. (2018) found that the major phyla in the sediments were *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Acidobacteria*. The bacterial communities contained considerable proportions of *Crenarchaeota*, *Actinobacteria*, *Chloroflexi*, *Verrucomicrobia*, and *Planctomycetes*. In two renaturalized quarry lakes in Singapore, Kumar et al. (2019a) analyzed the microbial communities in the water and sediment and discovered that *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, *Firmicutes*, and *Bacteroidetes* represented for about 90% of the total reads. *Verrucomicrobia*, *Planctomycetes*, *Nitrospirae*, *Chloroflexi*, *Spirochaetes*, *Acidobacteria*, *Gemmatimonadetes*, and

Fusobacteria were the other main phyla, while *Thaumarchaeota*, *Crenarchaeota*, and *Euryarchaeota* were the three principal phyla that made up the domain Archaea. Bachran et al. (2019) studied the microbial diversity from Arava Valley and found that the bacterial diversity was mainly represented by the genus *Salinimicrobium* of the order *Flavobacteriales* within the phylum *Bacteroidetes*, from the gammaproteobacterial orders *Alteromonadales* and *Oceanospirillales* as well as members from the order *Bacillales* of the phylum *Firmicutes*. *Euryarchaeal Halobacteria* from the orders *Halobacteriales*, *Haloferacales*, and *Natrialbales* dominated the Archaeal diversity. Luo et al. (2020) studied the bacterial community structure along the Lancang River in southwest China and found out that *Proteobacteria* (primarily classes of *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*), *Bacteroidetes*, *Actinobacteria*, *Planctomycetes*, and *Firmicutes* were present in the sediment and water samples. Six archaeal phyla and a total of 28 bacterial phyla were spread throughout all samples from the three sites (Lakes Jackson and Talquin, and Pedrick Pond). *Actinobacteria*, *Proteobacteria*, *Cyanobacteria*, *Planctomycetes*, *Bacteroidetes*, and *Verrucomicrobia* are the primary phyla across all samples, according to changes in the relative abundance of dominating bacteria, while *Euryarchaeota* predominates in the archaeal phyla (Betiku et al. 2021).

According to a study by Sharma et al. (2016), 98% of the isolates found in Lonar Lake belonged to the phylum *Ascomycota*, subphylum *Pezizomycotina*. Rojas-Jimenez et al. (2017) examined the freshwater fungi that were found in ice-covered lakes in the McMurdo Dry Valleys of continental Antarctica and found sequences that, in descending order of dominance, represented taxa of the phyla *Cryptomycota*, *Chytridiomycota*, *Ascomycota*, *Basidiomycota*, traditional *Zygomycota*, and *Blastocladiomycota*. In their investigation on the diversity of fungi at the bottom of Lake Michigan and Lake Superior, Wahl et al. (2018) found that *Dothideomycetes*, *Eurotiomycetes*, *Leotiomycetes*, and *Sordariomycetes* accounted for about 84% of all isolates grouped by genus. A study by de Souza et al. (2021) evaluated the fungal diversity in two lakes of the South Shetland Islands and found 34 fungal taxa of the phyla *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Chytridiomycota* and *Rozellomycota*. Mwirichia (2022) explored the diversity of fungi in the soda lakes of Magadi, Elmenteita, Sonachi, and Bogoria in Kenya and discovered 107 genera belonging to the phyla *Ascomycota*, *Basidiomycota*, and *Glomeromycota*, including *Aspergillus*, *Penicillium*, *Acremonium*, *Phoma*, *Cladosporium*, *Septoriella*, *Talaromyces*, *Zasmidium*, *Chaetomium*, *Aniptodera*, *Pyrenochaeta*, *Septoria*, *Juncaceicola*, *Paradendryphiella*, *Phaeosphaeria*, *Juncaceicola*, and *Biatriospora*.

According to a survey done in the Jiulong River Estuary that connects to Xiamen Sea harbour, the two most prevalent phages were HTVC010P and HMO-2011, and *Caudovirales* was the dominant viral group in the viromes (Cai et al. 2016). The viral diversity of Lake Baikal's shoreline water was researched by Butina et al. (2019), who found that the virotypes belonged to six families (*Myoviridae*, *Poxviridae*, *Mimiviridae*, *Siphoviridae*, *Phycodnaviridae* and *Podoviridae*).

3.2.3 Microbial Diversity of Glaciers

A glacier is a sizable, slowly moving deposit of hard ice that has developed over the course of thousands of years of snow accumulation. The cryosphere, which is a vital component and sensitive indicator of climatic and environmental changes, includes the glacier as one of its constituent parts. Glaciers are regarded authentic biomes (Anesio and Laybourn-Parry 2012), in which microbial life persists despite a hostile environment and plays a crucial part in the functioning of the glacial ecosystems (Hodson et al. 2008). Glaciers harbor autotrophic, chemolithotrophic, and heterotrophic bacteria because of the intense solar radiation and low oxygen levels in the atmosphere (Lutz et al. 2017). These microorganisms have been specifically reported in antarctic regions, polar arctic regions, and in high mountains (Larose et al. 2013; Hotaling et al. 2017).

Branchy bacterium sp., *Acinetobacter* sp. and *Agrococcus* sp. were among the various bacteria identified by Zhang et al. (2007) from the glacial ice of the Himalayas' East Rongbuk (ER) core. The study on bacterial diversity in a glacier foreland of high arctic on Ny-Å lesund (West Spitsbergen, Norway) by Schutte et al. (2010) found *Acidobacteria*, *Chlamydia*, *Nitrospira*, *Chloroflexi*, *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, *Spirochaetes*, *Actinobacteria*, *Cyanobacteria*, *Verrucomicribia*, and *Drinococcus-Thermus* but *Spirosoma*, *Sphingomonas*, *Terromonas*, *Hymenobacter*, *Gemmatimonas*, *Brevundimonas*, and *Sphingopyxis* as dominated ones. *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Chloroflexi*, *Actinobacteria*, *Firmicutes*, *Gemmatimonadetes* and *Verucomicrobia* were found to be the most prevalent bacterial phyla in a study by Huang et al. (2013) on East Antarctica, whereas *Deinococcus-Thermus*, *Nitrospira*, *Candidate Division OP10*, *Planctomycetes*, *Candidate Division TM7*, and *Fusobacteria* showed comparatively lower abundance in snow meltwater samples. Peter and Sommaruga (2016) revealed the presence of *Sphingobacteria*, *Flavobacteria* and *Betaproteobacteria* as the abundant phyla on the Austrian Central Alps at glacier melting. In Livingston Island of Maritime Antarctica, Hodson et al. (2017) discovered *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Acidobacteria*, *Cyanobacteria*, *Actinobacteria*, and *Verrucomicrobia* as the dominant bacterial phyla. The Garhwal Himalaya is very wealthy in terms of the occurrence of glaciers which includes one of the biggest, the Gangotri glaciers. The psychrophilic microbial diversity of glaciers in the Garhwal Himalaya, India was studied by Kumar and Sharma (2021) and they found that most of the bacteria and actinomycetes isolated from the ice samples of the glaciers (Satopanth, Bhagirathi-Kharak and Gangotri) were gram positive and most of them were in form of rods, non-spore forming, non-pigmented and with filamentous branching. *Vibrio harveyi*, *Pseudomonas fluorescens*, *Microbacterium paraoxydans*, *Microbacterium scheliferi*, *Serratia marcescens*, *Paenibacillus azatofixans*, *Ralstonia eutropha*, and *Staphylococcus cohnii* were the bacterial species isolated, the actinomycetes included *Microbacterium avium*, *Streptomyces rangoon*, *Arthrobacter sulfonivorans* and fungal species included *Aspergillus nidulans*, *Cladosporium cladosporioides*, *Verticillium nubilum*, *Curvularia lunata* and *Phanerochaete chrysosporium*. Glaciers represent a key linkage between coasts and their downstreaming tidewaters and are of immense significance in land-to-ocean fluxes. Garcia-Lopez et al. (2019) studied the microbial communities in coastal Glaciers and Tidewater Tongues of Svalbard Archipelago, Norway and found that the glacier microorganisms mainly corresponded to the phylum *Proteobacteria*, *Bacteroidetes* and *Cyanobacteria* and the seawater microorganisms belonged to *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. Campen et al. (2019) carried out study on Taylor Glacier (Antarctica) that revealed *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* were the dominant bacterial phyla. In a glacial-fed Tibetan lake, bacteriological study conducted by Liu et al. (2019a, b) revealed *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* (Alpha and Beta), *Firmicutes* and *Cyanobacteria* were dominant phyla in the samples. Sharma et al. (2020) carried out research in the Polar Regions and the Tibetan plateau and concluded that *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Verrucomicrobia*, and *Actinobacteria* were the most dominant bacterial phyla. Study of two Tibetan Plateau ice cores revealed common glacier-ice lineages including *Janthinobacterium*, *Polaromonas*, *Herminiimonas*, *Flavobacterium*, *Sphingomonas*, and *Methylobacterium* as the dominant genera (Zhong et al. 2021).

3.3 Application of Microbes Isolated from Cold Water Reservoirs

Over 90% of the oceans have temperatures of 5 °C or lower and the potential use of psychrophilic bacteria as biotechnological tools remained a promise for decades. Despite actuality, they were more well known for the damage they inflicted, such as the spoilage of refrigerated food. Certainly, psychrophiles (and the metabolites they produce) are currently regarded as sustainable and priceless resources for the development of a wide range of biotechnological processes and/or products, many of which are already protected by patents or are subject to other forms of industrial secrets (Feller 2013; Arya et al. 2022).

The main areas of research and development of new processes and/or products related with psychrophilic microorganisms are listed below:

- Psychrophilic enzymes (food technology, molecular biology, bioremediation, and medicine)
- Psychrophilic biomolecules (dietary supplements, therapeutic, cosmetic, bioremediation, nutrition, and other uses)
- Pharmaceutical and medical uses generally focused on screening of new antibiotics, anticancer drugs, and cosmeceutical products
- Biostimulation or bioaugmentation in bioremediation: degrading contaminants such as unintentional petroleum spills or old waste disposal techniques

The microbial diversity of cold-water reservoirs and its applications are depicted in Fig. 3.1 and described in following sections:

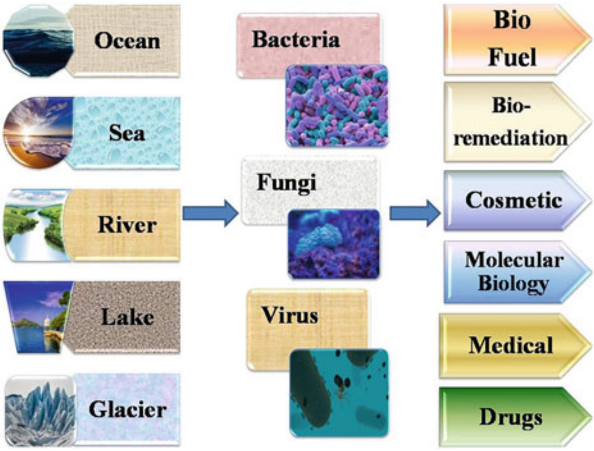

Fig. 3.1 Microbial diversity of cold-water reservoirs and their prospective applications in different fields

3.3.1 Bioremediation

A wide range of microbes may use hydrocarbons as their exclusive source of carbon and energy, that is, biodegradation. Regional psychrophilic microbial populations in cold habitats accomplish for low-temperature biodegradation of organic pollutants. They convert or mineralize organic contaminants into less toxic, nonhazardous chemicals (mostly through hydrocarbon bioremediation), which are subsequently incorporated into the biogeochemical cycles of the environment (Brakstad 2008; Filler et al. 2008). Cold climates present unique difficulties for hydrocarbon degraders, increased viscosity of liquid hydrocarbons, including decreased enzymatic reaction rates, decreased volatility of toxic compounds, restricted bioavailability of contaminants, low nutrient levels, and occasionally extreme salinity and pH (Margesin 2004; Aislabie et al. 2006). Various bioremediation techniques have been used to increase microbial activity during pollutant breakdown at cold temperature.

Ex-situ bioremediation and in situ bioremediation, two generally defined approaches, have been widely used for pollution remediation in cold climates, according to the implementation locations. The most popular ex situ bioremediation methods comprise biopiles, bioreactors, composting, and land farming, whereas in situ bioremediation includes natural attenuation, biostimulation, phytoremediation, bioaugmentation, and bioventing. There are some drawbacks to using psychrophilic in bioremediation in addition to their benefits. Before a bioremediation technique is implemented, a number of elements must be evaluated. Frigid temperatures, restricted access to contaminated areas in a hostile environment, and Geographic isolation make it difficult to successfully remove toxins. Therefore, while developing a soil treatment plan, it is important to take into account the primary limiting elements including pH, temperature, salinity, hydrocarbon bioavailability, nutrient availability, microbial population, soil moisture, and oxygen level (Chaudhary and Kim 2019).

A specific genetically modified organisms (GMO) psychrophile is renowned for bioremediating organic pollutants (such as hydrocarbons and toluene) from cold environments. It was developed by transforming the TOL plasmid from *Pseudomonas putida*. Examples include the recombinant expression in the Antarctic *Pseudoalteromonas haloplanktis* of the gene encoding a monooxygenase involved in the breakdown of aromatic hydrocarbons from the mesophile *Pseudomonas stutzeri* (Siani et al. 2006). Due to their capacity to break down large quantities of organic compounds in a little period of time at cold elevations, psychrophilic bacteria and fungi also function as inocula for wastewater treatment. One such cold-adapted *Arthrobacter psychrolactophilus* strain increased the biodegradability of organic molecules while completely clarifying a synthetic wastewater turbid medium by hydrolyzing proteins, carbohydrates, and lipids (Gratia et al. 2009). Further evidence is the complete phenol degradation by psychrophilic bacteria (*Rhodococcus* sp.) and yeasts (e.g., *Rhodotorula psychrophenolica*) at 10 °C in fed-batch cultivation (Margesin et al. 2005).

Owing to their physiological adaption to cold temperatures, psychrophilic enzymes are also utilized in bioremediation. Despite those challenging circumstances, cold-active enzymes, which are ten times more active than mesophilic enzymes, help to break down xenobiotics. They demonstrate the three benefits of biotechnology listed below:

1. High activity: A full reaction may be achieved with a lower dose of psychrophilic enzymes, which lowers overall experiment costs.
2. Cold activity: Since they continue to function at room temperature, processes do not require high temperatures.
3. They are heat labile and so quickly become inactive at higher temperatures.

These psychrophilic microorganisms can be utilized in the appropriate consortia throughout the winter to remove contaminants from soil or wastewater. They have the capacity to operate effectively in cold habitats. This enzyme's broad-spectrum activity makes it acceptable for use in a variety of industrial processes as well as in the bioremediation of soil that had been contaminated by effluents from food-processing plants (Kumar et al. 2019a, b).

3.3.2 As a Source of Novel Drugs

Through synthesizing and excreting secondary metabolites, many free-living microbes can colonize a certain habitat by eliminating, impeding, or distracting their rivals and/or predators (Mojib et al. 2010). The majority of actinomycetes, bacteria, and fungi are recognized for producing a variety of secondary metabolites that are significant for medicine, including antibiotics, antifungal, antiprotozoal, antiviral, and antihelminthic substances (Baltz 2007). Numerous psychrophilic microorganisms have been demonstrated to possess such antibacterial properties recently, including *Candida albicans* and methicillin-resistant *Staphylococcus* *aureus* that were suppressed by spore-forming and cold-adapted bacteria (Vollú et al. 2014). However, certain unprocessed extracts of various Antarctic microorganisms have antibacterial properties, such as ethanolic extracts of Antarctic fungus and organic extracts of Antarctic bacteria (Gonçalves et al. 2015; Godinho et al. 2015). Gesheva and Vasileva-Tonkova (2012) isolated glycolipids and lipopeptides (antimicrobial/antifungal compounds) from *Nocardioides* sp. A-1 (halophilic Antarctic actinomycete) that inhibit growth of *Candida tropicalis*, *Bacillus subtilis*, *S. aureus*, *Sarcinalutea*, and *Xanthomonas oryzae*. Actinomycetes bioactive metabolites prevent the growth of *Pseudomonas aeruginosa*, methicillin-resistant *S. aureus*, and *C. albicans* (Lee et al. 2012). Gram-positive and Gram-negative phytopathogens are both inhibited by the metabolites generated by *Streptomyces* species, including aromatic polyketides, glycopeptides, and polyenes (Encheva-Malinova et al. 2014; Giudice and Fani 2015). The antifungal and antimicrobial properties of Amphotericin B from *Penicillium nalgiovense* Laxa are comparable. It inhibits the growth of *C. albicans* and *S. aureus* (Svahn et al. 2015). In 2013, Bhattarai et al. isolated the pseudodepsidone metabolite, an antibacterial chemical from *Stereocaulonalpinum* lichens that inhibits the growth *of S. aureus* and *B. subtilis*. Similar to this, the cold-tolerant killer toxin from the *Mrakiafrigida* yeast has antifungal action against the fungi *Metschnikowiabicuspidata*, *C. tropicalis*, and *Candida albicans* (Hua et al. 2010; Liu et al. 2012).

Since many years ago, colored bacteria have also been known to produce antimicrobial substances. For example, *Janthinobacterium* sp., which was discovered in Antarctica in 1991, produces violacein, a violet pigment with multiple therapeutic properties, including antitumoral, antibacterial, antileishmanial, and antiviral activities (Shivaji et al. 1991; Soliev et al. 2011). According to this, *Mycobacterium smegmatis* and *M. tuberculosis* development are inhibited by *Janthinobacterium* sp. Ant5-2 (Mojib et al. 2010). This inhibitory action was linked to additional substances that may work in concert with the pigment and was not only a result of the pigment being present. Antarctic yeasts of the *Dioszegia*, *Cryptococcus*, *Exophiala*, *Microglossum*, and *Rhodotorula* genera produce pigments such as carotenoids and mycosporines to defend themselves from UV exposure (Vaz et al. 2011). Melanin, a different photoprotective pigment, has been isolated from the bacteria *Lysobacter oligotrophicus* (Kimura et al. 2015). An enzyme from *Chlamydomonas* sp. called photolyase is also employed to protect against light (Li et al. 2015).

Some marine isolates have also been found to produce bioactive compounds. For example, *Burkholderia cepacia* is entirely inhibited from growing by *Pseudoalteromonas* strains obtained from the Ross Sea through the production of different volatile organic compounds (VOCs) (lipophilic chemicals) (Lo Giudice and Fani 2015). Parallel to this, bacteria found in sponges produced microbial VOCs that have antibacterial effects on *S. aureus, P. aeruginosa*, *Xanthomonas campestris*, and *Clavibacter michiganensis* (Chávez et al. 2015). Similarly, four asterric acid nitro derivatives from *Pseudogymnoascus* sp. that were isolated by Figueroa et al. (2015) have potential uses in medicine. High and specialized antibacterial activities were exhibited by fungi isolated from different microalgae. For example, the dangerous fungi *Cladosporium sphaerospermum* and *Trypanosoma cruzitrypomastigotes* were both suppressed by *Penicillium* sp. (Godinho et al. 2013). Furthermore, a *Penicillium steckii* ethanoic extract reduced interferon alpha (IFN-) activity by 68% and yellow fever virus proliferation by 96% (Furbino et al. 2014).

Numerous research organizations have worked on the antioxidant capabilities of natural products in light from some promising findings suggesting that regular eating foods high in antioxidants might prevent or delay the development of such diseases. With similar approach, current research efforts have been concentrated on the quest for novel microbial metabolites with improved antioxidant properties from cold biological resources (Yarzábal 2016). For example, lobaric acid (depsidone), which was derived from the lichens *S. alpinum* and *Cladonia* sp., shows high antioxidant capabilities and gastroprotective effects (Bhattarai et al. 2013). Also, ramalin [γ-glutamyl-*N*′-(2-hydroxyphenyl) hydrazide] from *Ramalinaterebrata* (lichen) (Paudel et al. 2011) and β-carotene, ergosterol, torulene, torularhodin, and CoQ10 from *Sporobolomyces salmonicolor* AL1 (yeast) (Dimitrova et al. 2013) inhibit the tyrosinase enzyme activity with low cytotoxicity in human. Some microbes with their metabolites along with its applications are summarized in Table 3.2.

3.3.3 Probiotic Potential

Probiotics are defined by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." According to the U.S. Food and Drug Administration (FDA), the basic guidelines for storage include a working refrigerator temperature at or below 4 °C, while the freezer compartment should be −18 °C (Mojikon et al. 2022). Therefore, all household and laboratory refrigerator temperatures should operate at 4 °C as a means to prevent or slow down the growth of foodborne pathogens such as *Salmonella, L. monocytogenes*, and *C. botulism*. Low temperatures slow down metabolism and extend the log or stationary phase of the mesophilic bacterial growth curve (Madigan et al. 2005; Mojikon et al. 2022).

As probiotics, psychrophiles are believed to have potential for use as dietary supplements in aquaculture to improve health and nutrition of livestock. While studies in this are scarce, their adaptation to low temperatures is suggested to be beneficial for a more efficient utilization in the marine habitat as compared to currently available terrestrial and/or moderate temperature adapted probiotic organisms (Collins and Margesin 2019). *Bacillus*, Lactic acid bacteria (LAB), and *Bifidobacteria* have been intensively employed as probiotic strains due to their recognition as members of the indigenous microflora of the animals, safety, and the evidence supporting their positive role (Lee et al. 2012). Marine cold-adapted bacterium, *Psychrobacternamhaensis* SO89, is used as putative dietary probiotic in Nile tilapia (*Oreochromisniloticus*) feeds (Makled et al. 2017). Biomel, GT's Organic Kombucha, KeVita Apple Cider Vinegar Tonics, and VitaCup Immunity

Table 3.2 Applications of microbes isolated from cold water reservoirs

Microorganism	Metabolite	Application	References
Streptomyces sp. LPB2019K190-4	–	Antimicrobial	Pereliaeva et al. (2022)
Rhodococcus sp. LPB2019K201-3			
Microbacterium sp. LPB2019K198-2			
Aeromonas veronii NS07	Amylase	Detergent and textile industries	Bauvois et al. (2008)
Marine Bacterium Strain YS-80-122	Protease	Peeling of leather	Wang et al. (2010)
Pseudoalteromonas haloplanktis	Cellulase	Biodegradation	Violot et al. (2005)
Rhodococcus erythropolis BZ4	Primary and secondary metabolites	Bioremediation (*n*-alkanes, phenol, anthracene, pyrene)	Margesin et al. (2013)
Rhodococcus cercidiphyllus BZ22			
Arthrobacter sulfureus BZ73			
Pimelobacter simplex BZ91			
Pseudomonas peli N3–6P	Primary and secondary metabolites	Bioremediation (petroleum hydrocarbons)	Cai et al. (2014)
Streptomyces venezuelae N3-6A			
Halomonas variabilis N3-8A			
Rhodococcus phenolicus N1–1P			
Bacillus subtilis N2–3P			
Acinetobacter oleivorans P7-1A			
Rhodococcus erythropolis N2–4P			
Rhodococcus erythropolis P6–5P			
Alcanivorax venustensis N3-7A			
Exiguobacterium antarcticum N4–1P			
Acinetobacter calcoaceticus P9-1A			
Roseovarius NS163			

Table 3.2 (continued)

Microorganism	Metabolite	Application	References
Halomoans NS159	Primary and secondary metabolites	Bioremediation (crude oil, TPH, PAH)	Chronopoulou et al. (2015)
Glaciecola NS168			
Nocardioides sp. A-1	Glycolipids and lipopeptides (antibiotics)	Antimicrobial	Gesheva and Vasileva-Tonkova (2012)
Janthinobacterium sp. Ant5-2	Purple violet pigment	Antimicrobial	Huang et al. (2012)
Flavobacterium sp. Ant342	Flexirubin	Antimicrobial	Mojib et al. (2010)
Pseudoalteromonas sp.	Microbial volatile compounds	Antimicrobial	Maida et al. (2015)
Guehomyces pullulans	Crude and ethanolic extract	Antimicrobial	Furbino et al. (2014)
Metschnikowia australis			
Dipodascus australiensis			
Pseudogymnoascus sp.			
Penicillium steckii			
Geomyces sp.	Methanol crude extracts	Antimicrobial	Henríquez et al. (2014)
Aspergillus sydowii	Organic extracts	Antimicrobial	Godinho et al. (2015)
Penicillium allii-sativi			
Penicillium chrysogenum			
Penicillium rubens			
Stereocaulon alpinum	Lobaric acid, lobastin	Antimicrobial	Bhattarai et al. (2013)
Dioszegia, Cryptococcus, Exophiala	Carotenoids and mycosporines	UV protection	Vaz et al. (2011)
Microglossum, Rhodotorula			
Janthinobacterium sp.	Violacein (purple violet pigment)	Antimicrobial	Mojib et al. (2011, 2013)
		UV protection, cryoprotection	
Ramalinaterebrata	Usimine-like compounds	Antiaging (fibroblast cell proliferation)	Lee et al. (2010)

Coffee Pods contain *Bacillus coagulans*, which can reproduce by spore formation. The use of spores is beneficial in the manufacturing and storage process of probiotic drinks, as the *B. coagulans* spores are resistant to heat, cold temperatures, and stomach acid and remain dormant in the juice but germinate once it passes through the gastric compartment (Mojikon et al. 2022). *Lactobacillus algidus* sp. nov. was isolated from vacuum-packaged refrigerated beef (Kato et al. 2000). *L. casei*, *L. plantarum*, and *L. delbrueckii* were isolated and identified as probiotic culture (Yoon et al. 2006). Kasimin et al. (2020) isolated two psychrophiles strains of bacteria (*Lactobacillus* sp. strains CA1 and CA4) capable of producing antimicrobial substances from dairy products and raw milk.

3.4 Conclusion and Future Perspectives

Microbes isolated from cold reservoirs produces more novel bioactive primary and secondary metabolites compounds viz. polypeptides, macrolactones, polyketides, isocoumarins, etc., compared to terrestrial. Although majority of bacteria, fungi, and some viruses isolated from cold-water reservoirs produce metabolites with potential biotechnological applications. Nevertheless, one of the main challenges to guarantee a sustainable use of cold biological resources is still related to how to allow positive benefits from bioprospecting without incurring significant harm to the environment. Uncontrolled prospecting activities, including the logistical efforts necessary to access the natural resources, and the impact associated with them could prove in fact to be unsustainable and to severely compromise Antarctic microbial habitats. On the other hand, commercially oriented research activities and the secrecy surrounding them may compromise one of the main pillars.

The psychrophiles were formerly considered as potential resources for the advancement of several biotechnologies. Today, a significant portion of this promise has materialized, in part due to the research of psychrophilic microbes. This promise looks to be far bigger than for thermophiles when taking into consideration the broader psychrophilic biodiversity, which includes bacteria, plants, and animals, as well as the extensive regions of application. The sustainable and intelligent use of these biological and genetic resources can help other disciplines of knowledge, such as bioremediation, food, agriculture, nanotechnology, medicine, and energy production. However, the sustainable exploitation of these resources necessitates the creation of new regulations to mitigate the potential environmental damage that unregulated prospecting operations may create. Last but not least, the majority of psychrophile biotechnological applications are energy and environmentally friendly, both of which are becoming more and more important.

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Overview of Microbial Associations and Their Role Under Aquatic Ecosystems

4

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Abstract

Water plays a vital role in regulating the lives of living beings on earth. Life cannot be imagined without water. Aquatic plants inhabit the littoral or shallow water zones of waterbodies like river, ponds, lakes, and oceans. These plants exhibit a mutual coexistence, pathogenic infestation, commensalism, or in symbiotic association with the microbes. These plants also serve as powerful tools for removal of contaminants in the form of heavy metals in water and soil sediments. The phyllosphere consists of various microbes such as bacteria, viruses, protists, ecto- and endoparasites, nematodes, protozoa, etc. Although the zone between the microbes and roots of aquatic plants is not well defined in terms of nutrients due to their diffusion in water, still there exists a zone of interaction between them. Microbes coevolved with the plants for the fulfillment of their nutrient deficiencies in their fundamental niches. Thus, the aquatic micro biome plays a vital role in influencing and promoting the aquatic ecosystem. The plant–microbe interaction is also affected by the environment: both biotic and abiotic factors have a concomitant effect on the marine ecosystem, thereby leading to change at molecular and gene expression levels causing production of compounds by these microbial–host–environmental interactions. This chapter focuses on how the aquatic microbiomes influence the structure, growth of plants, and their diversity. It also encompasses the the molecular strategy adopted for production of

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metabolites and biofilm, siderophores, system of quorum sensing, cell to cell signaling, signal transduction, etc.

Keywords

Aquatic plants · Phyllosphere · Microbial interaction · Microbiome

4.1 Introduction

At some point of aquatic plant decomposition, plant residues launch vitamins into the water frame (Zhang et al. 2018). Some plant remember bonds with biofilm populating benthic surfaces, making submerged sediments the very last material receptor for plant residue (Wan-Lei et al. 2018). The discharge of this cloth and its succeeding migration alters benthic cloth composition (DeBusk and Reddy 2005), and drives benthic sediment network turnover (Pratiksha et al. 2020) and inhibits the performance of nutrient Elimination in sediments. Destroyed particularly by species opposition, predation, and mutualism, community turnover is pondered inside the network meeting (Yang et al. 2018). This herbal succession is Crucial to benthic geochemical balance, even though variation inside the External surroundings results in complicated, multilevel community Responses (Rocha et al. 2019). As pioneer indicators of aquatic outside pollution, benthic Prokaryotic microbial groups (archaea and microorganism) play an essential position in the elimination and stream of key vitamins (carbon, nitrogen, phosphorus, and sulfur) (Le et al. 2016) and assist in maintaining the stability of the aquatic biosphere (Mendonça et al. 2017). Long-term or sudden material entry from plant residue Release or the pollution of exogenous Escherichia coli (Gu et al. 2020) appreciably alters the composition, distribution, and biogeochemical capabilities of the benthic community. Variant of benthic microbial network in pond ecosystems, but, is confined by way of water frame length (Verpoorter et al. 2014). Scattered small ponds are broadly dispensed in agricultural areas (Holgerson and Raymond 2016). As a matter of reality, these ponds nevertheless have complicated ecological restorative capabilities, in spite of their small length (Mendonça et al. 2017). But, external pollution might result in pond surroundings dysfunction. Water bodies in agricultural regions are threatened by using wastewater from aquaculture and animal husbandry, that may be rich in excessive *E. coli* (Jiang et al. 2015). Previous researches advocate that *E. coli* can live to tell the tale and accumulate in benthic environments for a giant period following infection (Kadir and Nelson 2014). Additionally *E. coli* can reproduce in secondary habitats (Jang et al. 2015; Sadowsky and Whitman 2011), which include surface water and sediments (Bergholz et al. 2011). Under a regime in which *E. coli* invasion is constant and steady, interspecific interplay (e.g., competitive absorption of materials) with indigenous benthic microbes (e.g., Geochemical purposeful groups) happens constantly (Wanjugi and Harwood 2012). To account for this regime, indigenous microbial groups searching for noncompetitive assets (Mallon et al. 2018). The environmental tolerance of *E. coli* improves which includes the secondary succession process of the Benthic community which will increase the length and depth of aquatic surroundings damage (Xiao et al. 2016; Maal-Bared et al. 2013). Even unsuccessful exogenous invasions produce lengthy structural variation in indigenous groups (Mallon et al. 2015). Exogenous *E. coli* has a sizeable impact on bacterial community Shape and the removal of nitrogen (N) in each growing (Gu et al. 2020) and decomposing plants (Wu et al. 2021). Thus caused hinderance in aquatic plant purification and recovery functions, and releases an abundance of vitamins to the water device (Bing et al. 2019). The blended effects of continuous Nutrient enter and *E. coli* pollution triggers the strain response of The benthic prokaryotic network (Mondav et al. 2017), and results In: versions in interactions (reciprocity and opposition between bacterial and archaeal network); the stability and Turnover of the benthic microbial community; and shifts in Geochemical niche shape. An analysis of benthic prokaryotes at the community-degree underneath exogenous *E. coli* pollution and accelerated nutrient input elucidates the complex mechanisms of Small-scale aquatic ecosystems, and has capability ramifications for Eutrophication (Li et al. 2021).

4.2 Aquatic Plants as a Natural Source of Antimicrobial and Functional Ingredients

Marine plants survive and thrive in a competitive and hostile environment by living in complex colonies and in close proximity to others. Aquatic plants have evolved physiological adaptations, such as the production of bioactive chemicals, which provide them protection against grazers. As a reaction to ecological pressures such as rivalry for space, predation, and tidal changes, they create complex secondary metabolites. Some secondary metabolites or chemicals generated by aquatic plants may be effective in preventing harmful bacteria from growing. Anticoagulant, antiviral, antioxidant, antihelminthic, antibacterial, antifungal, anticancer, and anti-inflammatory actions have been reported for photochemical substances generated from aquatic plants, crude extracts, and their partially purified or pure components (Pérez et al. 2016).

Seaweeds are also regarded as one of the potential aquatic plants, and have been proposed as a feasible and sustainable source of biofuel production that does not disrupt the world food supply, as well as having a variety of pharmacological, industrial, and biotechnological uses. Seaweeds are also beneficial to mankind in a variety of ways, including as a resource of medications, dietary supplements, toxic products, and a possible biofuel contender (Bast 2013).

4.3 Functional Foods and Nutraceutical Products

Due to its elevated nutritional and pharmaceutical values, seaweeds have long been used as food, sea vegetables, or herbal medicine to treat and prevent a variety of diseases and disorders (Cermeno et al. 2019; Kang et al. 2019; Okolie et al. 2018).

They are also utilized as animal feed, fertilizer, fungicides, herbicides, sauces, dietary supplements, and a reservoir of agar, alginate, and carrageenan for a variety of industrial and medicinal uses. It is generally known that seaweeds have been quite successfully used as a protein source for several decades, particularly in impoverished nations. Due to the availability of critical amino acids in greater proteins, seaweeds have now become a cheaper option source of protein in recent years (Pangestuti and Kim 2015; Peng et al. 2015). On a dry weight basis, the protein content of the principal seaweed species ranges from 10 to 40%. It was also mentioned that the largest protein content in seaweeds was discovered in the winter and the lowest in the summer.

Summer's low protein content might be attributed to the degradation of phycobiliproteins, which make up the majority of the seaweed's proteins. In comparison to green and brown seaweeds, red seaweeds are said to have a higher protein content. Red seaweed's protein level is sometimes compared to that of protein-rich foods like soybeans (Kim et al. 2011a, b). The majority of seaweed proteins are divided into two functionally active categories: lectin and phycobiliproteins (Pangestuti and Kim 2011). Light-harvesting pigments found in red seaweeds (phycocyanins, allophycocyanins, and phycoerythrins) are often utilized as fluorescent probes in scientific research (Glazer 1994; Sekar and Chandramohan 2008).

Seaweed proteins' structure and biological actions are still poorly understood. However, the majority of seaweed bioactive elements, including proteins, are intracellular and protected by a very stiff and structurally complex cell wall, making effective extraction and digestion of seaweed-derived protein fractions difficult. The presence of polysaccharides in seaweed protein makes it very cohesive, according to the researchers (Adalbjörnsson and Jonsdottir 2015; Admassu et al. 2018; Fleurence et al. 2012; Harnedy and FitzGerald 2013; Wijesinghe and Jeon 2012). As a result, novel emerging technologies like microwave-assisted extraction, supercritical fluid extraction, pressurized solvent extraction, ultrasound-assisted extraction, pulsed electric field–assisted extraction, and enzyme-assisted extraction have recently been used to extract proteins with higher yields and desirable functional properties (Jiménez-Escrig et al. 2011; Samarakoon and Jeon 2012).

4.4 Bioactive Compound from Aquatic Plants

In a wide range of sectors, including food, pharmaceutical, cosmetic, nutraceutical, and biomedicine, most aquatic plants (particularly seaweeds) are frequently employed as prospective and important sources of bioactive chemicals (Peng et al. 2015). Bacterial, antifungal, antimicrobial, antiviral, as well as other biological characteristics are all present in these bioactive substances (Khalid et al. 2018). Phenolics, sulfated macromolecules, organic acids, and complex combinations of phytochemicals with antibacterial and functional characteristics are the substances responsible for these actions (Gupta and Abu-Ghannam 2011). Chemical and biological processes (gastrointestinal digestion, food processing, or fermentation) can release seaweed-derived, protein-based bioactive peptides. The most frequent approach for releasing biocompatible hydrolysates and peptides from diverse sources is enzymatic hydrolysis (Rani and Pooja 2018; Rani et al. 2018).

The biological and highly functional actions of peptides are generally determined by the location and composition of their amino acids. Peptide bioactivity is also influenced by the protein's main sequence and the selectivity of the enzymes that discharge the peptides from the parent sequence (Pal and Suresh 2016; Rani et al. 2018). The patterns of biological and functional properties of peptides are most likely influenced by their structural features. Tyrosine, phenylalanine, tryptophan, proline, valine, leucine, lysine, isoleucine, and arginine all have a considerable impact on peptide binding to angiotensin-converting enzyme (ACE) (Pooja et al. 2017; Rani et al. 2018). Antimicrobial peptides' actions are linked to positively charged residues. Histidine, leucine, tyrosine, methionine, and cysteine amino acid residues are related to radical-scavenging activity (Pal and Suresh 2017).

The necessary amino-acid profiles of red, brown, and green seaweeds are well balanced. The glutamic acid concentration is higher in most brown seaweeds. To increase protein digestibility of seaweed fibers as well as refining process of bioactive peptides, plant, animal, and microbial enzymes can also be used. The commercialization of seaweed-derived bioactive peptides has potential applications in a variety of industries, including the nutraceutical and pharmaceutical industries (Rani et al. 2018; Samarakoon and Jeon 2012).

4.5 Categories of Macrophytes

Macrophytes are a diverse group of photosynthetic organism found in water bodies. They include bryophytes (mosses, liverwort, etc.), pteridophytes (ferns), and spermatophytes (flowering plants). An assemblage of macrophytic vegetation consists of emergent species whose vegetative parts emerge above the water surface, submerged and floating species, with each ecological group having specific features in morphology and physiological processes. A wide range of the adaptive mechanisms developed by aquatic macrophytes at the morphological, physiological, and biochemical levels enables them to inhabit various types of freshwater, brackish-water, and marine habitats. The aquatic ecosystem is composed of aquatic flora and fauna, which interact with each other in maintaining the aquatic ecosystem. The diverse and heterogeneous group of macrophytes has posed a challenge for definition and classification. Macrophytes can be loosely defined as all forms of macroscopic aquatic vegetation visible to the naked eye. This is in contrast to microphytes, that is, microscopic forms of aquatic plants, such as planktonic and periphytic algae. The fact that macrophytes live in aquatic environments, at least seasonally, makes them different from terrestrial plants that do not tolerate flooded environments. The macrophytes include taxonomically very diverse representatives: macroalgae (e.g., *Chara* and *Nitella*), mosses and liverworts (e.g., *Sphagnum* and *Riccia*), and vascular plants. Vascular plants represent the largest group of macrophytes including aquatic ferns (*Azolla, Salvinia*), Gymnosperms (rare), and Angiosperms, both monocots and dicots (Rejmánková 2011). Submerged macrophytes represent the

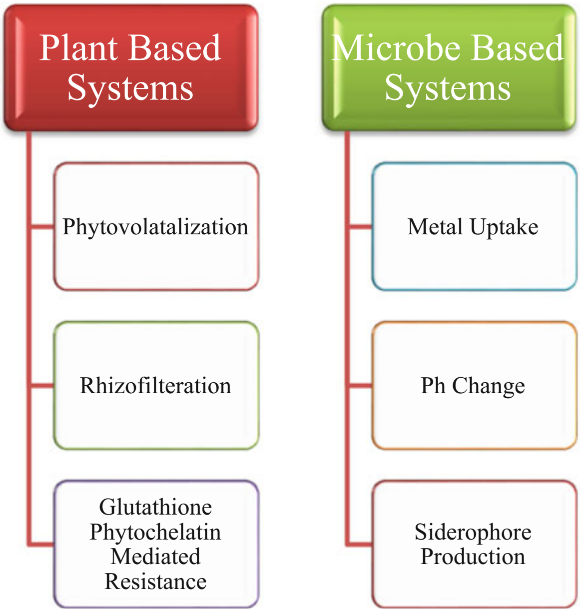


Fig. 4.1 Comparison between plant-based and microbe-based system in nutrient acquisition and accumulation

major component in aquatic ecosystems and help shape the physical and chemical environment, as well as the biota (Jeppesen and Søndergaard 1999). Aquatic macrophytes are aquatic photosynthetic organisms, large enough to see with the naked eye, that actively grow permanently or periodically submerged below, floating on, or growing up through the water surface. Aquatic macrophytes are represented by seven plant divisions: Cyanobacteria, Chlorophyta, Rhodophyta, Xanthophyta, Bryophyta, Pteridophyta, and Spermatophyta (Chambers et al. 2007). Arber (1920) and Sculthorpe (1967a, b) classified macrophytes into four different categories (Fig. 4.1) depending on their growth forms:

Emergent macrophytes: Plants rooted in soil and also emerging to a significant height above water. Submerged macrophytes: Plants grow below the surface water including few ferns, numerous mosses, and some angiosperms. Free-floating macrophytes: Plants that are not rooted to the substratum and float on the surface of the water. Floating leaf macrophytes: Plants occur in submerged sediment, and leaves are floating with long flexible petiole on the surface, which mainly include angiosperm. Besides the Arber and Sculthorpe ecological classification of macrophytes, several authors attempted to classify macrophytes into functional types (Boutin and Keddy 1993; Brock and Casanova 1997; Weiher et al. 1998). Plant functional types can be defined as sets of plants exhibiting similar responses to environmental conditions and having similar effects on the dominant ecosystem processes (Diaz and Cabido 1997; Lavorel et al. 1997). Macrophytes are important in the aquatic carbon cycle and play as primary producers a crucial role in carbon storage in aquatic systems. Macrophytes play an important role in the freshwater ecosystem functioning of many shallow water bodies: as primary producers, by providing structure in the habitat of many animal species, and provide shelter and food to invertebrates (Castella et al. 1984) and fish (Rossier 1995). Macrophytes, which are major primary producers in shallow freshwater systems, have been reported to contribute substantially to biodiversity at the ecosystem level (Zeng et al. 2012). Macrophytes are also involved in ecosystem processes such as biomineralization, transpiration, sedimentation, elemental cycling, materials transformation, and release of biogenic trace gases into the atmosphere (Carpenter and Lodge 1986). Recent studies have established the importance of aquatic macrophytes in regulating the nutrient availability in the water and enhancing the stability of lakeshores (Carpenter and Lodge 1986; Blindow et al. 2014). However, macrophytes are affected by increasing carbon concentrations (Reitsema et al. 2018). Aquatic macrophyte plants are widespread throughout the globe. Most macrophyte species are cosmopolitan, while groups of closely related species are known to replace each other in aquatic ecosystems of different parts of the world (Santamaría 2002., Zhang et al. 2019). Aquatic macrophytes are characterized by diverse growth forms and plasticity of physiological and metabolic processes, depending on changes in environmental conditions. Macrophytic vegetation includes deep-water and shallow-water species and species that actively grow in water in an emergent, submerged, or floating state. Some species are strictly aquatic, because they need to be submerged in water to complete their life cycle, while other species possess the ability to grow and reproduce in periodically inundated environments, can adapt to changes in water level, or inhabit the so-called ephemeral water bodies (e.g., floodplains, temporary springs, ponds, etc.) (Cook 1999., Jackson et al. 2009). Aquatic macrophytes play a significant role in freshwater ecosystems as they provide food and shelter to invertebrates (Rejmankova 2011) and stabilize sediments and shorelines, thus reducing turbidity of aquatic systems (Bamidele and Nyamali 2008). Submerged macrophytes affect nutrient dynamics, light attenuation, temperature regimes, hydrodynamic cycles, and substrate characteristics (Rooney et al. 2005).The macrophytes are responsible for the regulation and stabilization of mineral cycling in the water bodies, and hence, they serve as indicators for the possible degree of damage in the ecosystem (Pieczynska and Ozimek 1976). The aquatic plants are the drivers of ecosystem productivity and biogeochemical cycles, in part because they serve as a critical interface between the sediments and the overlying water column (Carpenter and Lodge 1986). Aquatic macrophytes influence metal retention indirectly by acting as traps for particulate matter, by slowing the water current, and favoring sedimentation of suspended particles (Kadlec 2000). Aquatic macrophyte also reduces sediment resuspension by offering wind protection (Brix 1997). Large aquatic macrophytes possess the ability to break down the human- and animal-derived pollutants in the water.

Macrophytes affect aquatic ecosystems in a variety of ways, especially the shallower ones where they colonize large areas. These plants change the water and physicochemical properties of sediments, influence nutrient cycling, may serve as food for aquatic invertebrates and vertebrates, both as leaves and dead biomass (detritus) and, in particular, change the spatial structure of the waterscape by increasing habitat complexity (Thomaz et al. 2004). Additionally, aquatic macrophytes are divided into several ecological groups according to their relation to environmental factors: hydatophytes (submerged species), pleistophytes (species with floating leaves), and helophytes (emergent aerial-water species) (Roshchyna 2018). Macrophytes growing at the margins of lakes or rivers and often facing challenges associated with fluctuations in water level are usually classified as a separate ecological group. These plants, commonly referred to as amphibious species, are capable of growth and reproduction in both aquatic and terrestrial environments. Amphibious plants have developed adaptations that allow them to withstand rapid emersion and submersion, including the plasticity of the leaf shape and photosynthesis process (Li et al. 2019; Maberly and Madsen 2002; Van Veen and Sasidharan 2021). Aquatic macrophytes are endowed with special adaptations to life in the submerged conditions or on the water surface. In addition to the climatic factors, such as light and temperature, inorganic carbon availability and specific factors related to aquatic environment determine the assembly and distribution of aquatic macrophytes (Barko et al. 1986). While limitations of the amount of CO_2 and sunlight for photosynthesis rarely occur in emergent macrophytes and those floating on the water surface, the growth of submerged macrophytes is strongly affected by the amount of penetrated light and the level of gas saturation (Hossain et al. 2017). Aquatic macrophytes are found mainly at depths of 0–4 m; however, their occurrence at depths exceeding 6 m has been reported, with vascular plants being found at maximum depths of about 12 m (Bornette and Puijalon 2011, Gradstein et al. 2018). In deep-water habitats, mosses frequently constitute the dominant vegetation owing to their better adaptability to lower light intensities and lower temperatures compared with vascular plants (Brönmark and Hansson 2017). An important restrictive factor for the growth of submerged macrophytes is the depth of light penetration. It is considered that the depth limit for their distribution occurs when water transparency allows <1–4% of light to reach plants (Sculthorpe 1967a, b). Temperature is an equally important factor determining the distribution of aquatic macrophytes (Barko et al. 1986; Bornette and Puijalon 2011; Santamaría 2002). Some species of macrophytes grow exclusively in tropical areas, while others are distributed only in the waters of the temperate climate zone (Chambers et al. 2008). Chemical composition of water, including salinity, strongly influences the growth and spreading of aquatic macrophytes. Macrophyte species inhabiting saltwater environments exhibit wide tolerance to osmotic stress and can grow at different salinities. For example, seagrasses, a unique plant group of approximately 60 species, which encompasses members of the families Hydrocharitaceae, Cymodoceaceae complex, and Zosteraceae, are the only angiosperms living fully submersed in the sea (Orth et al. 2006). Other plant species that have colonized saline waters (salt marsh plants, mangrove swamps, etc.) are members of several families, such as Potamogetonaceae and Ruppiaceae (Santamaría 2002). Aquatic macrophytes, similar to other plants, require a constant supply of nutrients and trace elements for growth and development (Rejmánková 2011). Nonrooted macrophytes receive ions and chemical compounds necessary for nutrition directly from water, while most aquatic plants rooted or attached to the bottom absorb them both from water and from bottom sediments (Barko et al. 1986).

4.6 Role of Macrophytes in Ecosystem

Macrophyte vegetation is an important component of various types of aquatic ecosystems. Together with phytoplankton species, these autotrophic organisms are the primary producers that provide the conversion of light energy into organic carbon compounds, thereby contributing to the formation of the trophic structure of aquatic ecosystems. During photosynthesis, macrophytes not only synthesize organic substances, but also release oxygen, which is necessary for the respiration of aquatic organisms and decomposition of organic matter. Macrophytes, especially submerged species, are important for aquatic food webs and affect the interaction among predatory, planktivorous, and benthivorous fish, as well as between fish and invertebrates (Hrivnák et al. 2006). According to available data, 37 freshwater herbivorous fish species belonging to 24 families feed on macrophytes (Opuszynski and Shireman 1995). Macrophytes, similarly to other aquatic organisms (e.g., phytoplankton, cyanobacteria), are producers and emitters of biologically active substances (allelochemicals), including low-molecular-weight volatile organic compounds, which play an important role in interspecies communication and competition. Allelochemicals released by aquatic plants perform a variety of functions, thereby affecting the composition and development of aquatic communities (Fink 2007; Li and Hu 2005; Zuo 2019). It is assumed that inhibition of phytoplankton and bacterioplankton, including cyanobacteria species, by allelochemical compounds secreted by macrophytes contributes to the stabilization of clear water states in shallow lakes (Chen et al. 2012; Mähnert et al. 2017). Additionally, macrophytes affect aquatic ecosystems through their influence on hydrological regime of water bodies (e.g., flow velocity, formation of surface waves, etc.), bottom sediment formation, and water quality (Dhote and Dixit 2009; Miler et al. 2014). Noticeable changes in pH values, dissolved gas concentrations, and ionic composition of water may result from their metabolism. Macrophytes that grow in near-shore areas can promote the stabilization of shores and contribute to reduction in erosion rates (Thomaz and da Cunha 2010).

4.7 Synergistic Action of Plant and Microbes in Aquatic Ecosystem

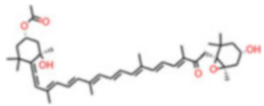
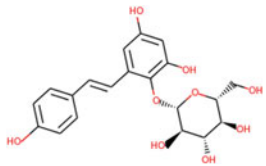
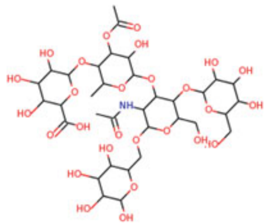
Microbes interact with plants in aquatic ecosystems mainly for the nutrition (organic carbon and oxygen), whereas plants developed defensive immunity and exchange of minerals. The nutritional conditions of the aquatic ecosystems depend on the distribution of the aquatic plants and microbes. Roots of the aquatic plants are directly interacting with microbial community, and provide continuous supply of oxygen, carbon, nitrogen, and nutrients. Stout (2006) reported that root-associated microbial communities of the *Lemna minor* negatively influence cadmium (Cd) acquisitions. Cd is a toxic heavy metal, which inhibits the growth and development of the *Lemna* plants (Haider et al. 2021). Several factors, such as pH, salt concentration, biological oxygen demands (BOD), chemical oxygen demands

(COD), dissolved oxygen electrolytic conductance, toxic heavy metals, organic pollutants, and available nutrients, are responsible for the plant–microbe interactions. In the aquatic ecosystems, very little information has been reported in the field of plant–microbes interactions. Moreover, some of the typical examples of plantmicrobe interaction and their roles are described (Table 4.1). The rhizospheric regions of aquatic plants are associated with maximum microbial interaction; therefore, this region exhibits different water chemistry than the other part of the aquatic plants (Stout and Nüsslein 2010). There are different types of plant–microbes interaction (terrestrial and aquatic) and these are associated with different types such as parasitic, symbiotic, mutualisms, and commensalism (Singh et al. 2019). Terrestrial plants secreted a group of chemicals, which act as signal molecules, and interact with microbes. On the other hand, an aquatic plant depends on organic carbon and oxygen (mainly rhizospheric regions) for survival of the microorganisms (Yadav et al. 2021). In general microbes make association with the plants: (1) endophytic in plant tissues and microbes provide nitrogen to host such types of microbes known as diazotropic microbes (Tripathi et al. 2013; Singh et al. 2018), (2) Ectophytic microbes (microbes associated out sides of the plants) are common example of ammonia-oxidizing microbes, methanogens, and metanotropic bacteria. The ectophytic interaction takes place in whole plants, including root, leaves, and plant trichome (Kandel et al. 2017). The transport of oxygen via diffusion through root to shoot via interconnected space. Oxygen is used as primary electron accepter for the energy generations, and this process is utilized by a group of oxidizing microbes in an oxidation processes (Morales-Olmedo et al. 2015). During digenesis process, the organic matters of sediment are degraded through anoxic microbial activities, and consumption of electron acceptor (Kristensen 2000). Under anoxic condition, bacterial cells utilized terminal electron acceptor from inorganic sources such as nitrate, sulfate, and carbon dioxides ions as terminal electron accepter to degrade the organic matters (Weber et al. 2006, Singh et al. 2018). Methane is produced by the methanogens by the reduction of carbon dioxide with hydrogen. It is poor energy-yielding process and predominant in complete combustion of all electron acceptors other than carbon dioxides (Chanton et al. 2005).

4.8 Plant-Microbe Interaction in Aquatic Plants

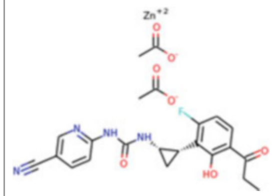
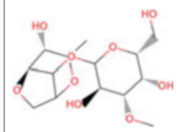
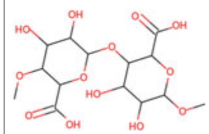
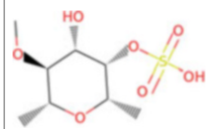
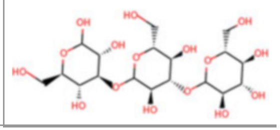
An aquatic environment provides a lot of opportunities for microbes to interact with the plants for their mutual survivals (Meshram and Chaugule 2018). Plant–microbe interactions are directly and indirectly involved for the mitigation of pollution known as bioremediations, and this topic has been more explored, in over of the world in the field of environmental and biological sciences (Dixit et al. 2021). Moreover, plant–microbe interactions are based on mutual benefits, whereas microbes provides minerals and metabolites to the plant in return plant provides shelter, organic carbon, and oxygen for growth and developments of the plants (Tripathi et al. 2013; Yadav et al. 2022).

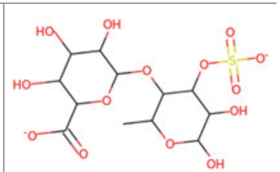
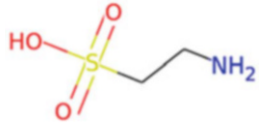
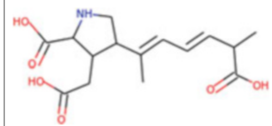
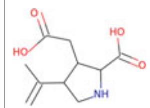
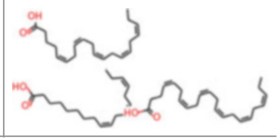
Table 4.1 Sea weeds as nutritional and functional food products

S. No.	Name of seaweed	Nutritional functional food	Bioactive compound	Structure
1.	*Himanthalia elongata*	Frankfurter sausages	Fucoxanthin	
2.	*Himanthalia elongata*	Reconstructed poultry steaks	Fucoxanthin	
3.	*Undaria pinnatifida*	Beef patties	Proteins	
4.	*Laminaria japonica*	Pork patties	Polyphenols	
5.	*Saccharina latissima, Sargassum pallidum*	Vegetarian burgers	Total polysaccharides	

(continued)

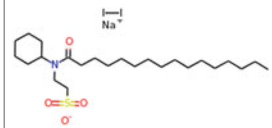
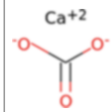
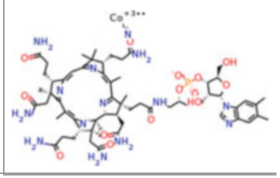
Table 4.1 (continued)

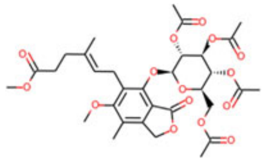
S. No.	Name of seaweed	Nutritional functional food	Bioactive compound	Structure
6.	*Chondrus crispus, Eucheuma cottonii*	Ice cream	Carrageenan	
7.	*Gracilaria cornea, Gracilaria domingensis*	Marmalade and Jam	Agar	
8.	*Laminaria digitata, Laminaria hyperborean*	Beverages	Aligns/alginic acid	
9.	*Fucus vesiculosus, Ascophyllum nodosum*	Soups	Fucoidan	
10.	*Fucus vesiculosus, Laminaria hyperborean*	Caprine feed	Laminarin	

11.	*Ulva lactuca, Ulva rigida*	Salads	Ulvan	
12.	*Saccharina latissima, Porphyra tenera*	Burger	Taurine	
13.	*Palmaria palmata, Digenea simplex*	Medicines	Kanoids (kainic and domoic acid	 Domoic acid  Kainic acid
14.	*Laminaria digitata, Saccharina latissima*	Medicine	PUFA (omega 3 fatty acids)	

(continued)

Table 4.1 (continued)

S. No.	Name of seaweed	Nutritional functional food	Bioactive compound	Structure
15.	*Laminaria digitata, Fucus serratus*	medicine	Carotenoids	
16.	*Laminaria japonica, Laminaria digitata*	Fertilizer	Iodine	
17.	*Porphyra tenera, Ulva lactuca*	Meat products	Calcium	
18.	*Ulva lactuca, Porphyra tenera*	Meat products	Vitamin B_{12}	

19.	*Monostroma nitidum*	Fresh noodles	Phenolic compounds	
20.	*Undaria pinnatifida*	Pasta	Fucoxanthin	

4.8.1 *Azolla-Anabaena* Symbiosis

Azolla is a heterosporus free floating aquatic macropyte frequently found on the water surface (Yadav et al. 2016). The macrophyte *Azolla* placed in the family a*zollaceae* has been reported. In India, seven species have been reported such as *A. caroliniana*, *A. maxicana*, *A. filiculoides*, *A. microphylla*, *A. rubra*, *A. nilotica*, and *A. pinnata.* Among them, *Azolla pinnata* is native strain of the India, and better sporulating and temperature sensitive (Mian 2002). *Anabaena-azollae* is an example of true endo-symbiont, on the dorshal leaf cavities; *Azolla* provides carbon sources and space for the growth and development of the cyanobiont, whereas cyanobiont synthesized nitrogen source in thick wall hetrocystes, and supply to macropytes. It is this unique symbiotic association between *Azolla* and prokaryotes partner *Anabaena azollae* (Adams et al. 2006). *Azolla* cavity provides natural-ecosystem and ecological defined structure, as result of an intimate contact between host and endosymbiont embedded in a mucilaginous cavity, helping in the exchange of nutrients as well as exchange of metabolites. *Azolla-Anabaena* associations are considered a successful coevolved organism, which makes important contributions to the food, feed, biofertilizer, phytoremediation, wastewater treatments, and several biotechnological applications (Yadav et al. 2021).

4.8.2 Degradation of Organic Pollutants

The major sources of contamination in India are organic compounds such as chlorinated hydrocarbon, poly-aromatic hydrocarbon (PAH_S), and poly-bromide biphenyl ether (PCBs), which are common environmental pollutants. Microbes are efficient bioremediation potential due to catabolic activities, and degrade almost all types of organic compounds (Kannikka et al. 2018). Cometabolism is another mechanism that follows the microbes to degrade recalcitrant organic compounds into simple organic compounds in aquatic and terrestrial zone of macropytes (Kumwimba and Meng 2019). The biodegradations rate follows second order kinetics in aquatic ecosystems; it is directly correlated with number of microbes and amount of xenobiotic compounds (Barragán et al. 2007), whereas the microbial population depends on species of the macropytes (Chao et al. 2021). In addition to that plant supplies organic carbon to the root associate microbes and degrade recalcitrant compounds (Hoffland et al. 2020) such as pyrenes and PAH_S (Mohapatra and Phale 2021). Golubev et al. (2009) found classical example of the mutualism between plant–microbe interactions whereby plants benefited in the form of plant hormone indole acetic acids (IAA) from microbes as a result microbial degradation of PAHs. Further, Golubev and his coworkers reported *Sinorhizobium meliloti* P 221 which form ectorhizospheric association with aquatic plants and mutual benefitted by the synthesizing IAA and PAHs degradations. The rhizospheric region of the aquatic plants rich sources of methanotrophs which is a groups of a and c proteobacteria used as methane as sources of energy and carbon. The methanotrophs (e.g., *Methylosinus trichosporium*, *Methylococcuscapsu-latus*) degrade wide range of toxic organic compounds by the synthesizing enzymes such as methane mono-oxygenase (Pandey et al. 2014). The xenobiotic compounds are mainly chlorinated ethene through cascades of the enzyme reactions; which involves the formation intermediate organic molecule formaldehyde, and finally produced terminal carbon dioxides (Yoon 2010).

4.9 Plant-Microbe Interaction Facing Environmental Challenges

Plants exist in a microbe-rich environment in nature, and they must interact with a diverse range of pathogenic, commensal, and helpful bacteria. Multiple components of the plant system are influenced by the microbiome. They influence plant health through antagonistic and synergistic interactions with the plants. Plants ability to utilize microorganisms beneficial activities while also combating microbial diseases has piqued the interest of generations of plant and microbial experts (Jones et al. 2016). Many studies looked into the fundamentals of plant-microbiota interaction, plant defense signals, and symbiotic responses to microorganisms, a microbe's genetic system for transporting signaling molecules and modulating host cell activities, the particular binary and community level conflict, as well as specific binary and community level conflict in the interaction (Hacquard et al. 2017). Environmental effect on plant and microbiome interaction is seldom studied at mechanistic and molecular levels, opening up new pathways in understanding how plants and microbes interact in nature to mediate ecological resilience.

A "holobiont" is made up of plants and microbial populations. In broad terms, a holobiont is an ecological unit formed by the assemblage or combination of a host with numerous species that surround it. Microbiota that interacts with plants has a wide range of locations, ecological functions in both the above-ground and below-ground environment, and may play a role in controlling climate tolerance and other stress responses. PGPR, fungus, actinomycetes, algae, yeasts, cyanobacteria, and others make up the microbial community in the soil system (Manzar et al. 2021). The Plant roots are home to a wide range of microfauna, both endophytic and rhizospheric (Tables 4.2 and 4.3).

4.10 Impact of Temperature on Microbial Mechanisms

Velásquez et al. (2018) have examined how pathogens are affected by environmental factors, highlighting that each pathogen has a temperature range in which it may develop and virulently. Elevated temperature has been demonstrated to suppress type IV secretion-associated pilus production and expression of virulence (vir) genes in *Agrobacterium* infection (Baron et al. 2001; Jin et al. 1993). At higher temperatures, however, the soft-rot bacteria *Pectobacterium atrosepticum* showed greater virulence, which is linked to increased production of plant cell-wall-degrading enzymes, quorum-sensing signals, and faster disease progression

Table 4.2 Common examples of the plant–microbe interactions, and its role in aquatic ecosystems

Symbiotic plants	Microbes	Role in aquatic ecosystems	References
Azolla filiculoids	*Anabaena azollae*	Nitrogen fixation	Yadav et al. (2016, 2019)
Lemna minor	*Pseudomonas sp*	Denitrification	Ying-ru et al. (2013)
Chlorella bulgaris	*Azospirillum braciliances*	Plant-growth-promoting activity	Gonzalez and Bashan (2000)
Typha latifolia	*Bacillus sp*	Nitrogen fixation	Biesboer (1984)
Scenedesmus bicellularis	*Pseudomonas diminuta*	Plant-growth-promoting activity	Mouget et al. (1995)
Nuphur sp.	*Mesorhizobiam lotti*	Nitrogen fixations	Wagner (2011)
Rooted macrophytes	*Sinorhizobium meliloti* P221	IAA production in root	Golubev et al. (2009)

(Hasegawa et al. 2005). Temperature changes also have an impact on beneficial plant-microbe interactions. It has a beneficial impact on arbuscular mycorrhizal fungus (AMF) hyphal development and plant colonisation in the majority of cases, owing to quicker plant carbon allocation to the rhizosphere, where AMF dwells (Compant et al. 2010).

4.11 Circadian Clock and Plant Immunity

External light and the internal circadian clock are involved in many areas of plant life. Interlocked transcription-translation feedback loops govern the circadian clock. Although, in addition to light, the circadian clock is a self-sustaining mechanism, certain parts of its operation may change in reaction to changes in external variables, such as temperature and humidity (Lu et al. 2017; Mwimba et al. 2018). According to Mwimba et al. (2018), humidity oscillation promotes ETI at night, implying that the host anticipates higher pathogen infection when the humidity is high at night. Effect of the circadian clock on ETI against the *Arabidopsis* oomycete pathogen Hyaloperonospora arabidopsidis (Hpa) has been studied. In order to commence infection, Hpa disperses the spores at daybreak. Through CCA1, the circadian clock has been demonstrated to modulate RPP4 (NLR)-mediated immunity against avirulent Hpa isolates—at dawn, RPP4 and RPP4-dependent genes had a modulated peak expression (Wang et al. 2011).

4.11.1 Light

Experiments including the genetic deletion of a circadian oscillator in B. cinerea, the use of continuous light to decrease fungal rhythmicity, and the use of out-of-phase light:dark cycles demonstrated that the fungal clock controls the result of the susceptible Arabidopsis-B. cinerea interaction. Light exposure may also be used

Table 4.3 Microbes present in phyllospheric and rhizospheric regions of different plant species

Microbial type	Microbial species	Plant species	References
Bacteria	Sphingomonads and Methylobacteria	Maize	Wallace et al. (2018)
	Pseudomonas	Apple, almond, tobacco	Aleklett et al. (2014)
	Pseudomonas, Erwinia herbicola	Sugarbeet	Thompson et al. (1993)
	Proteobacteria	Soybean, clover, rice, *Arabidopsis*	Vorholt (2012)
	Pseudomonas, Sphingomonas, Frigoribacterium	Grapewine, grape clusters	Compant et al. (2019)
	Cyanobacteria	Mangrove forests, Taiwan rain forests, tropical trees, rice	Lin et al. (2012), Venkatachalam et al. (2016), Zhu et al. (2018)
	Pseudomonas, Agrobacterium, Cupriavidus, Rhizobium	*Citrus* species	Xu et al. (2018)
	Proteobacteria, Actinobacteria, Acidobacteria, Bacteriodetes, Plantomycetes, Choloroflexi, Gemmatimonatedes	Grapevine	Faist et al. (2016), Samad et al. (2017)
	Proteobacteria, Actinobacteria	Cucumber, tomato, wheat maize	Reinhold-Hurek et al. (2015)
	Bacteriodetes, Actinobacteria	*Arabidopsis*	Bergelson et al. (2019)
	PGPR	Jerusalem artichoke	Montalbán et al. (2017)
	Acidobacteria, Alphaproteobacteria, and Gammaproteobacteria	Cottonwood	Timm et al. (2018)
	Calothrix, Scytonema	Paddy	Roger et al. (1993)
	Nostoc, Anabaena	Rice	Prasanna et al. (2009)
Fungi	*Undifilum* spp., Clavicipitaceous fungi	Legumes, Morning glory	Panaccione et al. (2014)
	Ascochyta species, *Colletotrichum gloeosporioides, Phomopsis* species	*Fagus* species, *Swida* species	Osono (2006)
	Ascomycota, Basidiomycota, Olpidiomycota	*Arabidopsis*	Bergelson et al. (2019)
	Orchidaceous m Mycorrhizal fungi	Orchids	Rudgers et al. (2020)

by pathogens to start an infection. For example, in the maize fungal pathogen *Cercospora zeae-maydis*, the blue light receptor Cercospora Regulator of Pathogenesis1 (Crp1) is required for sensing plant stomata and may mediate the biosynthesis of the light-activated toxin cercosporin, which disrupts stomatal guard cell membranes, allowing fungal infection through stomata (Kim et al. 2011a, b).

P. syringae's fitness and virulence may be influenced by light. The expression of coronatine toxin biosynthetic genes, for example, is suppressed by red light. Red light may limit bacterial entry through stomata, because coronatine is necessary for *P. syringae* to open stomata and enable bacterial entry. Light and the circadian clock, like temperature, impact both the host plant and the microbe during the creation of a plant–microbe connection (Santamaría-Hernando et al. 2018; Melotto et al. 2006).

4.11.2 Moisture

Water is necessary for life to exist on this planet. Too little water (osmotic stress) or too much water (flooding) can have a significant influence on many elements of plant and microbial life. Plants regulate the amount of the phytohormone ABA in response to a lack of water. Increased ABA activates a signaling cascade that leads to large-scale transcriptional reprogramming and physiological changes, including as stomata closing to minimize transpiration (Zhu 2016). ABA-induced stomatal closure may limit bacterial infiltration through stomata under drought stress. Increased ABA, on the other hand, can decrease the SA signalling pathway in the mesophyll cells inside the leaf, weakening SA-mediated resistance after invasion (Jiang et al. 2010).

4.11.3 Drought

Drought has an impact on plant-microbiome interactions. Drought changed the composition of microbial communities in all sampled compartments (bulk soil, rhizosphere, and root endosphere), and the closer the community is to the root, the greater the shift in composition in drought-stressed rice plants (Santos-Medellín et al. 2017). Drought increases the number of Actinobacteria and Firmicutes at the phylum level. The root endosphere shows the greatest decrease in community diversity and rise in Actinobacteria and Firmicutes transcript abundance, with specific enrichment in amino acid and carbohydrate transport activities. Drought stress produces a change in root metabolites on the host side. It needs to be seen if and how these drought-enriched metabolites "configure" root microbiome composition to boost plant stress responses.

Nonetheless, this intriguing link shows that during drought, molecular dialogues between plants and their associated microbiome may modify root microbiota in order to deal with drought stress. Deciphering this molecular conversation will help us get the essential information we need to use microbiota to improve drought tolerance in agricultural plants.

4.11.4 Humidity

Rain and/or high air humidity have long been known to be necessary conditions for many plant disease outbreaks. Plants typically die locally at the site of pathogen infection during ETI, a process known as the hypersensitive reaction (HR). The HR is hypothesized to inhibit biotrophic pathogen development and dissemination while also activating secondary immune responses. In a variety of plant-pathogen interactions, high atmospheric humidity reduces HR cell death, which might be one of the causes for increased plant sensitivity and epidemics in humid environments (Wang et al. 2005; Wright and Beattie 2004).

4.12 Plant–Microbe Interaction in Nutrient Acquisition and Accumulation

The rhizosphere in terrestrial structures is the area of soil surrounding plant roots where there is extended microbial activity; in aquatic plants, this definition may be less clean because of diffusion of vitamins in water, but there's still a quarter Of have an effect on with the aid of plant roots on this environment (Christensen et al. 1994). Within that Area chemical conditions differ from the ones of the encompassing Surroundings resulting from a variety of techniques that Had been brought about both immediately through the interest of plant roots or by way of The interest of rhizosphere microflora. These days, there are a Range of recent research related to rhizospheres of aquatic flora And specifically their multiplied capacity for remediation of Contaminants, mainly remediation of metals through Aquatic plant–microbial interaction (Stout and Nüsslein 2010).

4.13 Application, Benefits, and Barriers of Plant-Based Remediation

Phytoremediation, the use of plants and their associated Microbial groups to eliminate or inactivate pollution. From the environment, includes any of several technology For detoxifying the surroundings with genetically Changed or wild-type vegetation (Kraemer 2005). Phytoremediation of Aquatic environments can be used as an alternative or in Addition to standard remediation methods inclusive of Ion change resins and electrodialysis, chemical precipitation, Sedimentation, microfiltration, and reverse osmosis (Rai 2009). Organic remediation strategies provide powerful opportunity treatments that are often much less luxurious and are Taken into consideration extra environmentally pleasant and publicly Acceptable than traditional technology. Various phytoremediation technology encompass phytoextraction, Phytovolatilization, phytostabilization, and rhizofiltration and are summarized by several critiques (Flathman and Lanza 1998; Prasad and Freitas 2003; Jabeen et al. 2009). Current studies have carried out a number of those technologies to phytoremediation of heavy metals in aquatic or wetland structures. As an instance, Murakami et al. (Murakami et al. 2009) used rice cultivars that collected high ranges of cadmium to take away metal from contaminated paddy fields. In those conditions in which rice was well-tailored to Boom, one rice cultivar amassed ten times as an awful lot Cd as *Thlaspi caerulescens*, a terrestrial plant well known for its potential to accumulate heavy metals. Rhizofiltration, the use of plant life to put off heavy metals From aqueous environments, has been significantly examined As a manner to take away contaminants from solutions, and may Include aquatic or terrestrial flora in hydroponic structures. Recently, sunplant and bean vegetation have been examined for their competencies to dispose of u from contaminated groundwater. In laboratory batch experiments, bean plants eliminated greater than 70% and sunplant eliminated more than 80% of u however whilst sunplant was examined in a Non-stop rhizofiltration gadget, u removal was extra Than 99% (Lee and Yang 2010). Aquatic and wetland flora which include the Water hyacinth eichhorinia crassipes (Agunbiade et al. 2009; Mishra and Tripathi 2009), the invasive Reed phragmites australis (Ghassemzadeh et al. 2008), the duckweeds spirodela Polyrrhiza (Rahman et al. 2007), lemna minor (Hou et al. 2007; Uysal and Taner 2009), and lemna gibba (Khellaf and Zerdaoui 2009; Megateli et al. 2009), the aquatic fern azolla pinnata (Rai and Tripathi 2009), and yellow Velvetleaf limnocharis flava (Abhilash et al. 2009) have recently been studied For his or her abilities to eliminate metals from aquatic Structures and show promising effects.

Pinnata become discovered to dispose of as a good deal as 94% of Hg from A solution (Rai and Tripathi 2009), whilst eichhornia crassipes was located to acquire Cr in its shoots at 223 instances the attention Inside the water (Agunbiade et al. 2009), and removed 84% of Cr from water and 95% of Zn from water (Mishra and Tripathi 2009). At the same time as metals negatively Affected increase of lemna gibba, the plant s were capable of Dispose of 90% of Cd from answer after 6–8 days (Megateli et al. 2009).

4.13.1 Metal Tolerance and Resistance: The Bacterial Aspect

Bacteria have validated expanded tolerance to metals using many diverse mechanisms. They will hold metal homeostasis, preserving concentrations of critical metals together with Zn from attaining toxic ranges inside cells (Coombs and Barkay 2005) or they'll include resistance systems, active mechanisms for getting rid of or sequestering metals (Gadd 1992). For heavy metals such as Cd, Zn, Ni, Cr, Co, and Cu, there are numerous forms of resistance systems, such as efflux Pumps to remove metals from the cellular, and sequestration Mechanisms to bind metal in the cell. The two known Sorts of efflux systems are atpases, which pump out Metals the use of ATP to pressure the reaction, and proton antiports, which use the proton gradient to pump metals across the mobile membrane (Nies 2003). Some other mechanism of bacterial metal resistance, satisfactorily known in cyanobacteria, is sequestration by way of metallothioneins. Metallothioneins bind metals to sulfhydryl businesses of cysteine residues (Huckle et al. 1993; Ybarra and Webb 1999). Recently described cadmium tolerance in Stenotrophomonas maltophilia, and discovered now not only a Cd Efflux pump but additionally accumulation of Cds particles (Pages et al. 2008).

Some microorganisms expressing metal resistance produce greater extracellular polymeric materials (eps), which bind the metal, perhaps making the microenvironment across the plant less poisonous. Manufacturing of eps has been proven to

Increase with improved metal resistance. In different lines of *Rhizobium leguminosarum*, cd tolerant Traces showed expanded tiers of glutathione, indicating that this tripeptide allows the bacterium to deal with heavy metals, in preference to an efflux system (Figueira et al. 2005). The Biomarker glutathione is a critical antioxidant that may also protect against metallic toxicity related to oxidative Strain. Any other mechanism of dealing with poisonous Metals can also involve polyphosphates, lengthy chains of orthophosphates, which might also sequester metals (Alvarez and Jerez 2004). Perrin et al. Recently said that ni exposure promoted Biofilm formation in escherichia coli cultures, which may also Serve as a defensive tolerance mechanism. Ni appeared to Be worried in adherence by using inducing transcription of Genes encoding curli, the adhesive systems vital For biofilm formation (Perrin et al. 2009). The protecting fine of eps or these other mechanisms Provided by means of root-associated microorganism shows that enriching For positive bacteria might also replace the method of Amending plant root zones with synthetic go-related Polyacrylates and hydrogels to defend roots from heavy Metal toxicity (Blaylock et al. 1997) (Fig. 4.2).

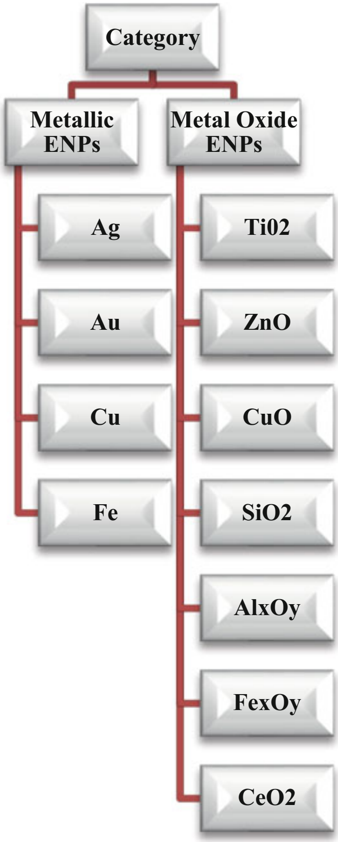


Fig. 4.2 Categories of engineered nanoparticles (Ebrahimbabaie et al. 2020)

4.13.2 Metal Tolerance and Resistance: The Plant Aspect

In plants, metal accumulation in the cells can be regulated by glutathione–phytochelatin-mediated resistance. On this system, glutathione, the identical cysteine-containing tripeptide defined above in *Rhizobium* sp., which also has several features in plant cells including coping with toxic oxygen species and amino acid delivery, Is used to synthesize phytochelatins, which chelate Heavy metals by means of formation of a thiolate. Thiolates may be transported to vacuoles for heavy metallic storage (Mendoza-Cozatl and Moreno-Sanchez 2006). Phytochelatins are activated by using heavy metals and scavenge heavy metals in plant cells (Blum et al. 2007). For a review of Plant metal tolerance mechanisms, see Jabeen et al. (2009). While some flora deal with mild degrees of toxic metals by using chelation, other plants have the potential to acquire extraordinarily high ranges of heavy metals and sequester them of their tissues. Flora that may accumulate extremely excessive concentrations of metals are termed "hyperaccumulators." There were extra than four hundred plant species recognized as such (Prasad and Freitas 2003), consisting of crop Species (Vamerali et al. 2010), and the quantity of hyperaccumulators among aquatic and wetland plants is growing (Prasad et al. 2001).

Hyperaccumulators can be defined based on bioconcentration Component (bcf), or the capacity to accumulate metals in plant tissues. As an instance, the potential to acquire more than one thousand instances the awareness of Cd (based totally on attention of metal in dry weight of Plant) than that inside the surrounding medium could be taken into consideration hyperaccumulation (Zayed et al. 1998). One of the maximum Studied hyperaccumulators is the terrestrial plant *T. caerulescens*, that's a Cd/Zn hyperaccumulator (Mijovilovich et al. 2009). Numerous aquatic vegetation have been located to have comparable abilities, along with *Salvinia minima* (Sanchez-Galvan et al. 2008), *Potamogeton natans* (Fritioff and Greger 2006), *Ceratophyllum demersum* (Robinson et al. 2006), and *S. polyrrhiza* (John et al. 2008). Metal hyperaccumulation is an adaptive manner between microbes exposed to heavy metals and flora, requiring continuous interactions a few of the cogoing on organisms. A recent proteomics look at by Farinati et al. (2009) indicated that the presence of a rhizosphere microbial Population, tailored to heavy-metallic-polluted websites, substantially enhanced the accumulation of metals in shoots Of the hyperaccumulator arabidopsis halleri. Aquatic environments consist of no longer simplest macrophytes, but also algae that may have interaction with microbes to take away contaminants from the environment. Algae can be produced in artificial structures and used to cast off contaminants. Loutseti et al. used a dried aggregate of microalgae and bacteria to dispose of cu and cd from wastewater. Munoz et al. efficaciously examined the mixture of the bacterium *Ralstonia basilensis* and the microalga *Chlorella Sorokiniana* on adsorption of Cd, Cu, Ni, and Zn. Moreover, mycorrhizal fungi associated with plant life can Beautify uptake of metals when critical metallic concentrations Are low and, vice versa, while metal quantities are too high mycorrhizae may be effective in assuaging metal Toxicity lowering plant uptake (Frey et al. 2000).

4.13.3 Mechanisms for How Aquatic Plant–Microbe Interactions Have an Effect on Phytoremediation Techniques

At the same time as many flora and microorganism have their personal mechanisms for handling heavy metal contaminants, the interplay of flora and microorganisms may additionally boom Or lower heavy metal accumulation in flora, depending on the character of the plant–microbe interaction. Due to the fact phytoremediation is an extraordinarily new era, information mechanisms of plant–microbe interactions in putting off contaminants from the surroundings is still no longer properly characterized. There have, however, been several ideas about the nature of plant–microbe interactions in metal accumulation. In their paper describing bacterial enhancement of Se and Hg uptake by wetland plants, De souza et al. (1999) proposed numerous possible mechanisms, such as bacterial stimulation of plant metal uptake compounds including siderophores; bacterial root increase promoting increasing the basis floor place; bacterial transformation of elements into greater soluble bureaucracy; or bacterial stimulation of plant transporters that could transport essential factors as well as heavy metals (within the case of selenate, The sulfate transporter. Van der lelie related the basis of this plant–microbe interaction to bacterial metal resistance, for the reason that bioavailability of metals may be altered by means of bacterial expression of resistance structures (Van der Lelie et al. 2000).

4.13.4 Plant Microbe Interaction in Perspective to Sustainable Agriculture Development

Microbes represent an important reservoir, which influence the macroorganisms. Plant health and its efficiency can be maintained by beneficial symbiotic bacteria such as *Bacillus, Pseudomonas, Rhizobium, Pantoea agglomerans, Bradyrhizobium, Actinobacteria, and Burkholderia* (Khati et al. 2018). Plant microbial interactions help in the growth of plants and increase the resistance toward stress conditions (Chaudhary et al. 2021a, b; Perreault and Laforest-Lapointe 2022). Microbes support plant rhizosphere functions via supportive expansion of roots and growth of plants (Kumar et al. 2021a, b; Chaudhary et al. 2022). In response, roots of plant secreted organic composites, which provide carbon sources and signals for microbial growth. Rhizospheric microbes adjust and affect the properties of soil/water ecosystem. Microbial activities help in transformation of nutrients, offer protection toward pathogens, and support the plant growth (Agri et al. 2021, 2022; Chaudhary et al. 2021c, d). Microbes respond rapidly to any change in the environment. Changes in microbial communities have been associated to the properties of soil under heavy metals/pesticides stress (Kumar et al. 2021a, b). Aquatic plants and their associated microbes are less studied. Application of denitrifying bacteria such as *Pseudomonas* sp. modifies the chemical conformation of root exudates in duckweed (aquatic plant) and improved excretion of stigmasterol, which is involved in N exclusion from aquatic plants via root and microbial interactions (Lu et al. 2021). *Acidobacteria* are extensively dispersed in different environments like mine water, marine sponges, and hot springs. It is also found in roots of some water plants such as *Scirpus juncoides* and *Lythrum* and increased plant growth (Tanaka et al. 2017). Coculture of *Acidobacteria* strains in aquatic duckweed species improved chlorophyll content and plant growth due to the colonization of bacteria on roots confirmed via fluorescence microscopy (Yoneda et al. 2021). It also showed the properties such as IAA and siderophore production and mineral solubilization. *Rhizobium unidicola* forms symbiotic association with aquatic plant *Neptunia oleracea* and improved their growth by production of phytohormones. Cable bacteria such as *Candidatus electrothrix* and *C. electronema* are sulfide-oxidizing and filamentous bacteria, which reduce the toxic sulfide and encourage the iron oxide development in oxygen-liberating plants, which act toward the toxic form of arsenic uptake in roots of aquatic plants (Sulu-Gambari et al. 2016; Scholz et al. 2021). These bacteria also promote the nitrogen availability to plants and present around the aquatic plant roots such as *Littorella uniflora*, *Potamogeton*, and *Equisetum* sp. (Kessler et al. 2019). Plant microbe interaction in aquatic water can be used in pollutant removal. *Bacillus* and *Microbacterium* sp. present in roots of *Lolium perenne* improved the remediation of nitrogen and phosphorus (Li et al. 2011). Presence of *Pseudomonas, Bacillus*, *Rhodococcus* sp., *Brachia mutica, Leptochala fusca* helps in remediation of iron, nickel, and Pb metals (Shahid et al. 2020). Remediation of benzene is increased in the presence of the aquatic plant *Syngonium podophyllum* and microbes such as *Enterobacter* and *Cronobacter* (Sriprapat and Thiravetyan 2016). Application of *Pseudomonas* sp. in the presence of *Lolium perenne* and *Arabidopsis* removed the total petroleum hydrocarbons from aquatic environment and improved the plant growth under stressful conditions (Iqbal et al. 2019). Nirophenols and phenolic compounds remediation occur with the help of microbes such as *Pseudomonas, Cupriavidus* sp., *Rhodococcus* sp., and *Pantoea* sp. and aquatic plant like *Sprirodela polyrhiza* and *Brassica napus* (Kristanti et al. 2016; Ontañon et al. 2014). Chlorpyrifos remediated by using planktonic bacterial communities with *Nymphaea alba* and *Phragmities australis* reported by Xu et al. (2018). Remediation of oil occurs by using *Ochrobactrum, Desulfovibrio* and *Halobacterium* sp. with *Halonemum strobilaceum* plant (Al-Mailem et al. 2010). Application of microbe-plant system *Bacillus* and *Pantoea* along with *Dracaena sanderiana* improved the bisphenol remediation to about 92%: this was reported by Suyamud et al. (2018). Removal of these pollutants occurs with the help of rhizofiltration and biostimulation.

4.14 Conclusion

Microbes coevolved with the plants for the fulfillment of their nutrient deficiencies in their fundamental niches. Thus, the aquatic microbiome plays a vital role in influencing and promoting the aquatic ecosystem. The plant–microbe interaction is also affected by the environment; both biotic and abiotic factors have a concomitant effect on the marine ecosystem. This leads to change at molecular and gene expression level causing production of compounds by these microbial-host-environmental interactions. Marine plants survive and thrive in a competitive and hostile environment by living in complex colonies and in close proximity to each other. Aquatic plants have evolved physiological adaptations, such as the production of bioactive chemicals, that provide them protection against grazers. Plants exist in a microbe-rich environment in nature, and they must interact with a diverse range of pathogenic, commensal, and helpful bacteria. Thus the aquatic microbiome plays a vital role in influencing and promoting the aquatic ecosystem. Aquatic phytoremediation of metals, the usage of plants to extract, contain and immobilize, or dispose of hazardous substances from aqueous environments is a completely promising vicinity, and several fairly green examples have proven the applicability of this technique to smooth business waste streams, to concentrate heavy metals, and to keep Drinking water and aquatic biodiversity. Limitations to this generation do exist and must also be taken into consideration. The utility of rhizofiltration is constrained by means of metallic availability, attention, and phytotoxicity. Environmental factors like mild, salinity, temperature, Ph, and presence of a couple of heavy metals might also have an effect on metallic uptake. Similarly limitations of phytoremediation Technology are seasonal increase of aquatic flora and contaminated biomass disposal problems. Phytoremediation centered on dissolved metals can be based totally on the software of each dead or live plant cloth or at the cultivation of aquatic plant s. No longer only aquatic macrophytes, but also algae and fungi, constitute a value-effective and green era for environmental Cleanup, a green answer regularly favored in Political decision-making. Rhizosphere microbes can lessen metal toxicity and enhance plant tolerance to dissolved metals, and may therefore be applied to deliver extended phytoprotection From dangerous results of the metals on plant life. In an immediate extension of this idea, the bacterial genes coding for metal resistance can be transplanted into the plant genome to confer elevated metallic tolerance to plants. In addition research in aquatic phytoremediation is wanted to increase knowledge of microbe–plant interactions. Such knowledge would boom the quantity of doubtlessly sizable programs and their effect together with the remedy of heavy metals from business effluents in Natural and built wetlands, or a wastewater metal stripping phase the use of rhizofiltration. A directed purposeful analysis has to inspect plant–Microbe interactions at complete organic hierarchy, starting with the genomic, transcriptomic, and proteomic analysis Of plant-associated bacteria and their extracellular enzyme activities, all of the manner to biochemical techniques And biking which might be energetic in the bacterially prompted rhizosphere. With this understanding the plant–microbe device may be carried out at discipline-scale, the use of clearly adapted indigenous microbes that have Been cultured and enriched in the laboratory. Any such multidisciplinary and included technique may also benefit aquatic metal phytoremediation the industrial significance in environmental biotechnology it deserves.

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Plant-Microbe Interaction in Freshwater Ecosystem for Improving Water Quality

5

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Abstract

In an aqueous environment, microorganisms and plants interact primarily for organic oxygen and carbon, while plants acquire resistance mechanisms and exchange of minerals. This interaction results in a strong link between the two that is heavily dependent on the mutual nutrient supply. Nowadays, water quality of freshwater resources is heavily polluted due to industrial waste, agricultural runoff, oil spillage, and several other factors. In addition to their mutual advantages, plant-microbe interactions have an impact on the water quality, particularly in the rhizosphere, which gives aquatic systems the innate capacity to reduce pollution from the water column. This chapter provides in-depth information and recent advances in the study of plant-microbe interactions, ecological and biochemical components, their role in fresh water ecosystems, and their potential to enhance water quality.

Keywords

Aquatic plants · Aquatic pollution · Plant-microbe interaction · Biofilm · Water quality

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5.1 Introduction

Wetlands are the transitional areas between land and water and are distinguished by soil surfaces that are waterlogged above them and support a wide variety of plant and animal species. The macrophytes and microphytes, including phytoplankton, diatoms, and other dominant algae, that make up the floral richness and diversity of aquatic ecosystems are incredibly diverse. Large plants known as aquatic macrophytes grow in water and in areas where land and water converge. The primary chemical components of surface water necessary for the correct development of macro- and microphytes are the optimal concentrations of key nutrients like Nitrogen and Phosphorus as well as organic Carbon and other nutrient elements. In addition to macro- and microphytes, microbial consortia exist at different community levels and are typically seen as detrital microbial mats, biofilms, and cluster of different microorganisms (planktons, bacteria, and microalgae) (Xie et al. 2015). They significantly contribute to nutrient cycling (nitrification, denitrification, sulfate reduction, methanogenesis, and metal ion (Noori et al. 2021). Consortia of microbes, especially those present in the rhizosphere and rhizoplane, and on the solid surfaces of sediments, form biofilms on submerged plants' leaves. Several environmental factors impact biofilm formation and its structure, including an abundance of nutrients (eutrophication), their supply, and the concentration of hazardous materials in the water (Giaramida et al. 2013; Calheiros et al. 2009). The eutrophication process, natural deterioration processes, and effects of human activity all affect the freshwater aquatic systems' water quality. Numerous academic studies have been conducted on water quality and their remediation by microorganisms (McGrane 2016; Ismail et al. 2019; Oliver et al. 2019; Bhatt et al. 2022) the remediation processes (Jalal et al. 2011; Kurniawan et al. 2021; Swaleh et al. 2022).

In addition, previous scientific studies appear to show that the majority of water quality improvement studies have focused on environmental contaminants and showed that these contaminants can be removed only by aquatic plant or by different microorganisms. Small number of publications are available implying direct effect of plant (macrophytes) and microbe interactions (Dhote and Dixit 2009; Sood et al. 2012; Kochi et al. 2020; Lu et al. 2014) and its potential benefits on water quality improvement (Stottmeister et al. 2003; Rodrigues 2007; Toyama et al. 2011; Chakraborty et al. 2013; Prakash et al. 2022). The researches on the interaction of microbes with aquatic macrophytes were discussed in this chapter. With an emphasis on their combined effect on the quality of freshwaters, several facets of microorganisms, microbial communities, and their involvement in aquatic ecosystems have been reviewed.

5.2 The Function of Microbial Communities (Biofilms) in Freshwater Ecosystems

Based on the nutrients rich and prevailing environmental scenarios, microorganisms account for the majority of all inland water habitats quantitatively and biochemically (Hahn 2006). This high microbial diversity supports the efficient functioning of freshwater ecosystems (Zehr 2010). The culturable bacterial group, which includes Actinobacteria, alpha-, beta-, and gamma-proteobacteria, firmicutes, and Bacteroides, constitutes the majority of the microbial biodiversity in freshwater ecosystems and archaea (Wang et al. 2008; Calheiros et al. 2009; Wei et al. 2011; Dash et al. 2021).

Microbial populations are present on solid substrates and plant surfaces as biofilm (Gagnon et al. 2007). The predominant bacterial groups frequently found in the assemblage, primarily in freshwaters, are depicted in Fig. 5.1. Extracellular polymeric material (EPS) is a porous slime matrix made of lipids, proteins, nucleic acids, and polysaccharides, which is used to produce biofilms, which are then filled with microbe cells (Wotton 2011; Branda et al. 2005). In a biofilm, microbial cells are confined to a specific microniche inside a complex, homeostatically stable community that exhibits strong metabolic coordination. This gives the microbes ecologically different characteristics (Costerton et al. 1995). When habitats and environmental factors change, the strong microbial assemblage in a biofilm is prone to significant change (Yannarell and Triplett 2004; Witteveen et al. 2020). According to Crump and Koch (2008), various plant types support various microbial species.

Additionally, molecular methods like terminal restriction fragment length polymorphism (TRFLP) fingerprints of PCR amplified 16S rDNA fragments and denaturing gradient gel electrophoresis (DGGE) can quickly provide knowledge about the general trend of the microbial population of Biofilm (Truu et al. 2009; Kumar et al. 2022). According to metagenomics research, consortia of microbes are good for their growth (Singh et al. 2021; Suyal and Soni 2022). Bacterial cells in a biofilm have numerous possibilities to share genetic info via horizontal gene transfer (HGT), which confers tolerance, resistance, and capability of chemical degradation. Bacterial survival in natural habitats is commonly attributed to HGT (Ventura et al. 2007). The genetically stable populations of microorganisms produce diverse responses and sensitivities to different anthropogenic disturbances in a biofilm (Mcclellan et al. 2008). The vulnerability of the bacterial population in Biofilm to toxicants is mainly influenced by PO_4^{3-} ions (Kamaya et al. 2004; Guasch et al. 2007; Tlili et al. 2010). In particular, when there is food scarcity, Tlili et al. (2010) showed how the microbiota changes in reaction to toxicants like Cu and diuran (herbicide).

Water quality is deteriorating nowadays due to increasing anthropogenic activities such as commercial as well as industrial. Freshwater resources are sinking and the condition is very harsh as far as the water quality is concerned. Different studies have been conducted on the water quality of freshwater resources, that is, rivers, lakes, groundwater, etc., globally: these studies have exposed the increasing

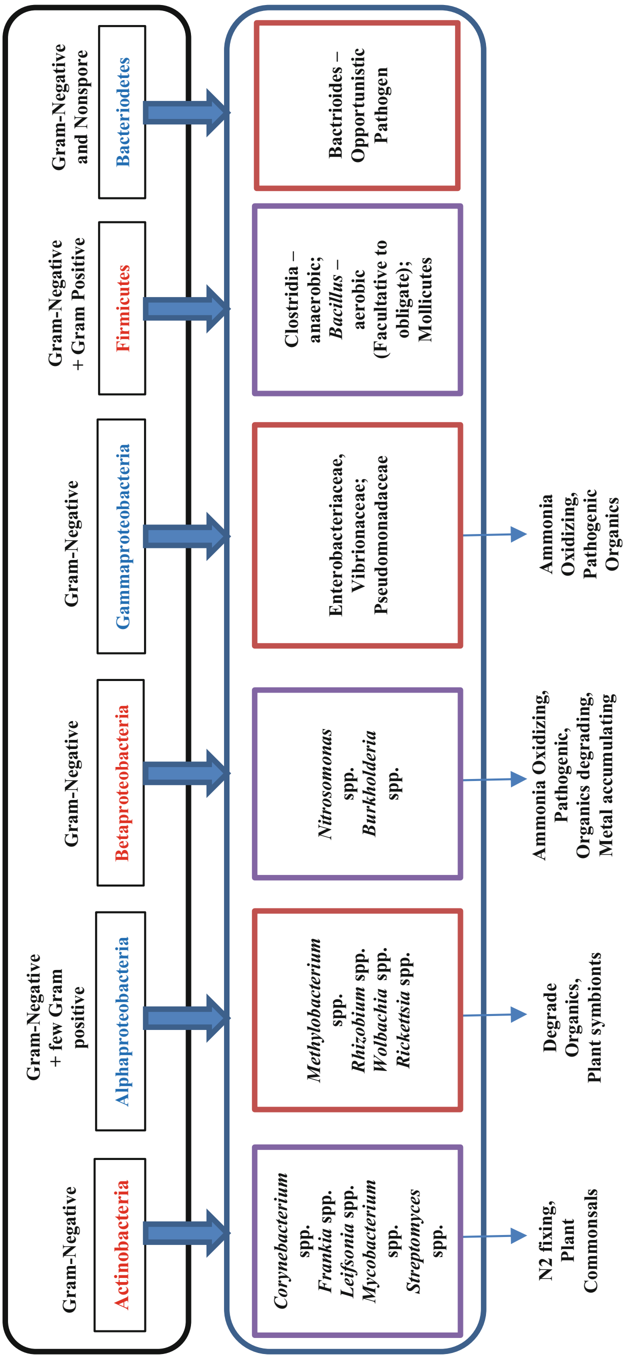

Fig. 5.1 Common bacterial group in aquatic environment

levels of contamination in the water in terms of change in physicochemical parameters (Matta et al. 2018d, 2020b, c, 2022a, d; Gjyli et al. 2020; Kumar et al. 2021; Bensoltane et al. 2021) and heavy metal toxicity (Matta et al. 2018b, c, 2020d, c) due to industrial and agricultural runoff and other sources. It was also observed in several studies that decreasing water quality has negative impact on the planktons diversity (Matta et al. 2018a, 2020a) and limnological condition including species diversity in the water (Matta and Uniyal 2017). However, in the UN agenda of 2030 of Sustainable development Goals (SDGs), water, health, and hygiene practice is very important for making a healthy environment (Matta et al. 2022b).

5.3 The Importance of Aquatic Plant-Microbe Interactions in Freshwater Ecosystems

The macrophytes in the freshwater ecosystem include members of 4 distinct groups: (a) free-floating (e.g., *Pistia stratiotes*) (b) Floating (only leaves) (e.g., *Hydrilla* spp.), (c) submerged macrophytes (e.g., *Chara* spp.), and (d) Emergent (e.g., *Phragmites australis*) and are only found in the macroscopic flora. According to various studies, it was revealed that the distribution of aquatic plants and microbiological species is significantly influenced by the freshwater's nutritional quality (Buosi et al. 2011; Wu et al. 2007; Debbarma et al. 2021) in the increasing order from oligotrophic to hypertrophic. Many microbial communities in water and soil directly interact with macrophytes in the rhizoplane. There is no negative effect on micriobial community's structure by the macrophytes. This has been proved by several studies even in sediments too (Munch et al. 2007; Ahn et al. 2007). The prolonged surface that aquatic plants' roots give for the benthic microbial community to rest and the unique niches they provide as for individual microbes ensure a constant supply of oxygen, nutrients, and organic carbon (Stottmeister et al. 2003). Microbes provide mineral nutrients and protective immunity to aquatic plants in exchange, building solid interactions between the two. To prevent the entry of this hazardous metal into the plants, Stout (2006) showed how the interaction between plants and microbes on Lemna minor negatively affects the uptake of Cd metal ions. Water chemistry (salt concentrations, pH, dissolved oxygen, electrical conductivity, dissolved organic matter, and hazardous organic pollutants), redox conditions, and nutrients availability are a few elements that affect how plants and microbes interact in freshwater bodies (Buosi et al. 2011; Ahn et al. 2007).

However, less studies are available on the importance of plant-microbe interactions in aquatic ecosystems, some typical examples of these interactions and their function in the aquatic system are shown in Table 5.1. The connection between aquatic macrophytes and microbes is also seen in Table 5.1, with the nitrogen cycle being the primary beneficiary. A zone of influence for aquatic plants, the rhizoplane has distinct water chemistry from the rest of the water column due to vigorous microbial activity (Stout and Nüsslein 2010). According to this, regardless of the plant and microbe species, aquatic plants and microorganisms interact in most aquatic regimes, including artificial wetlands, in parasitic and symbiotic ways.

Table 5.1 Environmental perspectives of plant-microbe interaction in aquatic ecosystem

Microbe	Plant	Interaction type	Environmental significance	References
Methylosinus trichosporium	Rooted macrophytes	Ectorhizospheric	Degrade trichloroethylene	Tsien et al. (1989)
Mycobacterium gilvum	*Phragmites australis*	Ectorhizospheric	Degradation of bezo[a]pyrine	Toyama et al. (2011)
Hydrogeno phaga S1; *Agrobacterium radiobacter S2*	*Phragmites austrails*	Ectorhizospheric	Degradation of acid Orange-7	Davies et al. (2006)
Bacillus cereus GXBC1	*Pistia stratiotes*	Ectorhizospheric	Enhanced Cr (VI) uptake	Chakraborty et al. (2013)
a, b, c proteobacteria	*Typha latifolia* (L.)	Endorhizospheric	Reduction of Fe (III) into	Ye et al. (2001), Carranza-Alvarez and Alonso-Castro (2008)
AMF	*Ipomea aquatic*	Endorhizospheric	Enhanced cd uptake	Bhaduri and Fulekar (2012)
Microbacterium sp.	*Phragmites communis*	Endorhizospheric	Degrade Chloropyriphos	Chen et al. (2012)
Rhizoplane bacteria	*Eichhornia crassipes (Mart.)*	Rhizospheric	Reduce the toxicity of heavy metals	Duraivadivel et al. (2020)

Unlike aquatic plants, which rely more on resources like organic carbon and oxygen (particularly at the rhizoplane) that are needed largely by microbes to thrive, upland plants also produce a variety of chemical messages to link with other species (Badri et al. 2009). Commonly, there are two different kinds of symbiotic relationships that microbes can have with plants: (1) endophytic, which involves the colonization of a plant's internal tissues, including nitrogen fixing diazotrophs and other nutrient assimilation AMF and (2) ectophytic, for example, ammonia-oxidizing bacteria and methanotropic bacteria (Nielsen et al. 2001; Sorrell et al. 2002; Srˇaj-Krzˇicˇ et al. 2006; Wei et al. 2011). An essential plant association is an ectophytic, which involves roots and leaves. Ectophytic interaction affects the elemental cycles in aquatic ecosystems by influencing many biochemical events that take place at the interacting surface (Laanbroek 2010).

The oxygen is transferred from stem to root via interlinked lacunae in the plant-microbe interaction within ectophytic zone of influence (ectorhizosphere) (Sand-Jensen et al. 2005), with some of it being expelled from the roots either through humidity-induced pressured flow by air venture method, generally considered as radial oxygen loss (ROL). The ROL is defined by vegetation types and the redox potential of water, with the maximum amount of oxygen stimulating the growth of aerobic nitrifying bacteria (Reddy et al. 1989; Wiessner et al. 2002; Stottmeister et al. 2003; Inoue and Tsuchiya 2008; Soda et al. 2007) and heterotrophic bacteria aerobically decomposing organic materials present as plant extracts. Oxygen is primarily used as a principal electron acceptor in energy production (Bodelier 2003) and in various beneficial oxidation activities (Laanbroek 2010). Furthermore, organic matter diagenesis in sediments occurs via oxic and anoxic microbiological activities, with the use of electron acceptors such as oxygen resulting in an oxygen deficiency region. To disintegrate organic material under this kind of anaerobic condition, bacterial cells (facultative anaerobes) that can use NO^{3-}, SO_4^{2-}, and CO_2 as terminal acceptors of electrons become more effective (Steenberg et al. 1993). This increases the sediments' electron transport system (ETS) activity (Germ and Simcˇicˇ 2011). By reducing CO_2 with H_2, methanogens release methane gas (CH_4). Following the total utilization of all other electron acceptors besides CO_2, the lowest energy-yielding activity, CH_4 generation, dominates in freshwater environments (Rejmankova and Post 1996; Conrad 2008).With radial oxygen loss, which involves oxygen being released from the roots and traveling through interconnected lacunae, aquatic macrophytes replace the oxygen in the deep waters. By employing O_2 as an electron acceptor and organic secretions as carbon, ROL and root organic secretions trigger aerobic microorganisms to execute metabolic action for their survival. These essential conditions form the basis of most plant-microbe interactions (endorhizospheric and ectorhizospheric). Plants receive nutritional minerals in exchange and defenses against infections and hazardous contaminants. Anaerobic living forms use terminal electron acceptors like NO -, CO -, CH -, and SO_2- to break down organic matter in the lowest oxygen region or in sediments, producing minerals and gases. The rhizoplane, which includes both the interior and external rhizospheric zones, is made into a zone with a high electron transport system (ETS) and energy usage by bacteria.

5.4 Improvement of Water Quality Due to the Interaction of Plants and Microbes

As noted in the earlier segment, the interaction of plants and bacteria in the environment is extremely visible and impacts the general quality of media. The interaction between plants and microorganisms in freshwater ecosystems is abundant and necessary for their existence. Environmental pollution prevention, generally referred to as bioremediation and the most extensively studied area in environmental and biological sciences worldwide, is the combined impact of plant-microbe interactions in a larger sense (Pilon-Simts and Freeman 2006). Generally, the relationship between plants and microorganisms is based on mutually beneficial exchanges. For example, plants give microbes the minerals and metabolites they need to develop, while microbes give oxygen and organic carbon to plants. Table 5.1 gives some studies showing the plant microbe interaction in improving water quality.

5.4.1 Organic Pollutants Degradation

Freshwater ecosystems are the most sensitive of all ecosystems because of the widespread field application of organic substances like chlorinated organic compounds, poly-aromatic hydrocarbons (PAHs), poly-brominated biphenyls ethers (PBEs), and poly-chlorinated biphenyls (PCBs). There are well-known bioremediators due to their catabolic activity, which can break down almost all kinds of organic compounds (Hiraishi 2008; Fennell et al. 2011).

One of the primary tools used by bacteria to catabolically degrade resistant organic compounds and obtain organic carbon and electron acceptors from the rich rhizosphere of land and aquatic macrophytes is cometabolism (Stottmeister et al. 2003; Kour et al. 2021). While the microbial community relies primarily on the macrophytic species, the rate of microbial degradation is of second-order kinetics and related to the number of bacteria and amount of xenobiotics in water sources (Paris et al. 1981). (Calheiros et al. 2009). Additionally, the organic carbon supplied by plants to rhizospheric bacteria aids in degrading complex resistant organic substances, including PAHs and pyrenes (Mordukhova et al. 2000; Mori et al. 2005; Jouanneau et al. 2005; Debbarma et al. 2017).

A typical instance of this concerted mutual gain was reported by Golubev et al. (2009), in which plants receive the indole acetic acid (IAA), harmone responisble for growth, as a product of the rhizospheric microbial breakdown of PAHs. Other researchers have previously found similar findings on other aquatic species and sediments (Huang et al. 2004; Escalante-Espinosa et al. 2005). The microbe *Sinorhizobium meliloti* P 221, which forms an ectorhizospheric connection with aquatic plants and is capable of synthesizing IAA by degrading PAHs, was isolated and identified by Golubev and colleagues. Additionally, prior studies by Gasol and Duarte (2000) suggest that bacteria have the best chances of surviving in the productive aquatic environment of algae, where they can make efficient use of the carbon generated from the algae to grow and reproduce.

Problems with taste and odor are brought on by an upsurge in bacteria in freshwater (Okabe et al. 2002). The dissolved organic matter (DOM) (Tranvik 1998), which includes PCBs (polychlorinated biphenyls) (Ghosh et al. 1999) and atrazine, can be degraded by aquatic-plant-associated Biofilm, which primarily contains amines, aliphatic aldehydes, and phenolics (Guasch et al. 2007). A group of a and c proteobacteria known as universal methanotrophs, which use methane as a carbon source, are also abundant in the rhizoplane of aquatic plant species (Semrau et al. 2010). Many toxic organic compounds are degraded by Particulate methane monooxygenase (pMMO) generated by the methanotrophs (Pandey et al. 2014; Yoon 2010), especially chlorinated ethenes (Tsien et al. 1989; Yoon 2010) by a series of enzymatic reactions that first produce formaldehydes and then the terminal compound CO_2.

5.4.2 Inorganic Pollutants Removal

Aquatic biota are unaffected by the modest concentrations of metal ions that normally exist in aqueous ecosystems as a result of gradual leaching from rocks and soil (Zhou et al. 2008). In many regions worldwide, increased metal ions in water are mostly caused by industrial, agricultural, and municipal wastes. Numerous bio/chemical parameters, such as the pH and Eh (redox potential) of the water, the occurrence of hydrated iron oxides, metal carbonates, and plant-microbe interaction as a biofilm on the rhizosphere of macrophytes, affect the movement of metal ions in the water (Hansel et al. 2001; Carranza-Alvarez and Alonso-Castro 2008). Most metals in water occur in the form of cations, which stick to the negatively charged EPS of the biofilm matrix and stop metal ions from entering it and the plants.

Most freshwater macrophytes absorb metal ions from water and have iron plaque around their roots and submerged sections (King and Garey 1999; Hansel et al. 2001). When iron is oxidized by molecular oxygen or by bacteria that can oxidize iron, such as *Ferroplasma* sp. and *Leptospirillum ferroxidans*, a coating of iron (hydroxide) precipitate forms surrounding the plant components (King and Garey 1999). The plant's root porosity increases the oxygen level at the rhizoplane, which influences radial oxygen loss (Li et al. 2011). More iron plaques may grow as a result of iron-oxidizing bacteria. The process of root porosity, plaque development, ROL, and the toxic response to As (arsenic) metalloid have also been shown by Li et al. (2011); however, the latter was found to have significantly diminished.

Rhizoplanes are where interactions between plants and microbes are most active. The biofilm in the figure, which is assumed to be present on the rhizosphere of an aquatic plant, is involved in a number of bio−/physicochemical processes, including nitrification (1) denitrification, (2) sulfate reduction, (3) iron oxidation, and (4) methane oxidation by the appropriate microbes. The chemical ions produced as a result of these reactions enter the water column, where they trigger additional physicochemical metal sulfate (MS) precipitates, which are seen on the surface of water bodies and these are mostly formed when hydrogen sulfide combines with metal cations.

Iron plaques are formed on the surface of plants when $Fe^{3}+$ and water interact to generate Fe (OH) 3. Most facultative anaerobes break down organic materials and plant matter to obtain carbon in anoxic environments, and they use CO_2, SO_4^{2-}, and NO_3- as terminal electron acceptors at enhanced plaque formation. Sulfate reduction, which occurs when sulfate-reducing bacteria are connected with aquatic macrophytes as biofilm, is another significant metal-separating process in the aquatic environment after the oxidation of iron (Machemer and Wildeman 1992). This process lowers the pH, which is necessary for the microorganism cell to biosorbent the metal ions from the water column (Han and Gu 2010).

Additionally, metal ions sequester metal ions from the water column by reacting with hydrogen sulfide in water (as a result of sulfate reduction) to generate metal sulfide, which precipitates in acidogenic circumstances (Webb et al. 1998; Kumar et al. 2019). In order to remove contaminants from water, macrophytes and algae may work together. For instance, Munoz et al. (2006) observed that the microalga *Chlorella sorokiniana*, which is associated with the bacterium *Ralstonia basilensis*, enhanced the adsorption of toxic metals like cadmium, copper, nickel, and zinc, particularly for Cu (II) adsorption due to the presence of more copper binders. Most aquatic plants also associate with mycorrhizae as endophytic symbionts, which improves P absorption and nutrient transfer in the plants (Thingstrup et al. 2000; Srˇaj-Krzˇicˇ et al. 2006). Toxic contaminants like heavy metals are kept away from plants by mycorrhizal interactions. By obstructing the phosphorus membrane transportation system, a chemical counterpart of As, the mycorrhizal interaction in vetiver grass (a widespread wetland species of the Indian subcontinent, South East Asia, and Australia) protects from the As (III) (Meharg and Hartley-Whitaker 2002).

The growth of plants and microorganisms in an aquatic environment heavily relies on the presence of nutritional ions like different mineral elements, P, and N. Water bodies get eutrophicated as a result of too many nutrient ions, which is followed by a cyanobacterial bloom and the production of toxins (Giaramida et al. 2013). Overabundant nutrient ions from the water are taken up by aquatic macrophytes, which prevent algal growth. *Pistia stratiotes, Ipomea aquatica, Eichhornia crassipes*, and *Spirodela polyrhiza* are free-floating macrophytes that play a significant role in the removal of nutritional ions like dissolved inorganic nitrogen such as ammonium NH_4. *Nitrosomonas* and *Nitrobacter*, two aerobic chemoautotrophic bacteria, work together in the rhizosphere to oxidize ammonium to NH_4NO_2.

5.5 Future Perspectives

Characterizing the microbial population associated with the rhizoplane of aquatic plants using metagenomics and other technological advances will be crucial for future studies on plant-microbe interaction and its function in environmental cleanup and restoration. The behavior of plant-microbe interactions at the rhizoplane of free-floating aquatic macrophytes under increased CO_2 in the atmosphere and at increased ambient temperature, as well as the formation of new interactive combinations in freshwater regimes, would be fascinating to study, according to the second point. It is important to investigate further the content and role of the microbial community in a biofilm dealing with a specific plant species, including the effects of nearby harmful chemicals on microbial assemblages, how the microbial community changes in response to climate change, and the environmental implications of newly identified transgenic plant-microbe interaction.

5.6 Conclusion

Particularly on the rhizoplane, plant-microbe interaction is frequent in freshwater environments. Plants exude a variety of organic molecules (plant exudates) that include lipids, phenolic compounds, amino acids, polysaccharides, and nucleic acids to preserve the developing soft tissues, to absorb minerals according to the local electrochemical situation, and to attract bacteria that form a community of distinctive features that carry out specific functions. Based on the connections, these encounters might range from favorable to negative in character. Actinobacterial species, a, b, c, and d, proteobacterial species, bacteriodetes, firmicutes, and archaea species interact and coexist in a complex system of exopolymeric substance (EPS), which creates a matrix of microbial network (biofilm). The composition of the consortia of microbes changes among different species of plants depending on the type and amount of organic carbon as well as the Oxygen level at the rhizoplane. Known as radial oxygen loss, rooted macrophytes in deep seas continuously replace the oxygen lost by microbial and chemical intake by delivering through the plant's linked lacunae from the stem to the root where the O_2 is discharged (ROL). However, anaerobic life forms in the absence of or in low oxygen levels. The ROL at the rhizoplane delivers it a high electron transport (ETS) area where O_2 functions as electron acceptor requisite for the existence of aerobic life forms.

Each freshwater species of plants have a specific and predetermined pattern of microorganisms in addition to the bio- or physicochemical characteristics. While both species depend on one another for life, the interaction between flora and fauna makes the resources in the area available and, inadvertently, contributes to a greater degree to the removal of environmental pollutants. Examples include mycorrhizal interaction, which improves nutrient absorption and shields plants from hazardous metals by preventing their immediate access, and bacterial species, which break down PAHs to produce indole acetic acid (IAA), a hormone that promotes plant life.

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Microbial Interactions with Aquatic Plants

6

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Abstract

A fresh water ecosystem consists of rich floral diversity, which includes macrophytes, microphytes, diatoms, and many other algae spp. In addition, a fresh water ecosystem provides best niche for various levels of microbial communities such as biofilms and planktonic microalgal-bacterial consortia. Microbial accumulation as biofilms on aquatic plant surfaces is robust, and biofilms have the ability to change its structure with habitat and environmental changes. Adhesion of biofilms to plant surfaces is the main way bacteria interact with plant tissues. Through these interactions, aquatic plants provide nutrients, organic carbon, and oxygen to microorganisms and in return aquatic plants mainly receive mineral nutrients and defensive immunity. In the process, these interactions significantly contribute to nutrient recycling, energy flow in aquatic ecosystems, and removal of environmental pollutants. Thereby, also in terms of environmental perspective, aquatic plant-microbe interaction has substantially granted water quality.

Keywords

Bioremediation · Aquatic ecosystem · Biofilm · Quorum sensing

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6.1 Microbial and Floral Diversity in Aquatic Ecosystems

Microorganisms dominate all inland water habitats by both numerically and biochemically and proper functioning of an aquatic ecosystem is aided by the rich microbial diversity and it depends on the available nutrient content and prevailing environmental conditions (Baker and Orr 1986).

Bacteria are abundant on the surface of freshwater plants, ranging in density from 100 to 107 cm^{-2} (Fry and Humphrey 1978; Hossell and Baker 1979a, b; Kudryavtsev 1984). Epiphytic bacteria form an integral part of the leaf-surface community (Cummins and Klug 1979; Bhatt et al. 2022). Epiphytic bacteria are metabolically more active than planktonic bacteria present in the surrounding water (Fry and Humphrey 1978). Epiphytic bacteria serve passively as food for higher trophic levels, and as active members of the microbial community. However, bacteria are not distributed evenly over plant surfaces (Hossell and Baker 1979a, b; Ramsay 1974).

Wetlands are the transitional zones between land and water bodies. Shallow overlying water-logged soils harboring rich floral and faunal diversity are the characteristic features of wetlands. Floral diversity of freshwater ecosystem is rich in diversity of macrophytes and microphytes such as phytoplankton, diatoms, and other algae. The large plants growing in the water and wetlands are aquatic macrophytes. Microphytes are microscopic algae found in freshwater and marine water systems (Srivastava et al. 2017). Macroscopic flora of aquatic macrophytes includes four different groups: (1) emergent (e.g., *Phragmites australis*), (2) floating leaved (e.g., *Hydrilla* spp.), (3) free floating (e.g., *Pistia stratiotes*) and (4) submerged macrophytes (e.g., *Chara* spp.) (Srivastava et al. 2008).

The part of root remaining in contact with water or soil of all macrophytes is rhizoplane, and it is the most active zone due to the presence of various microbial communities (Davies et al. 2006; Munch et al. 2007). Extended surface for microbial community is provided by roots of aquatic plants ensuring continuous supply of nutrients, organic C, and O (Stottmeister et al. 2003). And at the same time aquatic plants are provided with mineral nutrients and defensive immunity in return from microbial community and this way both are forming an interrelationship (Srivastava et al. 2017).

The status of freshwater is one of the major dependent factors for the distribution of aquatic plants and microbial species in the following order: oligotrophic > mesotrophic > eutrophic > hypertrophic (Wu et al. 2007; Buosi et al. 2011). For the proper growth of both macrophytes and microphytes, they require optimum concentrations of major nutrients such as N (>45 mgL^{-1}) and P (>0.25 mgL^{-1}) along with organic C and other elements (Srivastava et al. 2008).

Microbial association exists at various levels of microbial community and can be observed as microbial mat, biofilms, and planktonic microalgal-bacterial assemblages and contribute to the nutrient cycling in nitrification, denitrification, sulfate reduction, methonogenesis, and metal ion reduction. And also serve in energy flow in aquatic ecosystem as a feed to zooplanktons. They serve in degrading environmental pollutants and alter the water quality (Paerl and Pinckney 1996;

Cotner and Biddanda 2002; Battin et al. 2003; Hahn 2006). Microbial biofilms generally occur on the leaves of submerged plants and rhizosphere. Some environmental conditions (excessive nutrients and their availability) and the toxic substances on the water affect biofilms and their structure (Giaramida et al. 2013; Calheiros et al. 2009).

6.2 Microbial Biofilms and Quorum Sensing in Aquatic Ecosystems

In aquatic environments, in order to survive in harsh environments, bacteria prefer to form microbial communities, which are attached to different surfaces and are embedded in an extracellular polymeric matrix. These specific microbial communities are called microbial biofilms (Nazir et al. 2019; Afonso et al. 2021; Jayaprada et al. 2020). In order to maintain a high density of bacterial cells in a biofilm, bacteria produce a matrix of substances called extracellular polymeric substances (EPSs) (Afonso et al. 2021). Polysaccharides, proteins, lipids, and extracellular DNA are the main components of EPS (Afonso et al. 2021). Biofilm formation occurs following several main steps, which are attachment to a surface, formation of microcolonies, colony maturation, and detachment phases (Davey and O'toole 2000; Gong et al. 2019). Biofilm formation is started by the attachment of planktonic cells to surfaces like aquatic plants, which are called adhesive biofilms. Some planktonic cells gather at an air-liquid interface and form floating biofilms. Next step is the propagation of cells followed by the aggregation of these newly formed cells to form microcolonies. EPSs formation occurs at this stage, which helps in the attachment and aggregation of cells with scaffolds gradually forming mature biofilms (Dang and Lovell 2016). Lumps of biofilms can be separated due to friction, pressure, and rapid water flow returning to their planktonic state, moving to find new niches. It is mentioned that biofilm formation and composition are affected by variables like temperature, salinity, pH, nutrient concentration of marine and freshwater along with other factors like geographical location, season, availability of light, depth of water, and availability of tides (Ghannoum et al. 2020).

Microbial biofilm is a consortium of both prokaryotes (bacteria and archaea) and eukaryotes (algae and fungi) (Zhang et al. 2019). This leads to a rich microbial community, creating a microenvironment where thousands of different species can exist together in a complex makeup, maintaining remarkable features like gene expression heterogeneity and division of functions in the community. These functions are carried out by cell-to-cell communication called quorum sensing (Antunes et al. 2019). In a nutshell, quorum sensing coordinates population-density-dependent changes in microbial behavior. During the process of biofilm formation, microorganisms communicate with each other through quorum sensing. It is reported that induction of biofilm formation is manifested through quorum-sensing-regulated metabolic activity of planktonic cells (Hmelo 2017; Lami 2019). Microbial biofilms are also controlled by factors like interactions with the environment, interactions with the geography of the location, cycling of nutrient and organic matter, and photosynthesis, which are harnessed through quorum sensing. As a result, biofilms are capable of adapting to dynamic environments influencing ecosystem processes and functioning (Bauer and Mathesius 2004; Joint et al. 2007; Stien et al. 2016). Quorum sensing and biofilm formation involve the biosynthesis of and response to diffusible signal molecules, which differ among different types of bacteria (Mooney et al. 2018; Saxena et al. 2019).

These signal-molecule-based quorum sensing is reported to be the basis behind microbial interactions with aquatic plants defined as interkingdom communication (Bauer and Mathesius 2004; Joint et al. 2007; Stien et al. 2016; Wijewardene et al. 2022). Hence, in both fresh and marine water environments, biofilms have been the subject of studies in terms of microbial-aquatic plants interactions. Therefore, in the following chapters, microbial-aquatic plants interactions will be discussed in terms of biofilms-mediated quorum sensing.

6.3 Aquatic Plant-Microbe Interactions and Its Role

6.3.1 Role of an Aquatic Plant in Plant-Microbe Interaction

Aquatic environment consists of numerous plants species with higher diversity. Aquatic plants existing in a particular water reservoir provide significant impact on microbial growth and development through forming symbiotic relationship (Yoneda et al. 2021). In an aquatic ecosystem, macrophytes are categorized into four different groups such as emergent, floating leaved, free floating, and submerged macrophytes (Srivastava et al. 2008). The surrounding area of macrophytes root is known as "rhizoplane," which is a highly active zone where most of biochemical reactions take place (Munch et al. 2007). Aquatic plants play a major role in structuring microbial assemblages (De Wolf et al. 2022). Such interactions are well described in terrestrial systems as well as in aquatic systems (Srivastava et al. 2017). Aquatic macrophytes directly influence water chemistry by altering pH, dissolved oxygen, and dissolved organic carbon concentration by releasing bioactive compounds (Vilas et al. 2017; Shahid et al. 2020). The neutral pH induced by vegetation encourages the settlement of dissolved contaminants (Borne et al. 2014; Debbarma et al. 2017). Therefore, microbial composition in the aquatic environment would slightly influence due to this alternated chemical nature, thus ultimately impacting its physicochemical condition of water (Collins et al. 2004). In addition, aquatic plants have significantly influenced nutrient uptake and storing of numerous trace metal(loid)s, thus controlling the competition for nutrients by algae and other phytoplankton (Liu et al. 2016). For instance, plants might remove phosphorous by direct uptake and inhibit growth of algae communities (Urakawa et al. 2017). Therefore, aquatic plants maintain the water quality and overwhelm algae blooms in water reservoirs, ensuring macrophyte-dominated clear aquatic ecosystem (Bakker et al. 2010).

Apart from chemical and microbial composition of adjacent water, plants' roots would influence on microbial biogeography in large scale, through providing surface for microbes to form "microbial biofilms" on the surfaces of roots and shoot parts

(Srivastava et al. 2017). Biofilm formation affects the special distribution of microbes under small-scale water reservoirs like lakes (Souffreau et al. 2018). Furthermore, the composition of plant-associated biofilms is species specific and varies with the surrounding water (Souffreau et al. 2018; Kumar et al. 2019; He et al. 2021). Root exudates secrete by macrophytes and deliver essential nutrients for microbes' growth (Ashraf et al. 2018). Furthermore, roots facilitate air circulation in rhizosphere through supplying of oxygen, thus accelerating the aerobic degradation of organic matter by rhizosphere bacteria. As a result of this degradation, nutrients are being accumulated in rhizosphere, which uptake by plants (Shahid et al. 2020). Simultaneously, Ijaz et al. 2016 reported roots assist in the reduction of biological oxygen demand (BOD) and chemical oxygen demand (COD) by enabling microbial populations to ingest the carbon molecules. Aquatic macrophytes have been widely used in constructed wetlands and floating treatment wetland (FTW) to remediate contaminants from polluted water artificially (Shahid et al. 2020). The selection of an appropriate plant species for these systems depends upon their availability, contaminant type, and climatic region. Moreover, the pollutant remediation potential of selected macrophyte should be good and ensure the ecosystem sustainability (Shahid et al. 2020). Furthermore, to develop an effective FTW, macrophyte with robust shoot growth, widely distributed root system, tolerance to toxic compounds, higher nitrogen removal capability, and availability of large aerenchyma in their roots and rizomes characteristics are highly concerned (Li et al. 2011; Lamers et al. 2013).

6.3.2 Role of Microbes in Plant-Microbe Interaction

Microbial communities in aquatic environment have a significant role in pollutant removal progression and plant growth promotion (Shahid et al. 2020). The microbial diversity depends on the nutrient and existing environmental circumstances (Zehr 2010). Freshwater microbial diversity includes actinobacteria, α-proteobacteria, β-proteobacteria, ɤ-proteobacteria, archea, bacteriodetes, cyanobacteria, fungi, etc. (Wei et al. 2011; Shahid et al. 2020). Among them, several bacteria have a distinct ability to form biofilms, which are known as epiphytic microbes; thus, microbial presence in the aquatic bodies can be categorized into biofilm-forming bacteria and water column bacteria (Shahid et al. 2020).

These biofilms have significant impact on continuation of biochemical cycles and other ecosystem processes in aquatic environment (Battin et al. 2003). A biofilm is mainly composed of aquatic plant and related bacteria communities. Biofilms are composed of extracellular polymeric substances (EPSs), proteins, comprised of polysaccharides, lipids, and nucleic acids, which embrace the cell together (Branda et al. 2005). The further development and maintenance of biofilms depend upon minor molecules secretion by microbes such as secondary metabolic compounds, antibiotics, and proteins (Lasa and Penadés 2006; López et al. 2010). Furthermore, the nature of biofilm is affected by growth conditions (e.g., water flow, light availability, nutrient conditions) and type of substrate (Shahid et al. 2020).

In addition, endophytes also contribute to strengthen the plant-microbe relationship through protecting plant from biotic stresses, relieving abiotic stresses, and facilitating metal uptake from contaminated water (Bacon and White 2016). Endophytic microbes hasten the organic and inorganic degradation process, which eventually produces nutrients essential for plant uptake (Wei et al. 2014; Peiris et al. 2022). For instance, rhizobacteria stimulate the growth of plants and restrict trace metal uptake, hence protecting plants from metal-induced toxicity (Ma et al. 2015). Moreover, endophytes involved in decontamination of other pollutants amplify metal translocation process (Babu et al. 2015). For example, endophytes in a FTWs system, vegetated with P. *australis* effectively, removed most of trace metal (loid)s from the polluted river (Shahid et al. 2019). According to previous research findings, it has been proven that prior inoculated plants with endophytes would reduce the disease incidents from microbial infections and biological agents such as insects and nematodes (Rasche et al. 2006). Endophytic response to stresses mainly occurs through production of siderophore, antimicrobial metabolics, phosphate solubilizing compounds, and phytohormones (Glick 2012; Pinheiro et al. 2013; Matsuoka et al. 2013; Jasim et al. 2014). Endophytes enable to mitigate stress conditions by accelerating of photosynthetic process, enhancing translocation, activating of antioxidative enzyme functions, etc. (Shahid et al. 2020).

6.3.3 Aquatic Plant-Microbe Interactions in Aquatic Ecosystem

Aquatic macrophyte-microbe interaction is a crucial aspect in maintaining of ecosystem equilibrium. However, very limited studies have been conducted to examine the significance of this relationship (Srivastava et al. 2017). However, symbiotic relationship between plant and microbe leads to positive outcomes for both organisms. Table 6.1 illustrates the several examples for plant-microbe interactions found in aquatic environment.

The nitrogen fixation by microbes occurs mainly in rhizoplane with the aid of numerous nitrogen-fixing bacteria (Suyal et al. 2018; Shahid et al. 2020). The plant root would supply oxygen and organic matter for nitrogen-metabolizing bacteria (Jun-Xing et al. 2012; Rawat et al. 2019; Wewalwela et al. 2020), whereas aquatic plant receives nutrients (e.g., Ammonium) from microbial degradation (Ormeño-Orrillo et al. 2013). Moreover, roots also reduce nitrogen concentration from the rhizosphere and regulate the pH and redox potential in the rhizosphere (Husson 2013). Scientists have identified significant N-fixing bacterial genera, namely, *Azospirillum*, *Pseudomonas*, *Enterobacter*, *Vibrio*, and *Klebsiella* (Lamers et al. 2012; López-Guerrero et al. 2012). Several aquatic plants such as P. *australis*, *J. effuses, J. balticus*, *Sagittaria triflolia*, and *Zostera marina* associated with nitrogen-fixing bacteria effectively fix nitrogen in aquatic reservoirs (Hiraishi 2008; Neori and Agami 2017). Similar types of interaction have been recorded by Stirk and vanStaden (2003), Rivas et al. (2003), Hay et al. (2004), and Wagner (2012) with different nitrogen-fixing species (see Table 6.1). Further, symbiotic relationship between nodulating bacteria of water legume (Ghosh et al. 2015) and

Table 6.1 Aquatic ecosystem interactions between plants and microbes

Plant species	Microbial species	Major function in ecosystem	References
P. australis	Nitrogen-fixing bacteria	Nitrogen fixation	Hiraishi (2008), Neori and Agami (2017)
J. effuses			
J. balticus			
Sagittaria triflolia			
Zostera marina			
Azolla filiculoides	*Arthrobacter* spp.		Stirk and vanStaden (2003)
Neptunia natans nov.	*Devosia neptuniae* sp.		Rivas et al. (2003)
Hemiaulus hauki	*Richelia intracelluaris*		Hay et al. (2004)
Nuphur spp.	*Mesorhizobium loti*		Wagner (2012)
Water legume	Root-nodulating bacteria		Ghosh et al. (2015)
Water fern	Cyanobacteria		Zheng et al. (2009)
Eichhornia crassipes	Bacteria	Denitrification	Gao et al. (2014)
Phragmites australis	*Nitrosomonas*	Ammonia oxidation	Okabe et al. (2012)
Ulva australis	*Pseudoalteromonas tunicate*	Allelopathic impact on algae	Rao et al. (2006)
Vitiveria ziznioides	*Arbuscularmycorrhiza*	Allelopathic impact on bacteria	Nautiyal et al. (2013)
Lemna aequinoctalis	*Acidobacteria F-183*	IAA production in roots	Yoneda et al. (2021)
	Acidobacteria TBR-22		
Chlorella vulgaris	*Azospirillum brasilense*	Plant growth promoting	Gonzalez and Bashan (2000)

cyanobacteria of water fern (Zheng et al. 2009) enhanced the nitrogen fixation in plants.

In addition, nitrogen fixation of some epiphytic bacteria is useful in denitrification and ammonia oxidation (Gao et al. 2014). For instance, *Eichhornia crassipes* remediates eutrophic water through generating of gaseous nitrogen (Gao et al. 2014). Moreover, ammonia oxidation by *Nitrosomonas*-associated *Phragmites australis* is also reported by Okabe et al. 2012. Some specific epiphytic microorganisms associated with aquatic macrophytes are involved with secreting with allelopathic compounds, which retard other algae growth (Rao et al. 2006; Srivastava et al. 2007; Hempel et al. 2008; Table 6.1).

Growth and development of aquatic macrophytes would enhance due to beneficial symbiotic bacteria; these are also identified as plant-growth-promoting bacteria (PGPB) (Rajwar et al. 2018; Pole et al. 2022). These organisms serve for host plant by supplying nutrients and plant hormones (ex: indole acetic acid-IAA). Furthermore, bacteria would provide nutrients through nitrogen fixation and phosphate solubilization (Shahid et al. 2020). For instance, Yoneda et al. 2021 revealed that *Lemna aequinoctalis* (Duckweed) associated with *Acidobacteria* strains F-183 and TBR-22 promoted healthy growth of duckweeds through IAA production. Simultaneously, tested *Acidobacteria* strains enhanced the chlorophyll content in the fronds of duckweeds. Similar of type results have been discussed by Gonzalez and Bashan 2000 (Table 6.1).

6.4 Environment Perspectives of Aquatic Plant-Microbe Interactions

6.4.1 Remediation of Pollutants by Microorganism-Plant Association

Aquatic ecosystem is adversely affected globally by enhanced anthropogenic activities during last few decades (Hill 2020). Industrialization and urbanization processes have accelerated the water pollution, which led to increase in numerous health hazardous resulting in deaths due to consumption of contaminated water (Xu et al. 2018a; Supreeth 2022). For instance, bioaccumulation of toxic pollutants exists in water, encourage trace metal(loid)s accretion in human body, and later change the physiological functions of the body. Simultaneously, when aquatic pollution increases, it negatively affects the ecological sustainability of the particular area (Supreeth 2022). Interestingly, due to epidemic COVID-19 pandemic situation prevailed in last 2 years has reduced the total environmental pollution (total environment including water, soil, and air ecosystem) significantly compared to previous years (Muhammad et al. 2020). However, consider to aquatic ecosystem, application of viable, economical and eco-friendly strategy would provide sustainable solution to mitigate water pollution from contaminants. Among the various remediation strategies use in contaminants removal in water; biological remediation or bioremediation has been widely used. Biological remediation usually relies with microorganisms and phytoremediation processes. However, according to previous research studies related to remediation suggest that microorganisms accompanied with plants would accelerate the remediation process in advance (Giri et al. 2017; Supreeth 2022). The plant-microbe combined system provides several advantages over each of these methods (Soni et al. 2022; Supreeth 2022). Through this combination, pollutants would be degraded and uptake by plants more effectively (Nasr 2019).

6.4.2 Remediation of Contaminants

Due to the phytotoxic impact, phytoremediation of pollutants by macrophytes and biodegradation of contaminants by microorganisms alone frequently lead to inadequate metal removal in aquatic ecosystems (Supreeth 2022). However, combination of these two methods showed significant impact on contaminants removal from the polluted water (Mishra et al. 2020). Nonetheless, the micro-environmental condition in polluted water quite differs compared to noncontaminated water. Mainly, plant required nutrient concentration and dissolved oxygen content are low in toxic water (Supreeth 2022); thus, both organisms might face unfavorable conditions to survive. Therefore, plants initiate to interrelate with root associated microbes through releasing several chemical substances which leads to create interactions (Supreeth 2022). Macrophytes would provide micro-aerophilic conditions and accelerate the degradation of contaminants through producing of numerous root exudates. In addition, plant roots stimulate microbe activities on contaminants through providing the substrates and then changing of media pH (Hussain et al. 2018). Simultaneously, microbes presence in rhizosphere or/and epiphytic bacteria involve in nutrient cycling and ensuring the vigorous plant growth (Clark et al. 2018; Kumar et al. 2021; Debbarma et al. 2021; Suyal and Soni 2022). Moreover, endophytic bacteria reside inside the plant tissues increase the plant's capability for cope with various biotic and abiotic stresses (O'Brien et al. 2020).

Presently, microbe-plant combined remediation system is widely used to remediate pollutants such as excess nutrients, heavy metals, petroleum-hydrocarbon, pharmaceuticals, and personal care products (PPCP) through artificially prepared systems, namely, constructed wetlands (CW) and floating treatment wetlands (FTW) (Zhang and Shao 2013; Shahid et al. 2020; Dash et al. 2021).

6.4.3 Removal of Excess Nutrients in Polluted Water

Nutrient accumulation (especially nitrates and phosphates) in water reservoirs result in severe environmental issues like "eutrophication" (Padedda et al. 2017). Several anthropogenic activities such as intensive catchment agriculture and industrialization could be reason for this situation and indirectly affect human health (Jiang et al. 2019). Hence, removal of these compounds is an essential process. However, efficiency of plant-microbe strategy depends on pollutant concentration, previous research experiments have proved beyond doubt that plant-microbe interaction in aquatic rhizoplane would enhance the cleaning process (Chen et al. 2012; Xu et al. 2018b). For instance, Organophosphorous compounds present in polluted water was removed by the plant species *Nymphaea alba*, *Phragmites australis*, and *Myriophyllum verticillatum* with the assistance of bacterial colonies in the plankton (Xu et al. 2018b). Furthermore, *Lolium perenne* plant combined with microbial species such as *Bacillus* sp. MOE1 and *Microbacterium* sp. MOE2 increased the remediation of ammonium nitrogen and phosphorous available in the eutrophic water (Li et al. 2011). Similarly, nitrogen presence in polluted water mitigated by immobilized nitrogen cycling bacteria (INCB) associated with water plants such as *Eichhornia crassipes* and *Elodea muttallii* (Chang et al. 2006). Similarly, Wu et al. 2016 reported several plant species (*T. augustifolia*, *P. australis*, and *A. calamus* L.) have removed nitrogen from water associated with microbial community.

Submerged plant species *Vallisneria natans* inoculated by *Pseudomonas putida* KT2440 enhanced carbon availability in water (Gan et al. 2018). Plant-microbes interaction effectively used in floating treatment wetlands technology to reduce nitrogen and phosphorous from polluted water. For instance, aquatic macrophyte, *Festuca arundinacea* combined with denitrifying polyphosphate accumulating microbes diminished the total nitrogen significantly; since improved the plant growth and biomass production (Zhao et al. 2011). Another study under FTW system revealed that aquatic plant named *Oenanthe javanica* interact with *Archaea* and Anaerobic ammonium oxidation bacteria resulted removal of NH_4^+-N, NO_3- N, and total nitrogen relatively higher in percentage (Wang et al. 2018). In addition, *Typha domingensis* and *Phragmites australis* plants associated with inoculated rhizospheric and endophytic bacteria improved the remediation performances of polluted river water through reducing nitrate and total nitrogen content (Shahid et al. 2019). Concurrently, some plants species such as *Cymbidium faberi*, *Ipomoea aquatica*, *Corbicula fluninea*, and *Thalia dealbata* also effectively used to remove excess nutrients from polluted water (Li et al. 2010; Zhao et al. 2011; Zhang and Shao 2013) and summarized results of these experiments shown in Table 6.2.

6.4.4 Plant-Microbe Interaction Relevant to Trace Metal(Loid)S Removal

In an aquatic ecosystem, phytoremediation process mainly occurs through phytofiltration technique (Islam et al. 2015). However, phytofiltration mechanism is encouraged by bacteria, thus enhancing the bioavailability of trace metal(loid)s (Khan et al. 2015). Several rhizospheric and endophytic bacteria promote trace metal (loid)s removal from contaminated water through absorbing metal(loid)s ions into their cell walls. Releasing of specific metal-binding proteins and peptides, enable microorganisms to accumulate toxic metals and this facilitates plant's hormone and redox signaling process due to toxic metal exposure (Cobbett and Goldsbrough 2002). Especially, endophytic bacteria involve with trace metal bioaccumulation and detoxification (Shin et al. 2015; Ijaz et al. 2016). For instance, *Pseudomonas fluorescence* G10 and *Micobacterium* sp. G16 on *Brassica napus* enhanced the Pd accretion in plant shoots (Sheng et al. 2008). On the other hand, some bacteria release extracellular polymeric substances (EPSs) to reduce metal(loid)s bioavailability (Rajkumar et al. 2009). For example, *Azobacter* sp. produced complexes with Cd through secreting of numerous EPS and decrease metal uptake by *Triticum aestivum* (Joshi and Juwarkar 2009). Furthermore, Shahid et al. 2019 revealed that few plants, namely, *B. mutica*, *T. domingensis*, *P. australis*, and *L. fusca,* remediated trace metal(loid)s (e.g., Fe, Ni, Pd, Cr, and Mn) associated with endophytic bacteria from contaminated river water by floating treatment wetlands. In addition, plant root and biofilm interaction enhanced remediation of Cu and Ni (Tanner and Headley 2011; Ladislas et al. 2015), Cr (Chakraborty et al. 2013), Cu (Munoz et al. 2006), Cr and Zn (Abou-Shanab et al. 2007) etc. displayed in Table 6.2.

Table 6.2 Plant-microbe interaction systems that improve the removal of pollutants and nutrients from aquatic ecosystems

Xenobiotics	Plant species	Relevant microorganisms	References
Organophosphorous compounds (ex: Chlorpyrifos)	*Nymphaea alba; Phragmites australis; Myriophyllum verticillatum*	Planktonic bacterial populations	Xu et al. (2018b)
	Phragmites communis	*Microbacterium* spp.	Chen et al. (2012)
	Nymphaea spp.	*Pseudomonas* spp.	
	Najas spp.	*Paenibacillus* spp.	
Ammonium nitrogen and phosphorous	*Lolium perenne*	*Bacillus* sp. MOE1 and *Microbacterium* sp. MOE2	Li et al. (2011)
	Eichhornia crassipes and Elodea muttallii	Immobilized nitrogen cycling bacteria (INCB)	Chang et al. (2006)
Nitrogen	*Typha augustifolia; Phragmites australis; Acorus calamus L.*	Microbial community	Wu et al. (2016)
	Festuca arundinacea	Denitrifying bacteria	Zhao et al. (2011)
Carbon dioxide	*Vallisneria natans*	*Pseudomonas putida* KT2440	Gan et al. (2018)
Ammonium nitrogen and nitrate nitrogen	*Oenanthe javanica*	*Archaea* and anaerobic ammonium oxidation bacteria	Wang et al. (2018)
Total nitrogen and total nitrogen	*Typha domingensis* and *P. australis*	Rhizospheric and endophytic bacteria	Shahid et al. (2019)
Excess nutrients	*Cymbidium faberi; Ipomoea aquatica; Corbicula fluninea; Thalia dealbata*	Bacterial biofilm	Li et al. (2011), Zhao et al. (2011), Zhang and Shao (2013)
Trace metal (Pd)	*Brassica napus*	*Pseudomonas fluorescence* G10 and *Micobacterium* sp. G16	Sheng et al. (2008)
Trace metal (Cd)	*Triticum aestivum*	*Azobacter* sp.	Joshi and Juwarkar (2009)
Trace metals (ex: Fe, Ni, Pd, Cr, and Mn)	*Brachia mutica; Typha domingensis; Phragmites australis; Leptochala fusca*	Endophytic bacteria	Shahid et al. (2019)
Trace metals (ex: Cu and Ni)	*Carex virgate; Cyperus ustulatus; Juncus edgariae; Schoenoplectus tabemaemontani*	Biofilms	Tanner and Headley (2011), Ladislas et al. (2015)
Trace metal (Cr)	*Pistia stratiotes*	*Bacillus cereus* GXBC1	Chakraborty et al. (2013)

(continued)

Table 6.2 (continued)

Xenobiotics	Plant species	Relevant microorganisms	References
Trace metal (Cu)	*Chlorella sorokiniana*	*Ralstonia basilensis*	Munoz et al. (2006)
Trace metals (Cr and Zn)	*Eichhornia crassipes*	*Ochrobactrum anthropi*	Abou-Shanab et al. (2007)
		Bacillus cereus	
Amines, phenolics, aliphatic aldehydes, polychlorinated biphenyles and atrazine	Aquatic macrophyte	Biofilms	Ghosh et al. (1999), Tranvik (1998), Guasch et al. (2007)
Methane	Aquatic macrophyte	*Proteobacteria*	Yoon (2010), Pandey et al. (2014)
Benzene	*Euphorbia milii, Hedera helix, Chlorophytum comosum*	Endophytic and Epiphytic bacteria	Sriprapat and Thiravetyan (2016)
Phenolic compounds	*Brassica napus*	*Pantoea* sp.	Ontañon et al. (2014)
Phenol	*Phragmite australis*	*Acinetobacter, Bacillus cereus, Pseudomonas* sp.	Saleem et al. (2018a, b)
	Lemna aoukikusa	*Acinetobacter, Calcoaceticus*	Yamaga et al. (2010)
	Chlorella sorokiniana	*Pseudomonas migulae, Sphingomonas, Yanoikuyae*	Borde et al. (2003)
Nitro-phenols	*Spirodela polyrhiza*	*Nitrophenols degrading bacteria*	Kristanti et al. (2014)
Decaclorobiphenyl PCB-209	*Ocimum basilicum L.*	*Acinetobacter, Bacillus, Lysinibacillus, Novosphingobium, Pseudomonas, Rhizobium* etc.	Sánchez-Pérez et al. (2020)
Bisphenol A	*Dracaena sanderiana*	*Bacillus thuringiensis* and *Pantoea dispersa*	Suyamud et al. (2018)
Carbamazepine	*Armoracia rusticana*	*Rhizobium radiobactoer* and *Diaphorobacter nitroreducens*	Sauvêtre et al. (2018)
Indusrial dye	*Phragmites australis*	*A. Juniistrain, Rhodococcus* sp. and *P. indoloxydans*	Tara et al. (2019)
Petroleum hydrocarbon	*Lolium perenne L.*	*Pseudomonas* sp.	Iqbal et al. (2019)
	A. thaliana		
	Halonemum strobilaceum	*Ochrobactrum* sp., *Desulfovibrio* sp., *Halobacterium* sp., etc.	Al-Mailem et al. (2010)

Fungi also have a potential to remediate toxic metals from the polluted water. Root exudates invite fungi to the rhizosphere (Shahid et al. 2020; Kumar et al. 2022). Fungi genera of *Penicillium*, *Aspergillus*, and *Rhizopus* are widely used in trace metal(loid)s removal (Ahalya et al. 2003). Fungi closely associate with wetlands plant roots and form symbiotic relationship, which leads to release of metal-chelating siderophores and concomitant detoxification of metal(loid)s (Liu et al. 2015; Saha et al. 2016).

6.4.5 Degradation of Organic Pollutants Through Plant-Microbe Interaction

Microbes act as excellent bioremediators, since they enable to digest all organic contaminant classes effectively (Hiraishi 2008; Fenchel et al. 2012). Microbes degrade these pollutants available in the rhizosphere of the aquatic ecosystem by a cometabolism process; whereas complex carbon-based compounds break down to organic carbon and electron acceptors (Shahid et al. 2020). However, success of degradation process depends on microbial population, concentration of organic pollutants/xenobiotics, and macrophyte species in natural aquatic ecosystem (Calheiros et al. 2009). In this plant-microbe relationship, plants provide organic carbon to microbes, which assist them to perform the degradation process and bacteria release indole acetic acid (IAA) to improve plants' development (Mori et al. 2005; Golubev et al. 2009). Interestingly, the biofilms attached to aquatic macrophytes are capable of digesting of amines, phenolics, aliphatic aldehydes, polychlorinated biphenyles, and atrazine (Tranvik 1998; Ghosh et al. 1999; Guasch et al. 2007). Moreover, methanotrophs containing a collection of proteobacteria in aquatic plant rhizosphere degrade various harmful organic complexes and produce methane that may be utilized for generating carbon and energy by microbes (Yoon 2010; Pandey et al. 2014). Sriprapat and Thiravetyan (2016) revealed that several macrophytes (*Euphorbia milii*, *Hedera helix*, *Chlorophytum comosum,* etc.) associated with both endophytic and epiphytic bacteria removed the benzene in hydroponic condition effectively. The bacteria strain *Pantoea* sp. inoculated into rhizosphere of *Brassica napus* showed phenol digestion is accompanied by Cr (V) reduction into Cr (III); hence, both parties receive benefits from the relationship (Ontañon et al. 2014). Another study relevant to phenol degradation through plant-bacteria synergism showed *Phragmite australis* associated with *Acinetobacter*, *Bacillus cereus*, and *Pseudomonas* sp. microbes accelerated the process (Saleem et al. 2018a, b). Similar type of study conducted by Saleem et al. 2018a, b, indicated above mentioned phenol-degrading bacteria associated with *Typha domingensis* successfully removed the phenols from the water (Saleem et al. 2018a, b). Moreover, Yamaga et al. 2010 and Borde et al. 2003 also provide evidences for phenol degradation through plant-microbe interaction (Table 6.1). Besides, water contaminated with various nitro-phenols (NPs) rhizoaugmented with NP-degrading bacteria allied with *Spirodela polyrhiza* roots completely removed available NPs from polluted water (Kristanti et al. 2014). A research conducted by

Sánchez-Pérez et al. 2020 described that Decaclorobiphenyl PCB-209 remediated by *Ocimum basilicum L.* associated microorganisms.

6.4.6 Degradation of Pharmaceuticals and Personal Care Products(PPCPS) Through Plant-Microbe Interaction

According to a study conducted by Suyamud et al. 2018 resulted microorganism-plant combined system increased the removal of Bisphenol A (BPA), which is considered as a toxic compound to human endocrine system. Here, *Bacillus thuringiensis* and *Pantoea dispersa* associated with *Dracaena sanderiana* plant root enhanced the BPA more than 90%. A disobedient contaminant Carbamazepine (CBZ) present in aquatic atmosphere was removed by a plant species *A. rusticana* in combination with *R. radiobactoer* and *D. nitroreducens* (Sauvêtre et al. 2018). The results of these experiments are given in Table 6.2.

6.4.7 Degradation of Industrial Waste Through Plant–Microbe Interaction

The inoculated bacteria species (e.g., A. *juniistrain*, *Rhodococcus* sp. and P. *indoloxydans*) associated with *Phragmites australis* degrade dye substances in FTWs system (Tara et al. 2019). Similar study revealed that parallel to the contaminant degradation, trace metal concentration was also removed by 87% in the treated water. Correspondingly, Iqbal et al. 2019 discussed that the inoculation of some bacteria would mitigate the stress conditions, which affect plant growth and development. For example, together with *Pseudomonas* sp., *L. perenne and A. thaliana* reduced petroleum hydrocarbon; simultaneously enhancing the plant growth (Iqbal et al. 2019). Furthermore, *H. strobilaceum* allied with several microorganisms to remediate oil compounds present in contaminated water (Al-Mailem et al. 2010).

6.5 Biotechnological Applications of Aquatic Plant–Microbe Interactions

Many studies have taken place in the context of aquatic plant microbiomes targeting many approaches. This has relied on development of various microbial biotechnological approaches to enhance our understanding of both aquatic plants as well as plant microbiomes.

6.5.1 Plant-Microbe Symbiosis in Transgenics

It is well known that the endophytic bacterial strains showing both pollutant removal and plant growth-promoting functions have been more efficient in phytoremediation.

This plant microbiome association has been successfully used in treating aquatic contamination of crude oil. *Pseudoarthrobacter phenanthrenivorans* (MS2) and *Azospirillum oryzae* have been successful in both crude oil removal and plant-growth-promoting function in terms of chlorophyll content, water potential, proline, amino acids, and antioxidant enzymes (Saeed et al. 2021).

However, developing transgenics has become another promising approach, which has been extensively used in plant-microbe symbiosis. In order to biodegrade toxic substances, many microorganisms and plants have been genetically engineered, which has been difficult to degrade by naturally occurring species.

One example depicts the expression of metal-tolerant genes in bacterial strains to increase Cd binding, and engineered bacteria strain associated reduced Cd toxicity in aquatic plant environment roots. Yeast has been genetically engineered by inserting the arsenic removal gene *WaarsM* from a soil fungi *Westerdykella aurantiaca,* and has been successful in rice-associated arsenic bioremediation (Verma et al. 2016). Recent efforts have also been taken to understand the association between aquatic rhyzosphere microbial communities and their associated root exudates using a systems biology approach involving metabolomics and metagenomics (Singh et al. 2021). Metabolomics and Metagenomics tools have been used in this study, which has been demonstrated using a system biology approach. This study reports of developing a RhizoFlowCell (RFC) system in order to check the dynamics of root exudation patterns of *Pandanus amaryllifolius* when it was exposed to naphthalene pollutant. The developed system was able to capture the complexity of root exudates in the aquatic rhizosphere and results were further analyzed using LC-qTOF-MS. The obtained metabolomic profile has significantly helped them to understand the response of roots to changing levels of naphthalene. They further have observed the formation of an active microbial biofilm during the process. Fluorescence in situ hybridization and Illumina Miseq Next-Generation Sequencing of metagenomic DNA experiments have revealed the ability of aquatic plant roots to attract bacterial communities. This has been facilitated through the metabolic compounds secreted by the root system (Lee et al. 2013).

6.5.2 Biorefineries: Cultivation Systems

Certain bacterial communities, especially plant-growth-promoting bacteria (PGPB), play a significant role in algae under phototrophic conditions (Lian et al. 2018): 10–70% increase in algae growth rate has been found in many instances in the association of PGPB. Therefore, many attempts have been taken on cultivation of algae and growth-enhancing bacteria together for better productivity. Further concern has been taken to maintain suitable levels of bacterial communities to achieve mass cultivation of microalgae with stagnant higher growth rates (Lian et al. 2018; Fuentes et al. 2016). In addition to that, several incidences have been reported regarding recycling of the harvested algal-bacterial community into High Rate Algal Ponds in order to safeguard the stability of the community (Lian et al. 2018; Makut et al. 2019). Cocultivation of microalgae and certain bacteria have been undertaken to enhance algal lipid production. This has been achieved by cocultivating microalgae *Chlorella sacchrarophila* and *Cellvibrio pealriver* together in a bioreactor. *Cellvibrio pealriver* was allowed to grow in an inner bag, which has been embedded in the bioreactor. This strategy has increased the lipid production by 825.34–929.79 mg·L^{-1} (Xie et al. 2021). Similar strategy between bacterium *Idiomarina loihiensis* and microalgae *Chlorella variabilis* has witnessed significant increase in both protein and lipid contents in algae (Rajapitamahuni et al. 2019).

Microalgae carry a significant potential in contributing to achieve clean energy by production of Hydrogen. Fakhimi N. and Tavakoli suggest the possibility of improving hydrogen production through the algae-bacteria cocultivation. Cocultivation of *Escherichia coli*, *Pseudomonas stutzeri*, and *Pseudomonas putida* bacterial strains along with *Chlamydomonas reinhardtii* enhanced hydrogen production significantly (Fakhimi and Tavakoli 2019).

Furthermore, certain studies report that during microalgae harvesting, the algal-bacterial interactions have caused a significant increase in algae flocculant size, thereby facilitating easy settlement, even in lesser flocculation contributive condition. The formation of the settleable flocs has been achieved by the bacteria surrounding the phycosphere of algae (Lee et al. 2013). Extracellular Polysaccharide Substances of bacterium, and the cell wall and secretory proteins of algae has contributed to this formation of settleable flocs. Quorum sensing between the two components also most likely causes this phenomenon. However, this is significantly important in algae biorefineries, since 20–30% of biomass production cost is taken by algae harvesting.

6.5.3 Other Biotechnological Applications

Development of light microbial solar/fuel cells is another application of the usage of synergism between electricity producing bacteria and algae (Feng et al. 2018). Periodic current generation with a significant energy conversion efficiency has been reported when green alga, *Chlamydomonas reinhardtii*, and an iron-reducing bacterium, *Geobacter sulfurreducens,* are cocultured together. *G. sulfurreducens* has yielded current by oxidizing formate, which has been produced by *C. reinhardtii.* This is a demonstration of the electricity generation through syntrophic interactions between phototrophs and electricity-generating bacteria (Nishio et al. 2013).

Biogas production is another biotechnological use of the algae-bacteria interactions. A study reports the successful production of both biohydrogen and biogas using mutulistic cocultivation of algae (*Chlamydomonas* sp. and *Scenedesmus* sp.) and *Rhizobium* sp. The study further shows that these mutually living bacteria have the innate ability to quench O_2, which led to the further utilization of spent algal-bacterial biomass for biogas production (Cantera et al. 2021).

Another study shows the importance in algal and prokaryotic community structure in an outdoors pilot High Rate Algal Pond (HRAP), which has been also used for the wastewater treatment and biogas upgrading. According to the study, the presence of NH_4^+_ and CO_2_tolerant algae *Chlorella vulgaris,* with certain prokaryotic bacterial strains, resulted in the production of biomethane with significant amounts of CH_4, CO_2, and no H_2S (Wirth et al. 2015).

An enriching advance of algal-bacterial coexistence is bioethanol production, which has also gained significant attention. A study shows the ability of certain marine bacteria to convert algae starch granules to ethanol (De Maia et al. 2020). Polikovsky et al. show that the consortium of genetically altered *Maribacter* sp. and *Roseovarius* sp. significantly affects the *Ulva mutabilis* growth rate and photosynthetic constituents and their contents. This has contributed to bioethanol production by causing significant increase in glucose and glycerol and decrease in xylose and glucuronic acid (Polikovsky et al. 2020).

Another high potential area of the use of bacterial-aquatic plant such as algae interactions is for the production of industrially important chemicals and fuels in biorefineries. For example, Liu et al. demonstrate the possibility of isoprene production using *Synechococcus elongates* and *Escherichia coli* coculture (Liu et al. 2021). In addition to that since algae-based food products are already a good option for food scarcity associated with increasing population and reducing fertile land area, scientists suggest the importance of using aquatic plant-bacteria interactions to produce high value food products such as nutraceuticals and also low value food products for aquaculture. The potential of using aquatic plant-microbes interactions for our benefit is enormous. With advancement of certain biotechnological tools and areas such as CRISPR-Cas, genomics, transcriptomics, metagenomics, proteomics, and metabolomics, the scientific community has taken more interest in unraveling more and more possibilities.

6.6 Conclusion

Microbial interactions with aquatic plants have immense importance. Microbial accumulation as biofilms in aquatic and marine water using quorum sensing led to many interactions such as to remediate pollutants by microorganism, removal of excess nutrient in pollutant water, trace element metal removal, organic pollutant removal, and pharmaceuticals industrial waste removal. Moreover, there are vast varieties of biotechnological applications using the plant-microbial interactions in aquatic and marine biosystems.

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Status of Microplastic Pollution in the Freshwater Ecosystems

7

Vaishali Bhatt, Neha Badola, Deepti Semwal, and Jaspal Singh Chauhan

Abstract

Only 7% of the total plastic produced worldwide is recycled, allowing the majority of plastics to build in the environment and pose a tremendous threat to living and nonliving things of the planet. These plastics in the form of fine particles [microplastics (MPs)] have nowadays emerged as a significant water pollutant. The MPs in the freshwater system are still a subject of research that has to be thoroughly investigated, because it has not yet been fully understood. The sources, circulation, and final disposition of MPs in the freshwater environments are the main topics of this chapter. Further, the movement and dispersion of MPs in the freshwater bodies are impacted by a number of variables, which are also discussed. For scientific communities all across the world, it is advised that a uniform technique be created for identifying MPs. The current chapter will help to better understand the origin, movement, fate, and distribution of MPs in the freshwater ecosystem.

Keywords

Pollution · Microplastic · Water · Ecosystem

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7.1 Introduction

Microplastic contamination is a problem that is getting worse and is widespread in the world's soil, air, and water. Because MPs are present in both natural and artificial water cycles, MP pollution is observed almost throughout the whole water cycle (hydrologic cycle) on the earth. The different aquatic ecosystems have been affected by MPs, with the oceans serving as catch basins and rivers as transporters of the material. The presence of small plastic objects (5 mm) accumulating in aquatic ecosystems was first noted in marine areas in the 1970s (Hays and Cormons 1974). Thompson et al. used the term MP to describe the tiny plastic pieces, and there has been an exponential increase in the number of scientific research on the topic of MPs since then (Thompson et al. 2004). There was not much research on MPs in the literature on freshwater ecosystems before the twenty-first century; however, Hays and Cormons (1974) reported MPs in the rivers of North America (Hays and Cormons 1974). The substantial artificial polymer contamination of rivers was previously unnoticed (or disregarded), but since these early papers, a lot more research has been done, and MPs have now been reported in freshwater systems, including lakes and rivers, all around the world. Researches show that microplastic is building up in freshwaters just as it is in the oceans. In a solitary waterbody, such as a lake or pond in a mountain range, the microplastic will persist forever, breaking down further into smaller MPs and nanoplastics and harming the ecology as a whole. Rivers and lakes provide water supplies to humans and act as habitats to aquatic species having significant ecological and economic importance as well as providing opportunities for recreation, and the production of aquatic goods. Therefore, understanding the prevalence, distribution, and impacts of MPs in freshwaters is crucial. This chapter describes the latest evidence on the occurrence, outcome, and influence of MPs in aquatic habitats to better understand the current state and fate of MPs in the freshwater ecosystems.

7.2 Sources and Fate of MPS in Freshwater Ecosystem

MPs can enter the environment through a variety of routes, and a route that is crucial in one area may not be so crucial in another. Particles from the diverse sources have a variety of morphologies, compositions, sizes, and other characteristics. The qualities of particles produced under carefully monitored industrial circumstances are often more uniform and homogenous. Intentional industrial manufacturing is one of the possible sources and entry points for designed MPs. For instance, plastic pellets used as feedstock in the production of plastic or microbeads purposefully made for cosmetic products. In 2012, it is projected that over 4000 tonnes of MP beads were utilized in cosmetics across Europe (Gouin et al. 2015). Although being only a minor portion of the anticipated total load of environmental MPs, primary MPs are a comparatively simple problem to manage and eliminate. However, unmanaged secondary sources of human origin are the primary causes of MP pollution. These sources include inadequately collected and disposed plastic garbage that is dumped directly into the environment or that is incorrectly collected and dumped in landfills and later on dispersed into the environment via wind or water. Additionally, synthetic paints, automobile tires, and industrial abrasion processes are anticipated to make contributions to the productions of MPs (Lassen et al. 2012). Other causes include pollutant from factories and building sites, which can enter aquatic environments through the wind and surface runoff. The use of plastic sheets for crop cultivation is one of the most significant causes of plastic pollution of agricultural soils and is regarded as a key agricultural emission (Xu et al. 2006). Synthetic textiles are a significant additional source, since they wash up with high amounts of MP fibers in waste-water (Napper and Thompson 2016). The fibers were always present in the several environmental compartments that had previously been studied, but the fragments were mostly found in urban runoff. These fibers may originate from a variety of places, such as landfills, trash incineration, degradation of macroplastics, synthetic fibers used in clothing and home furnishings, and landfills. The characterization indicates that the primary source of these fibers being clothing is the most likely theory. The fibers in the air, including MPs, may be carried by the wind and dumped on the land surfaces or transported to aquatic environments. These fibers, which include MPs, could be carried by the wind to the aquatic environment or left behind in terrestrial ecosystem. It is not feasible to determine whether the fibers are coming from nearby or far off sources when they are detected. MPs that have been discovered in the remote lakes imply that long-distance transfer may also be possible (Free et al. 2014; Zhang et al. 2016; Liu et al. 2021b). When looking into MPs in freshwater, it appears that atmospheric fallout may also be a substantial source for its availability. Other sources, such as fibers that may detach directly from persons strolling down the street while wearing their clothing, must also be taken into account in addition to atmospheric fallout (Fig. 7.1).

After entering the environment, MP does not stay in one place; instead, it is moved to different environments compartments, where it spends varied amounts of time. The flow of MPs from the land to the river system is influenced by the local weather, distance from the river, type of land use overland runoff, and dispersion into roadside ditches. The flow conditions, daily discharge, and the morphology at each individual site will control the migration of bulk plastics and MPs within the riverine system (Balas et al. 2001). Since MPs will be transported and dispersed to diverse environmental compartments more quickly than macroplastics, they are also vulnerable to varying rates of degradation. While the movement to sediments and the development of biofilms on MPs surfaces may also slow down the rate of deterioration by reducing light exposure. Most of the knowledge we presently have about the degradation of plastics comes from laboratory studies that recurrently concentrate on a particular mechanism, such as photo, thermal, or biodegradation, which restricts our knowledge of the degradation of plastics under environmental conditions where multiple mechanisms occur simultaneously. In contrast, no research has been done on MP formation rates. This is significant, because some polymers tend to depolymerize slowly and break down into smaller pieces (Lambert and Wagner 2016) and, as these fragments continue to fragmentate, MPs are eventually formed.

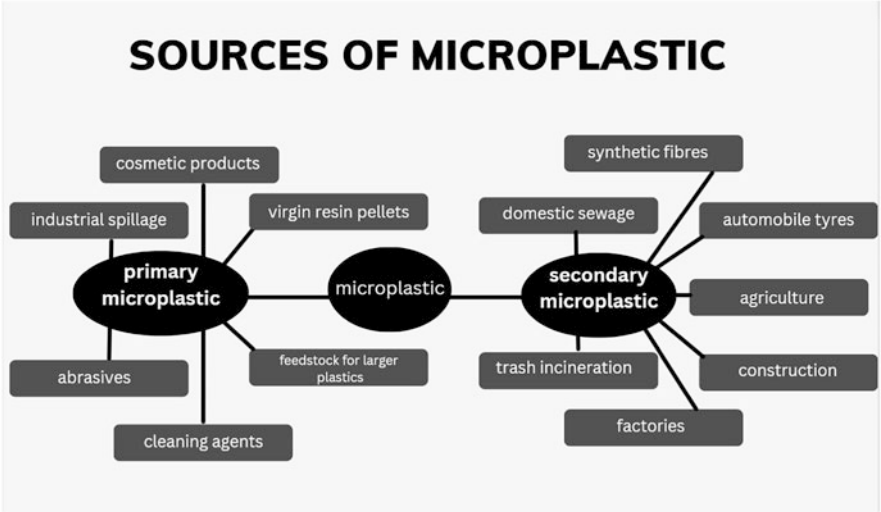

Fig. 7.1 Sources of MPs in freshwater ecosystems

Environmental factors, polymer characteristics, chemical additive type and quantity, and polymer properties are key factors that impact MP degradation and fragmentation. Crystallinity, a molecular characteristic that affects density and permeability of MPs due to more organized and tightly structured polymer chains, is a significant polymer attribute. This has an impact on how MPs behave when they are wet and their swelling, impacting bacterial binding to surfaces. While stabilizers like antioxidants and antimicrobials extend the lifespan of plastics, which leads to MP fragmentation into ever smaller particles, including nanoplastic formation, transformation of fragments, degradation into nonpolymer organic molecules, and further transformations/degradation into other chemicals (Lambert et al. 2013). Pollutants such as DDT, PCBs, metals, and dioxins interact with MPs and get adsorbed on its surface, affecting their mobility and bioavailability (Mato et al. 2001; Ashton et al. 2010). Pharmaceuticals, personal care items, and other industrial chemicals that enter the environment are likely to co-occur with MPs in the freshwater environment via physical, chemical, or pore-filling adsorption. It would be fascinating to know how much of the sorbed chemicals become accessible in the water column as a result of the MP's continuing deterioration as well as alterations in the environment (Fig. 7.2).

7.3 MPS in Freshwater Systems

Over the past 10 years, there have been numerous studies on MPs pollution in the marine environment; however, there have been few studies on MPs contamination in the freshwater ecosystem. There are around six times as many articles reported for

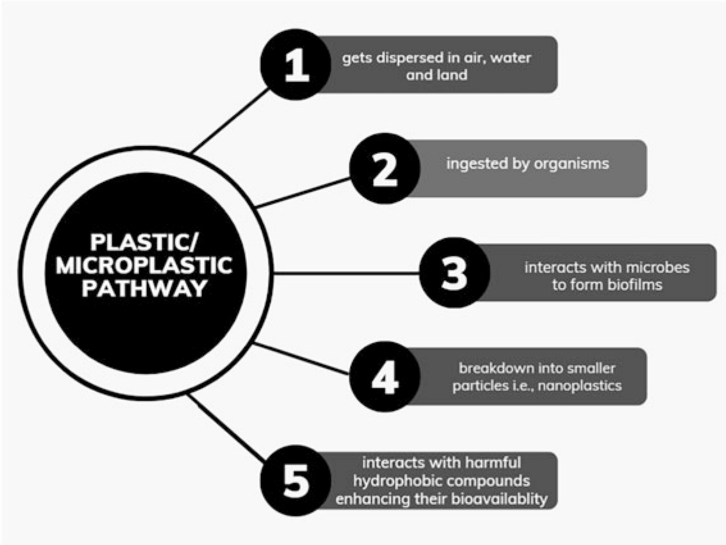

Fig. 7.2 Pathway of MPs in freshwater ecosystems

MP in the marine environment as there are in freshwater. Since freshwater systems, whether directly or indirectly, are the main source of drinking water, appropriate investigations must be conducted to look into the MPs pollution in freshwater too. Additionally, the release of land based as well as aerial MPs into freshwater ecosystems results in their transit to the marine waters. The study of MPs in freshwater systems is advancing, and MPs have now been found in freshwater systems on several different continents. Researchers have found MPs all over the world, from Asia to Antarctica, in rivers, lakes, ponds, wetlands as well as reservoirs. Despite having a very uneven distribution, MPs have been found in every compartment of the freshwater ecosystem. MPs are reported from fish, birds, amphibians, and other biota to river bottom sediments, beach sediments, surface water, and drinking water (Fig. 7.3).

7.4 MPs in River

MPs have been a growing subject in limnological research. Researchers have focused on MP pollution in the last decade in freshwater, beginning with lakes and later shifting to rivers, reservoirs, and wetlands. Among all the research on rivers, most were published in last 5 years. The geographical cover of these researches is not homogeneous and most of the researches are carried out in Asia. To summarize the research on rivers, more than 50 rivers along the globe are investigated for MPs. Most of these studies are carried out in China, among which the Yangtze River basin is the most studied worldwide (Xiong et al. 2019; Hu et al. 2018). Many European

Fig. 7.3 Status: Studies of MPs around the world

rivers are also reported to be polluted with MPs, among which the Rhine River has received most attention (Mani et al. 2019). In North America, St. Lawrence River system has been extensively studied (Castañeda et al. 2014; Crew et al. 2020). MP concentration in rivers is far less than marine and estuarine systems. MPs have been found in all the compartments of river system as surface water, bottom sediment, shore sediment, and biota reporting varying levels of contamination. The maximum number of MPs was reported from the Sinos river in Brazil (Ferraz et al. 2020), that is, 330.2 particles per liter and yellow river in China (Liu et al. 2021a), that is, 5358–654,000 particles per m^3. In river sediments, the most polluted rivers were river Yangtze (Hu et al. 2018) and river Fuhe (Zhou et al. 2021) in China and River Ganga (Sarkar et al. 2019) in India, that is, 35.76 to 3185.33 items/kg, 1049 $\pm$ 462 items/kg and 99.27–409.86 items/kg, respectively. In most of the rivers, fibers were the dominant type of MPs, while in some rivers like Han river in South Korea, Thames river in Canada, Ravi river in Pakistan, the Nakdong river in South Korea, Cisadane river in Indonesia, and Ottawa river in Canada, fragments were the most dominant type of MPs (Table 7.1).

MP concentration at water surface is highly dynamic and altered by flow regimes, precipitation, seasons, and proximity to point of entry. For example, the number of MPs in the surface water of 9 rivers in USA doubled after introduction of effluent from waste water treatment plants (Mccormick et al. 2016). Hydrological conditions and sampling season also play an important role in the abundance of MPs reported in the rivers. Seasonal changes can alter the occurrence of MPs in the rivers. For instance, MPs concentration reduced in the 10 urban, suburban, and rural river bed sediments in the United Kingdom and reached to 2812/kg from 6350/kg (Hurley et al. 2018). While in another study in a South African river, mean MP concentration

Table 7.1 MPs in rivers

Reference	Country	River	Sample type	Mean particle	Dominant Ttype
	Vietnam	Saigon river			
Ferraz et al. (2020)	Brazil	Sinos river	Surface water	330.2 particles L^{-1}	Fiber
Chauhan et al. (2021)	India	Alaknanda river	Water/ sediment	566/389 particles	Fiber
Dris et al. (2014)	Paris	Seine river	Surface water	62 and 101 fibers/L	Fiber
Vermaire et al. (2017)	Canada	Ottawa river	Surface water	0.05 and 0.24/L	Fragment
Hou et al. (2021)	USA	Illinois river	Fish	MPs present	Fiber
Martínez Silva and Nanny (2020)	USA	Magdalena river	Sediment	25.5 to 102.4 fibers/kg 10.4–12.7 fragments/kg	
Luo et al. (2019)	China	Suzhou river	Surface water	1.8–2.4 items/L	
Luo et al. (2019)	China	Huangpu river	Surface water	1.8–2.4 items/L	
Alam et al. (2019)	Indonesia	Ciwalengke river	Surface water	5.85 ± 3.28/L	Fiber
Sulistyowati et al. (2022)	Indonesia	Cisadane river	Surface water	13.33 and 113.33 particles m^{-3}	Fragment
Mani et al. (2019)	Europe	Rhine river	Bed sediment	0.26 ± 0.01 and $11.07 \pm 0.6 \times 10^3$/ kg^{-1}	
Deocaris et al. (2019)	Phillepines	Pasig river	Surface water	34 MP fragments	
Hu et al. (2018)	China	Yangtze river	Surface water	0.48 to 21.52 items L^{-1}	Fiber
Zhang et al. (2020)	China	Qin river	Sediment	0–97 items·kg^{-1}	
Chen et al. (2021)	Malaysia	Langat river	Surface water	4.39 particles/L	Fiber
Wong et al. (2020)	Taiwan	Tamsui river	Surface water	$2.5 \pm 1.8/m^3$ and $83.7 \pm 70.8/m^3$	
Wong et al. (2020)	Taiwan	Dahan river	Surface water	$83.7 \pm 70.8/m^3$	
Wong et al. (2020)	Taiwan	Keelung river	Surface water	$2.5 \pm 1.8/m^3$ and $83.7 \pm 70.8/m^3$	
Wong et al. (2020)	Taiwan	Xindian river	Surface water	$2.5 \pm 1.8/m^3$	
Napper et al. (2021)	India	Ganges river	Surface water	140 MP particles	Fiber
Eo et al. (2019)	South Korea	Nakdong river	Surface water	293 ± 83 to 4760 ± 5242 particles/m^3	Fragment

(continued)

Table 7.1 (continued)

Reference	Country	River	Sample type	Mean particle	Dominant Ttype
Xu et al. (2021)	China	Fenghua river	Surface water	300 N/m^3 to 4000 N/m^3	Fiber
Sarkar et al. (2019)	India	Ganga river	Sediment	99.27–409.86 items/kg	
Liu et al. (2021a)	China	Yellow river	Surface water	5358–654,000 N/m^3	Fiber
Yin et al. (2022)	China	Xiangjiang river	Sediment	288 ± 60 items/kg	
Sekudewicz et al. (2021)	Poland	Vistula river	Surface water	1.6 to 2.55 items L^{-1}	Fiber
Hu et al. (2018)	China	Yangtze river	Sediment	35.76 to 3185.33 items kg^{-1}	Fragment
Jiang et al. (2019)	Tibet	5 Tibetan rivers	Surface water	483 to 967 items/m^3	Fiber
Campanale et al. (2020)	Italy	Ofanto river	Surface water	0.9 ± 0.4 p/m^3 to 13 ± 5 p/m^3	
Irfan et al. (2020)	Pakistan	Ravi river	Surface water	190 ± 141 MPs/m^3 to 16,150 ± 80 MPs/m^3	Fragment
Constant et al. (2020)	France	Rhone river		6 kg km^{-2} $year^{-1}$	Fiber
Constant et al. (2020)	France	Tet river		6 kg km^{-2} $year^{-1}$	Fiber
Liu et al. (2021b)	China	Dafeng river	Surface water	3×10^{-4}-2.5×10^{-3} items/L and 4×10^{-5}-9×10^{-4} items/L	
Zhou et al. (2020)	China	Tuojiang river basin	Surface water	911.57 ± 199.73 to 3395.27 ± 707.22 items/m^3	Fiber
Zhou et al. (2021)	China	Fuhe river	Sediment	1049 ± 462 items/kg	
Simon-Sánchez et al. (2019)	China	Ebro river	Surface water	3.5 ± 1.4 MPs·m^{-3}	Fiber
Lechner (2020)	China	Danube river		316.8 ± 4664.6 items Per 1000 m^3	
Corcoran et al. (2019)	Canada	Thames river	Sediment	6 to 2444/kg	Fragment
Xiong et al. (2019)	China	Yangtze river		4.92 × 105 items/km^2	
Park et al. (2020a)	South Korea	Han river	Surface water	7.0 ± 12.9 particles/m^3	Fragment
de Carvalho et al. (2021)	France	Garonne river	Surface water	0.15 particles m^{-3}	
Wu et al. (2020)	Macao	Maozhou river	Surface water	4.0 ± 1.0 to 25.5 ± 3.5 items·L^{-1}	

(continued)

Table 7.1 (continued)

Reference	Country	River	Sample type	Mean particle	Dominant Ttype
Barrows et al. (2018)	USA	Gallatin river	Surface water	1.2 particles L^{-1}	Fiber
Dai et al. (2022)	China	Qiantang river	Surface water	1.5–9.4 items L^{-1}	Fiber
Patel et al. (2020)	India	Sabarmati river	Sediment	134.53 mg/kg to 581.706 mg/kg	
Lin et al. (2018)	China	Pearl river		379 to 7924 items·m^{-3}	Fiber
Wang et al. (2020)	China	Manas river	Surface water	21 ± 3–49 ± 3 items/L	Fiber
Huang et al. (2021a)	China	West river	Surface water	2.99 to 9.87 items/L	Fiber

changed from 6.3 ± 4.3/kg in summer to 160.1 ± 139.5/kg in winter (Nel et al. 2018). Most of the studies have reported MPs in the surface water of rivers, which may not be a reliable representation of entire river. For example, MP concentrations in six South Korean bays were four times higher at the surface than in the rest of the water column (Song et al. 2018). MPs have also been ingested by a wide range of aquatic organisms via feeding, respiration, drinking, or some other routes. MPs have been reported from biota in rivers from many rivers in the world. These organisms may act like reserves of MPs in the rivers. These particles can be egested back in the rivers, continuing its cycle in environment, or can be accumulated in the biota and transferred through the food web (Bhatt and Chauhan 2022), affecting the biological processes of organisms as well as communities and ecosystem.

7.5 MPs in Lakes

MPs have been found in lakes throughout Africa, Asia, Europe, and North America. MPs have been documented in North America from the Great Lakes (Zbyszewski et al. 2014), which have enormous watershed populations. In Europe, MPs have been found from heavily populated Swiss Lakes (Faure et al. 2015) to underpopulated lakes such as Lakes Bolsena (Fischer et al. 2016), and Lake Garda (Klein et al. 2018). MPs have been documented in Asia from the isolated Mongolian lake Hovsgol (Free et al. 2014) and the remote Chinese lakes on the Tibetan Plateau (Zhang et al. 2016). They have also reportedly been seen in China's Lake Taihu (Su et al. 2016), Qinghai (Xiong et al. 2018), Poyang (Jian et al. 2020), and Indian lake, Vembanad (Devi et al. 2020). The finding of MPs in fish taken from Lake Victoria in and Lake Ziway (Merga et al. 2020) was evidence of their presence in African lakes (Biginagwa et al. 2016). MPs have been found in all the compartments of Lakes as surface water, sediment, and biota reporting different levels of contamination. The maximum number of MPs was reported from the Lake Ziway, Ethiopia

(Merga et al. 2020), that is, 30,000 particles/m^3 with a range of 400 to 124,000 particles/m^3. In lake sediments, the most polluted lakes were Lake Onego, in Russia (Zobkov et al. 2020), that is, 2188.7 ± 1164.4 and lake St. Clair, Canada (Zbyszewski et al. 2014). Fiber is the dominant type of MP in most of the lakes in surface water as well as sediments, except for Lakes Michigan, Maggiore, Iseo, and Garda where fragments were the most dominant (Table 7.2).

7.6 MPs in Groundwater

MP pollution in groundwater has attracted less researches than that in other types of natural ecosystems, such as the ocean, rivers, lakes, and soil. In addition to serving as a wall between groundwater MP contamination, soil is also the most likely location for MPs to invade the groundwater system, which can have serious repercussions when groundwater is utilized for drinking purposes. Groundwater systems may get contaminated with MPs as a result of the transfer of MPs through soil migration, runoffs from garbage, industry, and urbanization. Groundwater is difficult to examine and maintain, since it is unseen. Some research papers on groundwater pollution with MPs have been published. In some of these researches, the issue of soil pollution was discussed, and it was found that the soil may serve as a pathway for MPs to reach groundwater systems. These investigations constitute the most recent contributions to the knowledge on groundwater contamination with MPs, and distribution comments and remarks have been made and published for those investigations. In a karst aquifer system in the United States, MPs (microfibers) were found at median and maximal concentrations of 6.4 and 15.2 n/L, respectively (Panno et al. 2019). In a similar study in China, mean concentration of MPs was 4.50 particles/L (An et al. 2022). While in another Karst system in Italy, 28 MPs were found in every liter of water on average (Balestra et al. 2023). MPs were absent in treated potable drinking water from taps in Germany (Weber et al. 2021), while a low concentration (0.48 particles/L) of MPs was reported in an aquifer from Iran (Esfandiari et al. 2022). Contrastingly, an outrageous amount (2103 particles/L) of MPs was found in Chinese groundwater (Khant and Kim 2022), which was higher than other observed concentrations of MPs in groundwater around the globe. The most prevalent MP polymer in groundwater systems are PE and PET (Huang et al. 2021b), while the most prevalent shapes are fragments and fibers (Table 7.3). The MP contamination of surface water is far higher than that of groundwater: it may be because anthropogenic activity has directly affected and contaminated it. When surface water sources are adjacent to WWTP and STP sites, these facilities act as entry points for MPs into the water (Park et al. 2020b). In soil as well as groundwater, MPs may operate as a carrier of these dangerous compounds by absorbing persistent organic contaminants and metals (Huang et al. 2021b). The above studies have provided data indicating that research on groundwater should receive international attention.

Table 7.2 MPs in freshwater lakes

Reference	Country	Lake	Sample type	Mean particle	Dominant type
Zhang et al. (2016)	Northern Tibet	4 lakes, Siling co basin	Shore sediment	8 ± 14 to 563 ± 1219 items/m	
Xiong et al. (2018)	China	Qinghai lake	Surface water	0.05105 to 7.58105 items km^2	Sheet and fibers
Xiong et al. (2018)			Shore sediment	50 to 1292 items m^2	
Xiong et al. (2018)			Fish	2–15/Ind	
Wang et al. (2017)	China	20 urban lakes	Surface water	1660.0 ± 639.1 to 8925 ± 1591 N/m^3	Fiber
Vaughan et al. (2017)	Uk	Edgbaston pool	Sediment	25–30/100 g	Fiber/film
Su et al. (2016)	China	Taihu	Surface water	3.4–25.8 items/L	Fiber
Su et al. (2016)			Sediment	11.0–234.6 items/kg Dw	
Su et al. (2016)			Asian clams	0.2–12.5 items/g	
Sighicelli et al. (2018)	Italy	Maggiore, Iseo, and Garda	Surface water	4000 ± 2700 to 57,000 ± 36,000/km^2	Fragments
Mason et al. (2016)	USA	Michigan	Surface water	17,000 particles/km^2	Fragments
Zobkov et al. (2020)	Russia	Lake Onego	Sediment	2188.7 ± 1164.4	Fiber
Mao et al. (2020)	China	Wuliangsuhai Lake	Surface water	3.12 to 11.25 N/L	Fiber
Li et al. (2019)	China	18 lakes (Yangtze river basin)	Surface water	240 items/m^3 to 1800 items/m^3	Fiber
Li et al. (2019)			Sediment	90 items/kg to 580 items/kg	Fiber
Jian et al. (2020)	China	Poyang lake	Surface water	1064 ± 90 Mp/m^3	Fiber
Jian et al. (2020)			Sediment	1936 ± 121 Mp/kg	Fragments
Klein et al. (2018)	Germany	Lake Garda	Sediment	108–1108	
Hu et al. (2020)	China	Dongting lake	Sediment	21–52 items/100 g	Fiber
Hu et al. (2020)			Surface water	0.62–4.31 items/m^2	Fiber
Fischer et al. (2016)	Italy	Lake Bolsena	Surface water	2.68 to 3.36 particles/m^3	Fiber

(continued)

Table 7.2 (continued)

Reference	Country	Lake	Sample type	Mean particle	Dominant type
Fischer et al. (2016)			Sediment	112 particles/kg dry weight	Fiber
Fischer et al. (2016)	Italy	Lake Chiusi	Surface water	0.82 to 4.42 particles/m^3	Fiber
Fischer et al. (2016)			Sediment	234 particles/kg dry weight	Fiber
Costello and Ebert (2020)	USA	Erie	Sediment	1 to 12 per m^2	Fiber
Costello and Ebert (2020)	USA	Ontario	Sediment	1 to 12 per m^2	Fiber
Biginagwa et al. (2016)	Tanzania	Victoria	Fish	MPs in 20% fish	
Anderson et al. (2017)	USA	Lake Winnipeg	Surface water	MPs present	
Devi et al. (2020)	India	Vembanad	Fish	MPs in 26% fish	Fiber
Grbić et al. (2020)	Canada	Ontario	Surface water	0.8/L	
Minor et al. (2020)	USA/Canada	Superior	Surface water	9000 to 40,000 particles/km^2	Fiber
Minor et al. (2020)			Sediment	0 to 55 particles/kg dry weight	Fiber
Merga et al. (2020)	Ethiopia	Lake Ziway	Surface water	30,000 (400–124,000) particles/m^3	
Merga et al. (2020)			Fish	MPs in 35% fish	
Free et al. (2014)	Mongolia	Lake Hovsgol	Surface water	20,264/km^2	
Zbyszewski et al. (2014)	Canada	St. Clair	Sediment	1.726×10^6	

7.7 Conclusion

With regard to MPs, freshwater habitats face similar challenges as marine ones. According to analysis, freshwaters are overwhelmingly probable to become contaminated with MPs. However, there have only been a few studies till date focusing on the presence of MPs in freshwater. Studies that are now available have shown that MPs are present in lakes, rivers, and ground water. Freshwaters are affected by MP pollution everywhere from remote, rural, and suburban regions with little to no population and urban areas with soaring populations. To lessen the concerns with MP contamination, waste management systems must be improved, because they are a generator as well as supplier of freshwater MPs. Since MPs are

Table 7.3 MPs in groundwater

Reference	Country	Sample type	Mean particle	Dominant type
Połeć et al. (2018)	Poland	Deep well (untreated potable water)	MPs present	Fragment
Panno et al. (2019)	USA	Karst system		Fiber
Ganesan et al. (2019)	India	Not mentioned	66 particle	Fragment and fiber
Samandra et al. (2022)	Australia	Drinking water		Fragment and fiber
Mintenig et al. (2019)	Germany	Well		Fragment
Selvam et al. (2021)	India	Wells and borewells		Fiber
Weber et al. (2021)	Germany	Tap (treated potable water)	No MPs	Fragment and fiber
Strand et al. (2018)	Denmark	Tap		Fiber, fragment and film
Shruti et al. (2020)	Mexico	Public drinking water fountains		Fragment and fiber
Kirstein et al. (2021)	Sweden	Drinking water		Fragment and fiber
Oni and Sanni (2022)	Nigeria	Borehole drinking water	206 to 1691 items m^{-3}	Fragment
Ledieu et al. (2022)	France	Landfill leachates	10.3 to 106.7 particles/L	
Alvarado-Zambrano et al. (2022)	Mexico	Borehole	12.3 particles/L	
Balestra et al. (2023)	Italy	Karst system	28 items/L	Fiber
Patterson et al. (2023)	India	Borewell and open wells	29.73 ± 3.27	Fiber
An et al. (2022)	China	Karst system	4.50 items·L^{-1}	Film and fiber
Wu et al. (2022)	China	Groundwater	17.0 ± 2.16 to 44.0 ± 1.63 n/L	Fiber
Cha et al. (2023)	Korea	Groundwater	0.02 to 3.48 particles/L	Fragment
Manh et al. (2022)	Vietnam	Well	2 to 21 particles/L	Fragment
Shi et al. (2022)	China	Groundwater	29 n/L	
Esfandiari et al. (2022)	Iran	Aquifer	0.48/L	Fiber
Khant and Kim (2022)	China	Groundwater	2103 particles/L	

transported to freshwater bodies such as groundwater, rivers, and lakes by soil migration, WWTP, air, surface water, landfill leachate, and other means, attention should be paid to the entire ecosystem as a single unit rather than to individual compartments when focusing on their reduction and removal. MP samples' characteristics, such as their forms, sizes, colors, texture, and polymer kinds, can be utilized to determine the origins and behaviors of the MPs in addition to their abundance and, hence, should be fairly analyzed. This chapter urges additional investigation to learn more about the origins and dispersal of MPs in freshwater bodies. Both biological and ecological consequences of MP exposure should be evaluated, particularly in situations where it is crucial. For the upcoming monitoring initiatives, protocols for sampling, pretreatment, and reporting of MPs should be harmonized and standardized.

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Heavy Metal Pollution in Water: Cause and Remediation Strategies

8

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Abstract

Heavy metals are naturally present in earth's crust, and some of them are essential to living organisms for carrying out life processes. Due to their high persistence and nonbiodegradable nature, heavy metal accumulation beyond recommended concentrations may lead to hazardous effect on various life forms and environment. Contamination of water bodies may be due to natural and anthropogenic sources. Unchecked discharge from industrial sites and agricultural runoff in to adjoining water bodies makes the water unfit for human consumption. Escalating levels of these pollutants pose a threat to aquatic life forms and surrounding environment. Heavy metals can execute various health problems that may range from mild to severe. They can be toxic to living organisms at very low levels of exposure. Excessive usage of heavy metals has raised concerns over time, and

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consequently, their impact on the overall environment is being studied by researchers extensively. To safeguard human health and environment, proper management and greener technologies for removal of heavy metal from water bodies is required. This chapter will discuss the source, toxicity, and permitted concentrations of some of the major heavy metals in water bodies. Remediation approaches for mitigation of these toxic compounds have also been described. Physical and chemical remediation processes for heavy metal cleanup are highly expensive and sometimes generate a significant amount of secondary pollutants; therefore, the focus has now shifted toward eco-friendly approaches such as bioremediation and phytoremediation. Further research needs to be carried out to maximize the applicability of the existing techniques and developing highly efficient technologies for heavy metal removal from water bodies.

Keywords

Bioremediation · Heavy metal toxicity · Phytoremediation · Water pollution

8.1 Introduction

Metals are elements with high atomic weight and density ranging from 3.5 to 7 g cm^{-3}. These are found to be deposited on earth's crust naturally as minerals in the form of sulfates, phosphates, oxides, etc. (Singh et al. 2022). In aquatic system, these are naturally present in low amounts, but a variety of natural and anthropogenic activities have unavoidably escalated metal concentrations in water bodies (Ansari et al. 2003). Expansion of industries that extensively utilize metals and metalloids, mining operations, improper e-waste disposal, transportation, and fossil fuel burning are some major human activities that significantly increase concentration of heavy metals in aquatic systems. Animals and plants use some heavy metals such as Fe, Cu, Zn, and Mn at low concentrations for carrying out their physiological processes, but they become hazardous at higher concentrations (Pratush et al. 2018). Some of them are important for activity of enzymes as cofactors and also help to maintain osmotic balance. However, their accumulation over time can harm human health and other life forms. Escalating levels of heavy metals in aquatic systems is one of the most serious global concerns. About 40% of the world's lakes and rivers have been contaminated with heavy metals (Zhou et al. 2020; Bhatt et al. 2022).

Due to their toxic nature, heavy metals are considered as potent pollutants in water bodies and soil (Duffus 2002). Drinking water contaminated with heavy metals is a potential threat to public health. Consumption of polluted water may lead to cardiovascular disorders and renal failure and in severe cases may also lead to life-threatening disease like Parkinson's disease, Alzheimer's disease, and cancer (Singh et al. 2022). These metals come into touch with human bodies via digestion and respiration. People who work or live close to industrial regions associated with these heavy metals and their equivalent compounds have a significant risk of exposure and cause increased mortality rates globally (Rehman et al. 2018).

Biodiversity in aquatic ecosystem is also hampered due to toxic effects of these metals, and reports indicate their accumulation and deposition within tissues of fishes and other aquatic life forms.

With increasing industrialization, generation of heavy metals also increases, and thus, their disposal is of paramount importance. Unlike organic pollutants, inorganic metal ions are resistant to degradation. Their persistence in environment and bio-accumulation in different organisms through food chain is major problem associated with heavy metals (Wuana and Okieimen 2011). Bioaccumulation of heavy metals and their toxicity level in food chain increases with time as their separation and purification is not easy. Although metal ions are resistant to degradation, their bioavailability and chemical forms can be changed. Heavy metals can easily run into water bodies via industrial effluents, agriculture runoff, and household, but there are many technologies and strategies that are employed to remediate heavy metals. Treatment of heavy metal-laden wastewater is an arduous job. The prominent strategies that are employed include thermal treatment, chlorination, electrokinetic, bioleaching, precipitation and coagulation, ion exchange, membrane filtration, bioremediation, heterogenous photocatalyst, and adsorption and are some commonly used methods for heavy metal removal from wastewater (Selvi et al. 2019). However, most of these methods have certain disadvantages and sometimes may generate toxic compounds; therefore, integrated approaches and safer technologies need to be introduced. This chapter highlights toxicity and remediation strategies for heavy metal removal from water bodies.

8.2 Sources of Heavy Metal Pollution in Water Bodies

In water bodies, main source of heavy metal contamination includes landfill leaches, petrochemical spillage, urban runoff, industrial and mining wastewaters particularly from the electronic, metal finishing, and electroplating industries (Khan et al. 2008). Mining and metallurgical operations are prominent cause of increased concentration of these hazardous metals. Heavy metals often reach the aquatic environment through natural physical sources such as volcanic eruptions, air deposition, forest fire, runoffs, and geological matrix erosion. Occurrence of metals in lakes, rivers, and ponds is related to the type of soil and waterflow through sewage and surface runoffs from soils. Additional natural sources of heavy metal contamination of water include the wet and dry deposition of atmospheric salts, water-rock contact, and water-soil interaction (Gautam et al. 2014).

Anthropogenic activities account for vast accumulation of heavy metals in water. Rapid urbanization and industrialization are the main anthropogenic factors that pollute water. The primary causes are mining, metallurgical activities, coal and oil combustion, and agricultural runoff entering waterways (Wang et al. 2004). Other anthropogenic sources include alloy production, atmospheric deposition, battery production, coating, explosive manufacturing, improper stacking of industrial solid waste, leather tanning, mining, pesticides, phosphate fertilizer, photographic materials, printing pigments, sewage, smelting, steel and electroplating industries, textiles, and dyes and wood preservation (Dixit et al. 2015). Untreated wastewater from municipal, domestic sewage, and industry directly discharged into the natural water system leads to water contamination, industrial effluents, water tank leakages, dumping beside water bodies, and atmospheric deposition, which are some major sources through which these heavy metals entry into aquatic ecosystem. Electroplating is a major contributor to pollution because it releases heavy metals via water, air emissions, and solid waste in an environment that has been reported to contain high levels of heavy metals such as nickel, iron, lead, zinc, chromium, cadmium, and copper (Baby et al. 2010). Mining and ore processing are important sources of heavy metal contamination in the soil, and the recovery of ecosystems from mining operations might take decades. These activities generated a vast number of piles and dumps, which are usually discarded without treatment, and these abandoned mines contaminate water system through chemical runoff (Adler et al. 2007). Pesticide overuse and misuse in response to rising food demand have resulted in greater pollutant burdens in the environment, including rivers, lakes, aquifers, and coastal waterways. Anthropogenic sources of heavy metal have been observed to be more ahead than natural sources. Besides, lack of awareness for proper disposal of metal waste and failure of implementation of strict government policies and not following the recommended guidelines also contribute to the problem. Table 8.1 enlist major sources of some common heavy metal pollutants.

8.3 Potential Risks Associated with Heavy Metals

Heavy metals generally have negative impact on the ecosystem and aquatic life, and even trace amounts of heavy metals in water can be hazardous to aquatic life and human health. Ayangbenro and Babalola (2017) defined toxicity of metal as its capability to cause undesirable effects on organisms. Metal toxicity is determined by the environmental factors such as pH, temperature, salinity and dissolved oxygen, the presence of other toxicants, the condition of the test organism, the kinetics of toxic reactions, etc. (Ansari et al. 2003). Metal ions frequently penetrate cells, get accumulated and interact with various elements of cells, and target molecules (Chiarelli and Roccheri 2014). Heavy metals have a negative impact on the health of humans and other living organisms in both the terrestrial and aquatic environments (Das et al. 2013). Toxicity of metal ions on any living system depends upon the duration of exposure and its dose. These cause oxidative damage to biological macromolecules and may end up in damaging DNA and even halt metabolic machinery of exposed life forms. High toxicity of heavy metals can be seen in fetus and newborn babies of industrial workers that are constantly exposed to its high concentrations. Metal ion exposure in newborn babies can harm brain memory and central nervous system, disrupt the function of red blood cells, and cause physiological and behavioral problems, and its severe toxicity may cause cancer. Plants exposed to heavy metals may undergo morphological and physiological changes, which can reduce the photosynthesis rate and may trigger mutagenic changes in several plant species. Heavy metal exposures also obstruct the microbial

Table 8.1 Sources of some heavy metal pollutants

Metal	Sources	References
Iron (Fe)	• Suspended sediment	Raiswell and Canfield (2012), Wang et al. (2015), Wells et al. (1995)
	• Aeolian dust transport	
	• Hydrothermal activity	
	• Recycling from shelf sediments	
	• Combustion of coal, petroleum, biofuel, fossil fuel, and biomass	
	• Wet and dry deposition of atmospheric aerosols, vertical mixing, and upwelling	
Zinc (Zn)	• Pigments and paints	Shah (2021), Rieuwerts (2015)
	• Coal burning, metal smelting, steel works, and waste incineration	
	• Pesticides (Zn-based fungicides) and phosphatic fertilizers	
	• Alloys and solders	
	• Sewage sludge	
	• Mining work and industrial effluents (e.g., from smelting and refining)	
	• Urban runoff from abrasion of galvanized roofs and tire rubber	
Lead (Pb)	• Drainage from mining sites	WHO (2017), Jaishankar et al. (2014)
	• Smelting, manufacturing, and recycling activities	
	• Lead batteries	
	• Pigments and paints	
	• Alloys and solders	
	• Lead plumbing system	
	• Drainage from leaded roofs and gutters	
	• Fossil fuel and waste combustion (PVC products)	
Cadmium (Cd)	• Phosphate mining and fertilizers	Wuana and Okieimen (2011), Gautam et al. (2014), Reichelt-Brushett (2012), Rieuwerts (2015)
	• Detergents and refined petroleum products	
	• Runoff from agricultural and mining activities	
Nickel (Ni)	• Domestic wastewater (detergents)	Gillmore et al. (2020), Cempel and Nikel (2006)
	• Combustion of oil and coal and incineration of waste and sewage	
	• Opencast mining of Ni laterite ore and metal smelting	
	• Phosphatic fertilizers and sewage sludge	
	• Leakage from plumbing	
Copper (Cu)	• Antifouling paints	Brown and Eaton (2001), Rieuwerts (2015)
	• Pesticides (algicides)	
	• Copper polishing, mining, smelting, discharge of wastewater	
	• The use of wood preservatives	
	• Dumping of sewage sludge	

(continued)

Table 8.1 (continued)

Metal	Sources	References
Chromium (Cr)	• Metal processing	Geisler and Schmidt (1991), Bielicka et al. (2005), Shah (2021)
	• Industrial effluents (tannery sludge, textile dyes)	
	• Chromate production and smelting	
	• Sewage sludge and phosphate fertilizers	
Arsenic (As)	• Smelting process of nickel, copper, zinc, and lead ores	Rieuwerts (2015)
	• Paints and wood preservatives	
	• Mining, pesticides, fertilizers	
Silver (Ag)	• Emissions from smelting operations	Shah (2021)
	• Manufacture and disposal of photographic and electrical supplies	
	• Combustion	
	• Industrial and municipal wastewater outfalls	
Mercury (Hg)	• Agricultural practices	Jaishankar et al. (2014), UNEP (2013)
	• Municipal and industrial wastewater (chlor-alkali industry) discharge	
	• Mining activities (artisanal gold mining) and combustion of fossil fuels	
	• Natural degassing and direct atmospheric deposition	
	• Sewage sludge and phosphate rock fertilizer	
Tin (Sn)	• Mining	Duan et al. (2012)
	• Coal and oil combustion	
	• Atmospheric deposition, riverine input, and sediment resuspension	
	• Antifoulant compounds (tributyltin)	

growth (Wase and Forster 1997). Presence of these heavy metals even at low concentration is toxic for humans and animals, for example, Pb exposure to human cause dysfunction in nervous system, reproductive system and kidneys. Cd is another heavy metals that get accumulated in aquatic environment from metal ore refining, alloy decomposition, and fertilizer input, which causes renal dysfunction, bone degenerations, and liver damage in humans (Dojlido and Best 1993). Cd is also associated with itai-itai disease in Japan. Hg is also one of common heavy metals that is associated with many human dysfunctions like Minamata disease. Hg toxicity has also been reported to cause abortion and physiological stress. The high concentration of even Fe and Mn in water bodies also reported to effect animal system although both of these metals are required by the body in consistent amount but in case of water contamination level of these metals tend to get increase, which poses threat to human health. Countries rapidly undergoing industrialization are found to have a high number of heavy metal-related disorders (Esslemont 1998). A correlation between historical events and the metal toxicity along with responsible metal is presented in Table 8.2.

Table 8.2 Historical events due to heavy metal toxicity

Year	Historical events	References
Late 1800s	Drainage water from Summerford Bing industrial area containing high concentration of chromium causing Cr toxicity in Scotland	Esslemont (1998)
1932	Minamata Bay in Japan is contaminated with sewage containing Hg, leading Hg poisoning	Harada (1995), Lenntech (2006)
1910s–1940s	Jinzu river in Japan contaminated with waste sludge containing Cd from Kamioka zinc mines, which gradually reached to drinking water and groundwater	Rieuwerts (2015)
1950s	Cd toxicity in Japan (Itai-itai disease)	Esslemont (1998)
1952	Minamata Syndrome (mass human mortality in Japan as a result of consuming Hg-polluted fish)	Lenntech (2006)
1986	At Sandoz in Germany, water used to put out a large fire releases 30 tons of fungicide containing mercury into the Upper Rhine, killing a huge number of fish	Giga (2009)
1998	A Spanish natural reserve has been contaminated by toxic chemicals from a burst dam	Lenntech (2006)
2010	Chemical spill into a river from a Cu mining-smelting complex resulting significant fish kill in China	Rieuwerts (2015)
2011	Illegal dumping of thousands of tons of waste tailings from the production of the tanning chemical chromium sulphate resulting Hexavalent Cr poisoning of drinking water in China	Rieuwerts (2015)
2014	8×10^4 tons of coal spilled into the Dan River in North Carolina, USA, causing As and Cr toxicity	Rieuwerts (2015)

Toxic effects of some heavy metals have been discussed under this section in brief.

8.3.1 Cadmium

Cadmium is considered to be one of the most toxic heavy metals. Its minute concentrations in food chain has been found to cause *itai-itai* disease. Cadmium is not an essential element for biological system unlike other heavy metals. Cadmium metal is used in the manufacturing of plastics, pigments, and nickel-cadmium batteries. It enters into the environment mostly through waste dumping and phosphate fertilizers. Due to its potential to be hazardous for both humans and animals even at minute concentrations, there is an increased concern over the function and toxicity of cadmium in the environment. The Restriction of Hazardous Chemicals (RoHS) directive of the European Union forbids the use of six substances, including cadmium, in electrical and electronic equipment, while allowing for some exceptions and exclusions from the rule (European Commission 2006). Numerous disorders, such as early atherosclerosis, hypertension, osteomalacia, cardiovascular conditions, and renal dysfunction, are all linked to cadmium exposure, including lung cancer (ARL: Cadmium toxicity 2016; Medinews direct 2009). In the 1950s, a large population near the Jinzu River in Japan is found to have kidney dysfunction and osteomalacia, resulting in deformed and fragile bones, which is caused by the ingestion of Cd for a long time. This disease is called as itai-itai named after the excruciating pain that people who had consumed Cd-contaminated rice had experienced. Klaassen et al. (2009) reported Cd to be a metallothioneins (metal-binding proteins) interfering metal that disrupts the homeostasis of an organism. Once ingested by an organism, Cd shows high bio-persistence without biomagnifying properties (Wuana and Okieimen 2011; Kumar et al. 2019).

8.3.2 Chromium

Chromium is a highly toxic metal that poses varying levels of harm to the ecosystem. Cr is mainly introduced to the environment through discharge of untreated or inadequately treated residues from tanning and leather industries, rubber manufacturing, and pulp and paper industries (Chiarelli and Roccheri 2014). Among various oxidation states of Cr, only +3 and +6 are chemically and biologically stable (Ducros 1992). Hexavalent Cr is the most toxic form of chromium for animal and human health, which is considered to be mutagenic and carcinogenic (Chiarelli and Roccheri 2014). High exposure to Cr may cause liver, kidney, and central nervous system damage. Hematological problems can be observed in fishes of freshwater contaminated with Cr.

8.3.3 Copper

Copper enters the water bodies mostly via mining, dumping of sewage sludge, and use of Cu-based pesticides in agriculture. Cu has been used by human civilization since pre-historic times. It is well known that Cu is an essential element for all living organisms. However, its high concentration may cause toxic effects (Bielmyer-Fraser et al. 2018). Excess Cu may alter enzyme functions, acid/base balance, iono-regulation, and endocrine disruption in aquatic organisms (Rieuwerts 2015). Cu shows bioaccumulation in organisms like plankton and oysters but does not magnify in the food chain (Rieuwerts 2015). At higher concentrations in human, it may damage kidney and stomach and cause diarrhea, vomiting, and loss of strength.

8.3.4 Lead

Leaded gasoline had been a significant contributor to the environmental dispersion of Pb. Tetraethyl lead was added in gasoline to boost its octane levels since the 1920s (ATSDR 2017). However, lead use has been banned in gasoline in the United States since January 1996, and USEPA has encouraged all the countries to do so (EPA 2017). Pb is a nonessential element that could be hazardous to most of the living forms. Lead can be present in water bodies in four different forms: ionic (mobile and bioavailable), organic complex (bound with limited mobility and bioavailability), strongly bound (attached to solid particles like iron oxides with limited mobility), and very strongly bound (attached to clay, dead remains, or solid particle with very low mobility) (ILA 2018). Exposure of high concentrations of Pb to human may cause severe toxicity to the central nervous system, damage the fetus, alter hemoglobin synthesis, and harm kidney and reproductive system. Poisoning of agricultural food may be caused by airborne Pb by its deposition on soils, water, and fruits (WHO 1984). Bio-magnification of Pb has not been observed in the food chain (De Pooter 2013).

8.3.5 Mercury

Mercury (Hg) is a nonessential, persistent and highly toxic element, which is present in the environment in elemental, organic, and inorganic forms that are all interconvertible and toxic (Jaishankar et al. 2014). Elemental Hg^0 is a volatile liquid at room temperature, inorganic Hg is present in mercuric (Hg^{2+}: HgS, $HgCl_2$) and mercurous (Hg^{1+}: Hg_2Cl_2) forms, and organic Hg is present in the form of methylated Hg (mono and dimethyl mercury) and phenyl mercury. Toxic effects related to Hg gained global attention due to the outbreak of Minamata disease in Japan in the year 1956 (Ye et al. 2016). Methylmercury containing waste from a fertilizer industry as a byproduct of acetaldehyde discharged into the Minamata Bay for a long time polluted the marine ecosystem harming organisms. Contaminated seafood consumption caused injury to the nervous system, brain, heart, eyes, kidney, and lungs. Methylmercury also shows bio-magnification, and it dissolves well in water and can persist fatty tissues by crossing biological membrane (Solomon 2008).

8.3.6 Nickel

Opencast mining for the extraction of nickel (Ni) ore Ni laterite leads to major landscape alteration and increased soil erosion rates (Gillmore et al. 2020). This can cause entering of Ni into water bodies such as rivers, later contaminating the sea (Clark 2001). Ni is an essential element that plays an important role in the synthesis of red blood cells. Trace amounts of nickel do not harm cells but at higher concentrations may damage liver and heart and decrease body weight. Nickel at very high concentrations may cause nervous system damage, reduced cell growth, and cancer (WHO 1984). Ni does not have bio-magnification properties and considered to be moderately toxic metal.

8.3.7 Zinc

Zinc (Zn) is found in earth's crust and an essential element for living organisms as it acts as a cofactor in more than 300 enzymes and contributes to the metabolism

(Morel et al. 1994). Excessive introduction of Zn into water bodies from rubber, plastic, cosmetic, and pesticide industries may cause phytoxicity in plants, anemia, lack of muscular coordination, and abdominal pain in humans. Bioaccumulation of Zn is not observed, but higher concentrations in environmental may lead to its bioaccumulation in organisms (De Pooter 2013).

8.3.8 Arsenic

Arsenic (As) has been considered to be one of the most concerning environmental pollutants around the globe (Kaur et al. 2011; Dash et al. 2021). Insecticides, herbicides, fungicides, and other pesticides are the largest source of As pollution in water bodies (El-Sorogy et al. 2016). Marine organisms are mostly contaminated with highly stable pentavalent form of As which is nontoxic and metabolically inert but may get bio-accumulated in some fishes and algae. Water contaminated with As may cause toxicity to blood and central nervous system, breathing problems, nausea, and lung and skin cancer. As is a geogenic issue that affects everyone, but man-made sources such as pesticide manufacturing and metal processing raise the environmental As content.

8.3.9 Silver

Silver (Ag) is a rare but naturally occurring metal that has been used by human civilization for a long time. Ag is extremely toxic in its +1 oxidation state (Ag^{1+}) to plankton and invertebrates (Luoma et al. 1995). Increasing use of Ag as a biocide in recent years raises concerns about its potential as a pollutant (Purcell and Peters 1998). Since Ag^{+} ion can be taken up by cell via cell membrane ion transporters, its high concentrations may bio-accumulate in some fish, algae, and shrimps (Clark 2001).

8.3.10 Iron

Iron (Fe) and phosphorus (P) along with some other trace elements act as a limiting nutrient for nitrogen fixation (Mills et al. 2004). As nitrogen-fixing organisms require higher concentrations of Fe, sometimes due to this higher concentration, microbes in remote waters may produce more dimethyl sulfide (DMS) and organic carbon, which in turn may have an impact on the radiative forcing in the atmosphere.

8.3.11 Manganese

Manganese being the 12th most abundant element in earth's crust is a broadly distributed metal in nature (Pinsino et al. 2012). Although low level of manganese intake is necessary for human health, exposure to high Mn concentrations is toxic. Excessive exposure to high levels of Mn for a long period may lead to neurological disorders (Manganism: a disease starting from feeling of weakness and lethargy and leading to speech disturbances, clumsy gait and a masklike face) and respiratory and reproductive problems. The individual metals determine the method by which heavy metals impact human bodies, but eventually, all metals create reactive oxygen radicals, which cause a variety of illnesses in living things. Therefore, setting a threshold at which harmful substances were deemed was crucial (Mahurpawar 2015).

8.4 Permissible Limits of Some Common Heavy Metals

The regulatory limits of heavy metals are defined by several well-known organizations based on their toxicities. Given that humans directly consume water, the residential water supply is regarded as the most significant use of water. The National Water Policy has prioritized drinking as the best use of water resources. Drinking water standards have been developed in India by organizations like the Indian Council of Medical Research (ICMR) and the Bureau of Indian Standards (BIS). Table 8.3 lists the drinking water requirements for hazardous and trace metals according to BIS code 10500-2012 (Hussain and Rao 2018). All water delivery systems must adhere to these restrictions. Some heavy metals are regularly present in naturally occurring water (both surface and groundwater) at concentrations 100 or 1000 times higher than the MCL standards. Additionally, drinking water standards have been established by the USA Environmental Protection Agency (USEPA) and World Health Organization (WHO), which are regarded as global standards. Table 8.4 contains some harmful heavy metal ions that have maximum allowable levels in surface waters in accordance to WHO and USEPA. These heavy metals are important resources for various industrial uses; thus, their removal, recovery, and recycling are more crucial than ever. Regulatory authorities have developed drinking

Table 8.3 Drinking Water Standards for Trace & Toxic metals (BIS-10500-2012; Hussain and Rao 2018)

S. no.	Toxic metal	Requirement (acceptable limit; mg/L)	Permissible limit in the absence of alternative source (mg/L)
1.	Cadmium	0.003	No relaxation
2.	Iron	0.030	No relaxation
3.	Chromium	0.05	1.5
4.	Lead	0.01	No relaxation
5.	Copper	0.05	1.5
6.	Zinc	5	15
7.	Lead	0.01	No relaxation
8.	Nickel	0.0	No relaxation
9.	Arsenic	0.01	0.05

Table 8.4 Based on WHO and US EPA rules, some harmful heavy metal ions have maximum allowable levels in surface waters is tabulated (Hussain and Rao 2018)

Toxicity rank	Heavy metals	USEPA (μg/L)	WHO (μg/L)
1	Arsenic	10	10
2	Lead	15	10
3	Mercury	2	1
8	Cadmium	5	3
17	Chromium	100	50
57	Nickel	100	70
75	Zinc	5000	No guideline
125	Copper	1300	2000

water standards in line with toxicity data gathered from human clinical examinations and numerous other research, such as animal tests. A succinct overview is provided in Table 8.5 in which various international regulatory bodies standards are mentioned.

8.5 Remediation Strategies

Different methods such as membrane filtration, adsorption, ion exchange, chemical precipitation, etc. are some of the most commonly used methods to eliminate heavy metals from wastewaters (Türkmen et al. 2022). Presently, many researchers are working on the remediation of heavy metals from water, soil, and air by natural methods rather than chemical methods. Remediation is the method of removing toxic compounds from environmental media or replacing them with less toxicones. The strategies used to reduce or remove pollutants from soil and other environmental mediums use both in situ and ex situ procedures. The three major categories of remediation techniques are (1) physical approaches, (2) chemical ways, and (3) biological methods (Fig. 8.1).

8.5.1 8.5.1 Physicochemical Methods

These methods involve electrodialysis, chemical precipitation, reverse osmosis, evaporation recovery, physical adsorption, ion exchange, etc. (Qasem et al. 2021). Some of these techniques have been described in Table 8.6. Although these conventional physicochemical methods can remove heavy metal ions, there are certain environmental considerations associated with them. These processes generate some secondary waste products that contaminate the environment (Diep et al. 2018). Besides, certain methods such as chemical precipitation and electrochemical treatment are not effective when concentration of heavy metals is low. Techniques that use membrane, activated carbon, nanoadsorbents, and ion exchange technologies are expensive (Dhankhar and Hooda 2011).

Table 8.5 Standards for drinking water quality for trace elements that might have an impact on public health (Hattingh 1977; Gupta 1999)

Parameter	Lead (Pb)	Arsenic (As)	Cadmium (Cd)	Mercury (Hg)	Zinc (Zn)	Chromium (Cr)	Barium (Ba)	Selenium (Se)	Copper (Cu)
Australia (1973)	50	50	10	–	5000	50	1000	10	10000
FRG (1975)	40	40	6	4	2000	50	–	8	–
Japan (1968)	100	50	–	1	100	50	–	–	10000
NAS (1972)	50	100	10	2	5000	50	1000	10	1000
SABS (1971)	50	50	50	–	5000	50	–	–	1000
US EPA (1975)	50	50	10	2	–	50	1000	10	–
USPHS (1962)	50	10	10	–	5000	50	1000	10	1000
USSR (1970)	100	50	10	5	1000	100	4000	1	100
WHO European (1970)	100	50	10	–	5000	50	1000	10	50
WHO Intern. (1971)	100	50	10	1	5000	–	–	10	50

All values are in μg/L
USPHS US Public Health Service, *SABS* South African Bureau of Standards, *USSR* Russia, *NAS* USA National Academy of Sciences

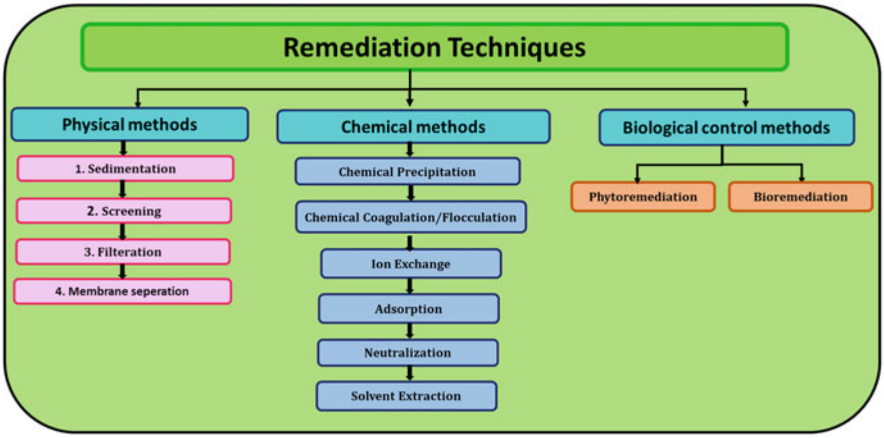

Fig. 8.1 Methods for heavy metal removal

Table 8.6 Physicochemical remediation technologies

S. no.	Physical methods	Description
1.	Screening	This method involves removal of large nonbiodegradable and floating solids
2.	Sedimentation	This technique involves gravity to remove suspended solids from water
3.	Membrane filtration	Membranes are in the form of complex structure which contains dynamic elements on the nanometer scale. In this method different types of membranes are included, namely, reverse osmosis, ultrafiltration, nanofiltration, and electrodialysis
4.	Membrane separation	This process involves membrane to separate the components in a solution by rejecting unwanted substances and allowing others to pass the membrane
5.	Chemical precipitation	This method changes the form of dissolved metal ions into solid particles to facilitate their sedimentation. The coagulant precipitates metal ions by changing pH, electro-oxidizing potential, or co-precipitation
6.	Chemical coagulation/ flocculation	Coagulation is the destabilization of colloids by neutralizing the forces that keep them parted, while flocculation is the agglomeration of destabilized particles.
7.	Electrochemical methods	This method involves the recovery of the heavy metals in the elemental metallic state by using the anodic and cathodic reactions in the electrochemical cell
8.	Ion exchange	This method is a reversible chemical reaction used to replace the undesirable metal ion in an ecofriendly manner
9.	Adsorption	Adsorption method is a solid-liquid mass transfer operation, where the heavy metal (adsorbate) is migrated from the wastewater to the solid surface (called adsorbent) and then bonded due to chemical or physical adsorption over the adsorbent surface

8.5.2 Phytoremediation Technique

Phytoremediation is a technique in which plants act as filters to remove contaminants from soil, water, and air. It is precisely a collection of plant-based technologies that cause removal of contaminants from environment by the use of plants and weeds (Ali et al. 2013). This technique is very cheap and alternative to present treatment methods. In several years, phytoremediation technique is gaining attention due to its cost-effectiveness, eco-friendly nature, and wide acceptance across the globe. Plants accumulate the toxic metals or contaminants from the environment through their body parts (shoots, roots, and stem). Phytoremediation is also identified by different names such as agro-remediation, green remediation, vegetative remediation, green technology, and botano remediation. It is very cheap method that requires technical strategy, knowledge of plants species and cultivars for particular contaminants, and regions. With the help of phytoremediation techniques, a variety of wastewater can be treated as municipal wastewater, industrial water, landfill leachate, paper industry, mine drainage, contaminated lands, and groundwater. This technique reduces the organic and inorganic pollutants from industrial wastewater by using plants. The performance of plants seems to be good in lowering the concentration of pollutants. Phytoremediation techniques follow different mechanisms such as phytoextraction, phytostabilization, phytovolatilization, phytodegradation, and rhizo-filtration during the uptake of heavy metals in the plant (Fig. 8.2). The summary of the phytoremediation techniques and mechanisms is shown in Fig. 8.2 and Table 8.7.

8.5.2.1 Bioremediation

Bioremediation is a technique of converting environmental pollutants into less hazardous forms using living organisms, primarily bacteria. It uses plants, naturally existing bacteria, and fungi to break down or detoxify pollutants that are harmful to the environment or to human health. This technique is advantageous over physico-chemical methods due to their eco-friendly nature (Gunatilake 2015). The basis of

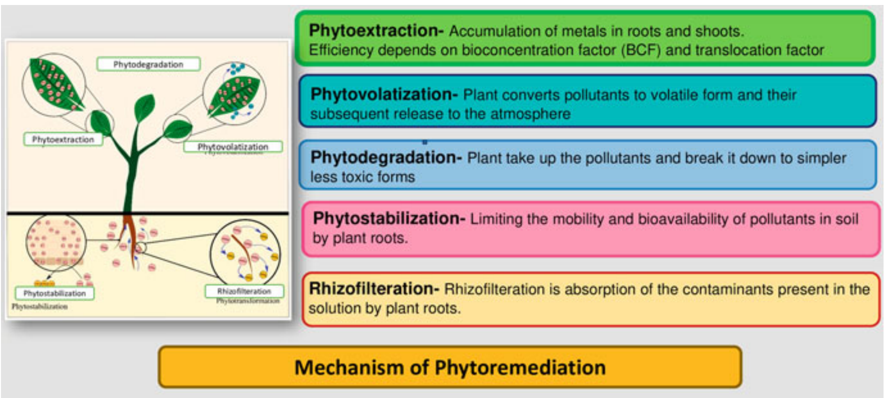

Fig. 8.2 Mechanisms of phytoremediation

Table 8.7 Phytoremediation for different heavy metals using different plant species

Phytotechnology	Pollutants	Plants	Metal-accumulated in plant parts	References
Phytoextraction	Pb, Hg, Cu, Cr, Ni, Zn	Water hyacinth	Roots and shoots	Molisani et al. (2006)
	As	*Hordeum vulgare*	Roots and shoots	Mains et al. (2006)
	Cr and Cd	*Cyperus rotundus*	Roots and shoots	Subhasini and Swamy (2014)
	Pb, Zn, and Cu	*Hordeum hirta*	Roots and shoots	Conesa et al. (2007)
	As	*Eleocharis acicularis*	Shoots	Sakakibara et al. (2011)
	Pahs	*Chrysopogon zizanioides*	–	Un Nisa and Rashid (2015)
	Pb	*Zea mays and Ambrosia artemisiifolia*	–	Shahandeh and Hossner (2000)
	Ni	*Alyssum heldreichii*	Leaves	Rizzi et al. (2004)
	As	*Tagetes minuta*	Shoots	Salazar and Pignata (2014)
	Cu	*Cannabis sativa* L.	Aboveground plant parts	Ahmad et al. (2015)
	Pb and Cd	*Betula occidentalis* and *Thlaspi caerulescens*	Shoots	Koptsik (2014)
	As	*Pteris vittata*	Shoots	Kalve et al. (2011)
	Cd	*Thlaspi caerulescens*	Shoots	Sheoran et al. (2009)
	Zn	*Euphorbia cheiradenia*	Shoots	Chehregani and Malayeri (2007)
Phytovolatilization	Se	*Salicornia bigelovii*	–	Huang et al. (2013)
	Se	*Typha latifolia* L.	–	LeDuc and Terry (2005)
	Se and Hg	*Chara canescens* (musk-grass) and *Brassica juncea*	–	Ghosh and Singh (2005)
	Zn, Mn, Co, Cd, Cr, Ni and As	*Typha latifolia* L.	–	Varun et al. (2011)
	Cd and Zn	*Sorghum bicolor* L.	–	Soudek et al. (2012)
	Cu, Pb, and Zn	*Agrostis castellana*	–	Pastor et al. (2015)

Phytodegradation	Trinitrotoluene	*Myriophyllum aquaticum*	–	Rajakaruna et al. (2006)
	Hg	*Azolla caroliniana*	–	Bennicelli et al. (2004)
	As	*Arundo donax* L.	–	Mirza et al. (2011)
Phytostabilization	Pb	*Sorghum halepense* L.	Reduction in rhizosphere	Salazar and Pignata (2014)
	Cu	*Spartina alterniflora*	Accumulation of metal in roots	Chai et al. (2014)
	Cd	*Phragmites australis*	Accumulation of metal in roots	Nunes da Silva et al. (2014)
	Se	*Salicornia bigelovii*	–	Huang et al. (2013)
	Zn	*Halimione portulacoides*	Accumulation of metal in tissues	Andrades-Moreno et al. (2013)
	Pb and Zn	*Suaeda salsa*	Accumulation of metals in roots	Wu et al. (2013)
	Cd	*Salicornia ramosissima*	Accumulation of metal in roots	Pedro et al. (2013)
	Cd	*Arthrocnemum macrostachyum*	Accumulation of metal in roots	Redondo-Gómez et al. (2010)
	Cu	*Commelina communis*	Accumulation of metal in roots	Wang and Zhong (2011)
	Cd, Ni, and As	*Salicornia brachiata*	Accumulation of metal in roots	Xu et al. (2010)
	Cd, Zn, Cu, and Co	*Sarcocornia perennis*	Accumulation of metal in roots	Lefèvre et al. (2010)
	As, Ag, Cr and Sb	*Solanum tuberosum* L.	–	Baghour et al. (2001)
Rhizofilteration	Cd	*Azolla pinnata*	–	Rai (2008)
	Pb	*Noccaea caerulescens*	Aerial part and shoot	Dinh et al. (2018)
	As	*Cynara cardunculus*	–	Llugany et al. (2012)
	Al, Fe, and Mn	*Pistia stratiotes*	–	Veselý et al. (2012)
	Sb	*Cynodon dactylon*	–	Xue et al. (2018)
	Fe	*Typha domingensis*	–	Hegazy et al. (2011)

removal and transportation of wastes for treatment basically is the two methods, namely, biosorption and bioaccumulation (Diep et al. 2018). Biological methods are sustainable and cost-effective in comparison to other methods. Figure 8.2 demonstrates bioremediation strategies commonly used for heavy metal removal.

8.5.2.2 Biosorption

This technique involves a rapid and passive adsorption mechanism. In this method, both living and dead biomass may be present for biosorption process. The biomass is derived from algae, fungi, plants, and bacteria (Ali Redha 2020). This strategy is inexpensive because it is possible to repeatedly renew and repurpose the biomass obtained from industrial waste. Various low-cost adsorbents are those derived from by-products of industry and agriculture such as rice husk, coconut shell, maize cob, etc. Biosorption involves two components: one is solid (biosorbent), and the other is liquid phase that contains an aqueous solution of contaminants such as heavy metals (Abbas Ali et al. 2016). Functional groups present on the solid phase are the major determinants for removal of a particular heavy metal.

8.5.2.3 Bioaccumulation

Bioaccumulation is a natural process by which microorganisms uptake metal ions using specific proteins from their surroundings (Diep et al. 2018). Researchers have also engineered some microorganisms to enhance their bioaccumulation process by introducing metal uptake proteins in their cell envelopes (Ueda 2016). Bioaccumulation utilizes live microorganisms in their metabolically active state, and major concerns associated with this process is the toxicity of wastewater to the organism, expression level of metal sequestering proteins, and exhaustive screening for selection of organism with high bioaccumulative potential. Genetic engineering helps to enhance the bioaccumulative potential of microorganism numerous folds and thus helps in efficient cleanup of water bodies contaminated with heavy metals (Fig. 8.3).

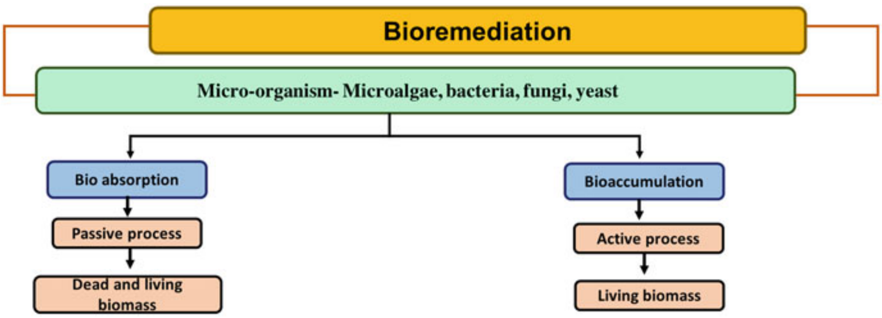

Fig. 8.3 Biosorption and bioaccumulation for heavy metal removal

8.6 Conclusion

Heavy metals become noxious when their concentrations exceed certain level. Increasing concerns regarding their toxicity and hazardous effect on aquatic as well as terrestrial life forms requires extensive study to better understand basic chemistry, toxicity, and selection of appropriate remedial options in order to restore environmental health. To choose an appropriate technique, it is important to consider the type of contaminant, degree of contamination, and cost-effectiveness of the strategy. Besides, efforts to improve their commercialization in developing countries considering environmental sustainability are also important. Implementation of biotechnological aspects and integrated approaches is gaining interest these days. Future research needs to focus on evaluation methods for assessing remediation effectiveness while developing new remediation technologies in future research.

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Recent Advances in Biological Wastewater Treatment

9

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Abstract

Water is a universal solvent and liquid required in most anthropogenic, natural, and biochemical processes. Once used, water will have chemical or microbiological pollutants (wastewater), and knowing that the amount of water on the planet will always be the same, a treatment process must be chosen that aims to eliminate the greatest amount of pollutants to be able to return it to the receiving bodies of water or to be able to reuse it, reducing the impact on the environment. These wastewater treatment processes can be grouped into chemical, physical, and biological. In the case of biological treatment, it consists of using enzymes, microorganisms, or plants to accumulate, transform, or degrade contaminants through their metabolism. Biological treatment systems can be divided into conventional and advanced. Conventional ones are activated sludge, rotating biological contactors, biofilters, nitrification, and denitrification, among others. As an example of advanced biological treatment systems, we can name all the processes that mineralize nitrogenous compounds to molecular nitrogen (such as ANAMMOX, CANON, OLAND), the bioaugmentation batch-enhanced process (BABE), the use of aerobic granular sludge (AGS), and constructed wetlands (CW), among others. Advanced biological treatment systems can offer some of the following advantages compared to conventional biological systems: decreased hydraulic retention times, fewer space requirements, mineralized contaminants, a better landscape, and nominal investment costs in equipment and infrastructure. The present chapter will focus on discussing the most recent

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advances in biological wastewater treatment and how these systems can be applied to deal with existing and emerging pollutants in wastewater.

Keyword

Advanced biological treatment

9.1 Introduction

There are different processes for wastewater treatment; commonly, the two main types are chemical and biological. In biological treatment, the use of microorganisms and plants is considered to transform pollutants either for their assimilation (metabolism) or disassimilation, for which the biological wastewater treatment systems are divided into two large types: microbiological treatment and wetlands (Buha et al. 2015; Pell and Wörman 2009). It is known that the biological treatment of wastewater removes organic matter (carbon), nitrogen, and phosphorus (Christensen et al. 2015) through processes based on the natural cycles of these elements carried out by microorganisms through the conversion of pollutants into gaseous compounds such as carbon dioxide, methane, and molecular nitrogen or to solid compounds such as biomass (Weissbrodt et al. 2020). In the case of wetlands, they are made up of plants, microorganisms, and a substrate; some plants can accumulate or transform heavy metals, degrade organic compounds, volatilize compounds, and remove pathogens, and in a wetland, the removal is done through the plant and microbial activity in conjunction with the substrate that serves as a filter and a support medium for roots and the adhesion of microorganisms (Kataki et al. 2021). As is known, natural cycles have been altered due to anthropogenic activity. This has led humans to novel approaches to understanding and applying these natural occurring processes and, in some cases, improving these processes. The current chapter will describe these processes and how they can be applied to enhance water quality and the environment.

9.2 Biological Nitrogen Removal

Nitrogen pollution of water is mainly due to the excessive use of synthetic nitrogen fertilizers to increase the growth and development of crops in agriculture as well as livestock, the production of fossil fuels, food supplements of animal origin, adhesives, cosmetics, paper, and no less important the increase in human waste as a result of the increase in the population that contributes to the eutrophication of receiving bodies of water due to the excessive growth of algae due to the high nutrient load causing a decrease in oxygen, light, and also of aquatic biodiversity (Bassin 2018; Cárdenas-Calvachi and Sánchez-Ortíz 2013; Huang et al. 2018; Ochoa-Hueso 2017; Tendengren 2021). In this regard, there are various transformation processes for biological nitrogen removal (BNR) through assimilation or

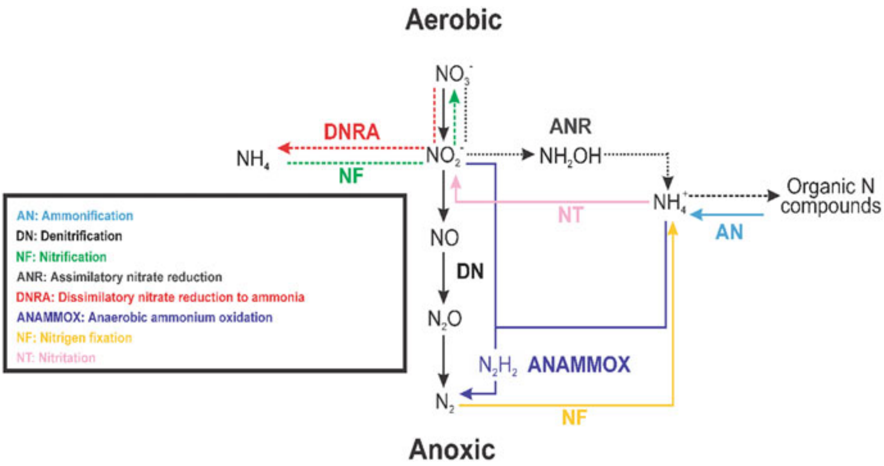

Fig. 9.1 Processes involved in the biological removal of nitrogen

dissimilation, such as biological nitrogen fixation (BNF), nitrification (NF), nitritation (NT), ammonification (AN), denitrification (DN), assimilatory nitrate reduction (ANR), dissimilatory nitrate reduction to ammonium (DNRA), and more advanced processes such as anaerobic ammonia oxidation (ANAMMOX) (Fig. 9.1) from which processes are derived and/or combined such as the single reactor system for high ammonia removal over nitrite (SHARON), completely autotrophic nitrogen removal over nitrite (CANON), oxygen-limited autotrophic nitrification denitrification (OLAND), SHARON/ANAMMOX, partial NT-ANAMMOX (deammonification), denitrifying ammonium oxidation (DEAMOX), simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD), single stage removal using anammox and partial nitritation (SNAP), among others (Bassin 2018; Sanabria et al. 2009; Sanchez and Sanabria 2009; Wang et al. 2017). These processes derived or combined from ANAMMOX activity have aroused much interest due to the toxic effect of ammonia on all life forms (Bagchi et al. 2012). Additionally, other technologies can potentially improve BNR processes, such as bioaugmentation batch-enhanced BABE (Gatti et al. 2015) and aerobic granular sludge AGS (Sarma and Tay 2018).

9.3 Advanced Biological Processes for Nitrogen Removal

9.3.1 ANAMMOX

The process based on lithoautotrophic denitrification, known as ANAMMOX, refers to the anaerobic oxidation of ammonium. This process is carried out by bacteria belonging to the phylum Planctomycetes, which are slow-growing and chemolithoautotrophic bacteria that use nitrite as an electron acceptor (although some species of bacteria involved in ANAMMOX have been described that can use organic acids such as propionic as an electron acceptor instead of nitrite) under anoxic conditions producing molecular nitrogen. This process can be implemented in wastewater with high nitrogen content and low carbon content (ANAMMOX process is known to be little tolerant to the presence of organic matter). In other words, it works best in environments with a low carbon/nitrogen ratio, which is one of the factors that govern the nitrogen removal processes and has a major effect on microbial populations. Although the C/N ratio should be low, the combined partial NT-ANAMMOX process has been used to remove nitrogen from carbon-rich wastewater such as leachate from landfills and sludge digesters, coupling processes from DN (where organic matter is used as an electron donor for the reduction of nitrogenous compounds such as nitrate or nitrite to nitrogen) or from anaerobic digestion where most of the organic matter is transformed into methane (Ahmad et al. 2020; Akgul et al. 2013; Bassin 2018; Cervantes-Carrillo et al. 2000; Figueroa et al. 2012; Saha et al. 2022; Sanchez et al. 2014; van Loosdrecht 2008). It should also be considered that ANAMMOX bacteria have good activity in a pH range of 6.7–8.3 (8.0 optimal value) and at a temperature range of 35–40 °C; temperatures over 45 °C may cause cell lysis (Bassin 2018). The ANAMMOX process presents some disadvantages, among which are that the bacteria that oxidize ammonium grow slowly (not only because it is an autotrophic process but also because ANAMMOX bacteria have a low intrinsic conversion rate of ammonium to nitrite or because the enrichment of the culture does not have the ideal conditions for growth). In some cases, the process startup can take over a year, they are sensitive to visible light, and the presence of heavy metals can inhibit the process, high salinity, organic compounds, sulfides, and phosphates; in addition the accumulation of substrates such as nitrite and free ammonia can also be problematic (Bassin 2018; Lacroix et al. 2020; Xie et al. 2017).

9.3.2 SHARON

SHARON comprises a combination of two processes in which aerobic microorganisms participate in the partial NT (ammonium to nitrite) process and facultative anaerobic microorganisms in the DN (nitrite to molecular nitrogen) process. Due to the nature of both processes, they must be carried out in separate reactors (Sanabria et al. 2009; Sánchez and Sanabria 2009). This process is an alternative for wastewater with high ammonium and low organic matter content, which requires low aeration costs and, in some cases, an additional carbon source such as methanol. Among the factors that govern this process are the pH and the redox potential (ORP), which indicate whether the predominant phase is aerobic or anoxic (Claros et al. 2012), and the type of bacteria present in the process, among others. A submerged biofilter (using PVC as carriers) with a partial-SHARON activity for synthetic wastewater treatment (simulating anaerobic digester leachate with a high ammonium concentration and low organic matter concentration) was operated at two hydraulic retention time HRT (0.4 and 0.5 days) in order to know its effect on bacterial biodiversity. At an HRT of 0.5 days, bacterial biodiversity was lower but more specialized since it managed to convert all the ammonium into nitrite, most of the biofilm was formed by *Nitrosomas* sp., while at an HRT of 0.4 days, this biodiversity was higher since the biofilm was formed by *Nitrosomas* sp., *Nitrospira* sp., and *Nitrosovibrio* sp., but the conversion of ammonium to nitrite decreased substantially (González-Martínez et al. 2013). The partial-SHARON activity process has been reported to be used to treat effluents generated during syngas (this type of effluents contain CO, CO_2, H_2S, CH_4, NH_3, organic acids, ammonium formate, phenols, and cyanides synthetic gas) production with a stirred tank reactor (without biomass recirculation and with an induced microbial consortium with synthetic wastewater rich in NH_4^+ at an HRT of 1.25 days), and despite the presence of phenols and cyanides, the NH_4^+ (NT) removal efficiency was maintained, generating an effluent suitable for an ANAMMOX process (Milia et al. 2015a). Also, the partial-SHARON activity process was studied to determine the effect of different Ci (inorganic carbon)/N ratios (1, 1.5, 1.75, and 2) in the sour water treatment (ammonium-rich refinery wastewater) to determine if the effluent from this treatment was suitable for further ANAMMOX (autotrophic) or DN (heterotrophic) process; the study carried out in a water-jacketed continuous stirred tank reactor (CSTR) under the following conditions temperature of 35 °C, pH of 7.0 ± 0.5, HTR of 1.25 days, DO above 2 mg/L and inoculated with activated sludge (AS) from municipal wastewater plant (WWTP). The sour water used in this study contained dissolved organic carbon (DOC), cyanides, sulfides, and phenols (2000 mg NH_4-N was added). The results showed that the Ci/N ratio affects the regulation of SHARON performance; a Ci/N = 1 ratio produces an adequate effluent for an ANAMMOX process, while a Ci/N = 2 ratio produces an adequate effluent for a DN process. Additionally, using adapted biomass showed a reduced inhibition effect of toxic compounds (Milia et al. 2015b).

9.3.3 CANON

CANON is a combination of processes between partial NT (ammonia to nitrite) carried out by aerobic ammonia-oxidizing bacteria (AOB), and the ANAMMOX process (ammonia + nitrite → molecular nitrogen) carried out by anaerobic ammonia-oxidizing bacteria (AnAOB), both processes in a single reactor (Sánchez and Sanabria 2009; Wang et al. 2017). The microorganisms involved in these processes are autotrophs that can remove high ammonium concentrations; therefore, adding an organic carbon source is unnecessary. Other advantages of this process are that it requires less aeration and the production of sludge is lower (Bassin 2018; Wang et al. 2017). The factors that must be considered in this process are: aeration control is very important since a DO concentration of 1 mg/L is the ideal value, the thickness and density of the biofilm, the concentration of organic matter, and the temperature in a range between 30 and 35 °C as has been established in different studies, resulting in greater growth of microorganisms (Bassin 2018; Sanabria et al. 2009).

9.3.4 OLAND

OLAND is a similar process to CANON, and even some researchers mention that they are the same process; the reactions are carried out in a single biofilm or biofilter reactor where AOB (ammonium to nitrite) and ANAMMOX anoxic bacteria (Sanabria et al. 2009; Sánchez and Sanabria 2009). The biofilm allows partial nitritation and the ANAMMOX process to occur in a single reactor, reducing costs (capital, operational, and aeration) in the treatment process (Sivalingam et al. 2020). Some important factors of this process are the DO levels and the absence of organic electron donors that ensure that the NT occurs, and later, due to the low concentration of DO, the nitrite is consumed in the oxidation of ammonium to nitrogen; therefore, the coexistence of AOB and ANAMMOX can be carried out in the OLAND reactor (Bassin 2018). OLAND is a process that has been used for the treatment of wastewater with very high or very low ammonium contents, above 1000 or less than 66 mg N L^{-1} depending on the concentration of dissolved oxygen (DO) for which it is recommended that does not exceed 0.2 mg L^{-1} (Sanabria et al. 2009; Sánchez and Sanabria 2009; Hien et al. 2017). This recommendation has been derived from studies where, when using an OLAND process, different DO ranges have been applied (0.4–0.8, 0.2–0.4, and 0.1–0.2 mg L^{-1}) for wastewater from a latex processor treated by a Upflow Anaerobic Sludge Blanket (UASB) reactor, which contained 100–200 NH_4^+ mg L^{-1} and 50–80 mg L^{-1} of chemical oxygen demand (COD); the best performance was detected in a DO range of 0.1–0.2 mg L^{-1} with the removal of total nitrogen (TN) and COD of 94% and 61%, respectively (Hien et al. 2017).

9.3.5 DEAMOX

This process combines in a single reactor the ANAMMOX reactions carried out by AnAOB with the autotrophic denitrification carried out by bacteria belonging to the genera Thiobacillus and Thiosphaera, which oxidize reduced forms of sulfur using nitrate as an electron acceptor to produce nitrite in an anoxic/anaerobic environment. and with low organic carbon content (Masłoń and Tomaszek 2009). Among the advantages of this process is that the production of nitrite does not need to be controlled; the formation of granules is stimulated, which is very appropriate for the ANAMMOX process; and high nitrite levels are not detected due to the conversion to molecular nitrogen; however, the process can be limited by a high level of sulfide, which can inhibit the bacterial activity of the DEAMOX process (Kalyuzhnyi et al. 2006; Wang et al. 2018a). Ammonia removal through the DEAMOX process has been applied in industrial wastewater, dewatered sludge, and leachate from sanitary landfills (Masłoń and Tomaszek 2009). The process has been used in continuous flow reactors with AnAOB immobilized in polyethylene glycol in order to reduce the sludge washing in ANAMMOX and with denitrifying bacteria, resulting in a complete nitrate removal and total nitrogen removal of up to 88.5% of synthetic wastewater that contained ammonium, nitrite, nitrate, and sulfate

(Wang et al. 2018a). The DEAMOX process has been compared using planktonic bacteria and bacteria in biofilm (using an SBBR—sequencing biofilm batch reactor—packed with polyurethane foam cubes) with synthetic wastewater (60 mg/L of NH_4^+ and NO_3^-, a C/NO_3^- ratio of 2.5), at a temperature of 30 °C under anoxic condition. The author reported that the ANAMMOX activity (NH_4^+ removal) and TN removal efficiency were significantly higher in the biofilm reactor. This is due to ANAMMOX and denitrifying bacteria occupying different environmental niches, and the increased biofilm thickness creating an environment that favored the ANAMMOX bacteria (due to low O_2 level and COD mass transfer was limitations within the inner layer of the biofilm; these conditions are favorable to ANAMMOX bacteria) (Zhang et al. 2019).

9.3.6 SNAD

This process is the sum of three nitrogen removal processes: NF, ANAMMOX, and DN, in which ammonia and organic carbon contained in wastewater can be removed (Wang et al. 2017). It has been used to remove nitrate formed by AnAOB involved in the CANON process. Through this process, a high nutrient removal has been obtained as a result of heterotrophic denitrification and autotrophic nitrogen removal and has been used to remove nitrogen from wastewater with a low C/N ratio and with a high ammonia content, such as swine wastewater, sludge-digestion liquids, leachate from landfills, and, in a few cases, for domestic wastewater, which contains a low concentration of ammonia (Daverey et al. 2013; Li et al. 2019).

9.3.7 SNAP

Similar to the CANON process, it is carried out in a single reactor, combining the ANAMMOX and partial NT processes, allowing the removal of nitrogen without adding organic carbon and reducing investment and energy costs (Zhang et al. 2014). A membrane-aerated biofilm reactor (MABR) inoculated with AS was tested to remove nitrogen contained in synthetic wastewater through a SNAP process; the results showed a rapid startup of the reactor. In addition, ANAMMOX activity was detected at 48 h, and the removal of NH_4^+ via nitritation was also verified when DO levels were below 0.7 mg/L (it was observed when DO levels increased, there was an imbalance in the microbial community, decreasing the efficiency of MABR), and the total nitrogen removal of 84% was achieved (Augusto et al. 2018). Other reports showed a rapid startup of the SNAP process (46 days), and high removal percentages of 86.9% and 76.8% for NH_4^+ and total nitrogen, respectively, were also obtained in an SBBR inoculated with AS in the synthetic wastewater treatment; the ANAMMOX activity was detected from day 12, and throughout the monitoring, changes in microbial diversity were observed until the SNAP process was established (Cai et al. 2020).

9.3.8 Use of the Combination of Processes to Remove Nitrogen

In the production of carbon shell, large amounts of wastewater are generated, which contain a high concentration of ammonium (600 mg L^{-1}), TN (900 mg L^{-1}), COD (4500 mg L^{-1}), and phenolic compounds (2000 mg L^{-1}), and these waters must be treated in several stages, including the biological removal of nitrogen with advanced processes such as SHARON, ANAMMOX, OLAND, and CANON (Bargieł and Zabochnicka-Świątek 2018). SHARON and ANAMMOX can be potential alternatives to treat the wastewater mentioned above because they are considered economical and sustainable processes for removing ammonia-rich wastewater, such as leachate, from sanitary landfills; they save energy, reduce investment costs, and require less space and operating costs due to lower oxygen consumption and carbon (Shalini and Joseph 2012). Another study focused on the treatment of leachate from landfills, and the treatment was carried out in a multistage system in order to achieve a removal of the high content of organic matter and nitrogen; the multistage process was integrated by a UASB reactor, a membrane bioreactor (MBR), a SHARON reactor, and an ANAMMOX reactor, and it has reported a 99% and 90% removal of BOD_5 and COD, respectively, in the UASB and MBR reactors, while in the SHARON and ANAMMOX reactors, the removal of nitrogen was 92% and 78% for NO_2^- and NH_4^+, respectively (Akgul et al. 2013).

A system consisting of three UASB + SHARON + ANAMMOX reactors was used to know the dynamics of the microbial population during the treatment (314 days) of piggery wastewater with a concentration of 5500–8500 mg/L COD and 500 at 1500 mg/L NH_4^+. The reactors were started up separately to induce the specific activity of each one (gradual increase in the concentration of NH_4^+ and NO_2^- through synthetic residual water), and after 229 days, they were connected and fed with piggery wastewater. In the ANAMMOX reactor, nitrogen removal was detected after 38 days of operation (at this same time, ANAMMOX bacteria were also detected in the biomass samples from the reactor) and increased in time (in the same way the bacteria ANAMMOX and its diversity increased). In the SHARON reactor, bacterial diversity decreased when it was fed with synthetic wastewater (206 days); however, when the influent was changed (change to the effluent from the UASB reactor that treated piggery wastewater), the bacterial diversity increased slightly after 14 days. Finally, it was observed that the microbial composition in the ANAMMOX and SHARON reactors was influenced by the presence and concentration of NH_4^+, NO_2^-, and NO_3^- in addition to other factors such as pH, DO, and COD concentration (Du et al. 2016; Huang et al. 2018).

In the treatment of municipal wastewater, the removal efficiency of organic and nitrogenous compounds, the use of energy, and the production of biosolids of four treatment systems were compared: (a) aerobic organic oxidation AOO + NF followed by the addition of COD and a posterior DN; (b) anaerobic organic removal AOR + AOO followed by an NT process and a subsequent ANAMMOX; (c) DN followed by AOO + NF; C: DN followed by AOO + NF followed by NT and ANAMMOX. The best option was the system described in point "b" due to the lower energy requirement (required oxygen) and production of biosolids in addition to the production of energy in the form of methane (McCarty 2018).

9.3.8.1 BABE

The BABE process is important since nitrifying organisms are very slow growing and sensitive to changes in temperature and pH (Yu et al. 2012). BABE combines the bioaugmentation of nitrifying organisms due to the return of autotrophic nitrifying bacteria from activated sludge that is washed (sludge liquor) from the main treatment process, thereby increasing the NF rate by up to 60%. In addition, the nitrifying organisms grow in flocs of the activated sludge, and without this bioaugmentation, the nitrifiers will tend to grow suspended in the medium; when bioaugmentation is combined with the deammonification process, the negative effects of the nitrogen load of WWTPs are reduced as well as the loss of biomass, so it can be considered that the deammonification process is efficient for the removal of nitrogen and cultivation of nitrifiers for bioaugmentation (Berends et al. 2005; Muszyński-Huhajło et al. 2021; Nsengiyuma et al. 2021; Van Loosdrecht and Salem 2006). A high concentration of nitrite can inhibit denitrifying microorganisms; however, by using the bioaugmentation process in an sequencing batch reactor (SBR) with continuous feeding of nitrite and acetate, the denitrifying bacterial community was enriched, and the nitrite denitrification rate increased from 10 to 275 mg/L h, showing an increase in the microbial biodiversity involved in the denitrification process from 2.16% to 84.26% during the enrichment period (Yao et al. 2019). An SBR operated at 3 different temperatures (20, 15, and 10 °C) was bioaugmented with archaea resulting in up to 20% removal of total nitrogen contained in municipal wastewater compared to other SBR that was not bioaugmented (Szaja and Szulżyk-Cieplak 2020). In addition, SHARON, ANAMMOX, deammonification, and BABE, among other processes, are considered sidestream nutrient removal since they remove a high concentration of nutrients (nitrogen) from the sludge (biosolids) and or the centrate of a treatment plant (Izadi et al. 2021; Nsengiyuma et al. 2021; Van Loosdrecht and Salem 2006).

9.3.8.2 AGS

The AGS is a technology with fewer spatial (lower volume reactor) requirements than activated sludge; due to the compacted biomass, it produces less sludge, requires less energy, and is more tolerant to toxic contaminants and high contaminant loads. In short, it is more effective than AS technology. AGS can simultaneously remove carbon, nitrogen, and phosphorus (Nancharaiah et al. 2019; Sepúlveda-Mardones et al. 2019). The formation of AGS granules is achieved through an adequate establishment of parameters such as the organic load rate (OLR), hydrodynamic shear forces, aeration, temperature, type of microorganisms, and wastewater composition. The granules are formed by different microorganisms distributed in different layers; in the outermost layers is where aerobic microorganisms are located, such as nitrifying bacteria that form nitrate, which will be furtherly denitrified inside the granule where anoxic microorganisms are located. The granules have good sedimentation, so the settling time is concise, which is a great advantage over wastewater treatment systems with activated sludge. Good sedimentation allows the granules to separate between one phase and another (Sepúlveda-Mardones et al. 2019; van der Roest et al. 2011).

9.4 Bioelectrochemical Systems

Bioelectrochemical systems (BES) are a type of technology that allows the conversion of chemical energy in various types of organic substrates to electrical energy through the biological activity as a catalyst. The biological activity is usually provided by microorganisms (bacteria and some yeasts), although, in some cases, the feasibility of some systems to convert solar irradiance to usable energy has been reported (Gul and Ahmad 2019). These types of systems cover different types of cells, and operational configurations, from double-compartment microbial fuel cells (MFC-DC), used almost exclusively for laboratory-scale experimentation, to plant-based microbial fuel cells (PMFC), which allow taking advantage of the photosynthetic processes of plants for the production of electrical energy (Maddalwar et al. 2021). This type of technology presents various advantages over conventional bioenergy processes, among which are its sustainability, zero emissions of greenhouse gases, wide operational range of temperature and pH, and ability to tolerate high organic loads (Cecconet et al. 2020). Currently, interest in this type of technology has increased considerably due to the capacity of this type of system to remediate contaminated water and soil (Varjani 2022), treat persistent pollutants (Bagchi and Behera 2020), and degrade emerging pollutants (Yan et al. 2019).

9.4.1 Types of BES

Bioelectrochemical systems can be classified in different ways, although the most practical is based on the direction of electron transfer and the type of reaction carried out (Zheng et al. 2020).

9.4.1.1 Microbial Fuel Cell (MFC)

Microbial fuel cells are bioelectrochemical systems that allow electrical energy to be generated from the metabolization of organic compounds by electrochemically active microorganisms capable of transferring the generated energy to an external load (Wang et al. 2018b). This process can be described as follows: (1) biocatalyst microorganisms oxidize the organic substrate at the anode, which produces electrons and protons; (2) protons are transported to the cathodic compartment through the cation exchange membrane (CEM); (3) electrons flow out of the system and are redirected toward the cathode compartment; and (4) protons and electrons react with the oxygen present in the cathode compartment to obtain water as the final product (Gajda et al. 2018). The performance of these systems depends on multiple factors; among the most important are the conductivity and microbial biocompatibility of the electrodes (Zhang et al. 2020), nature and concentration of the substrate (Heidrich

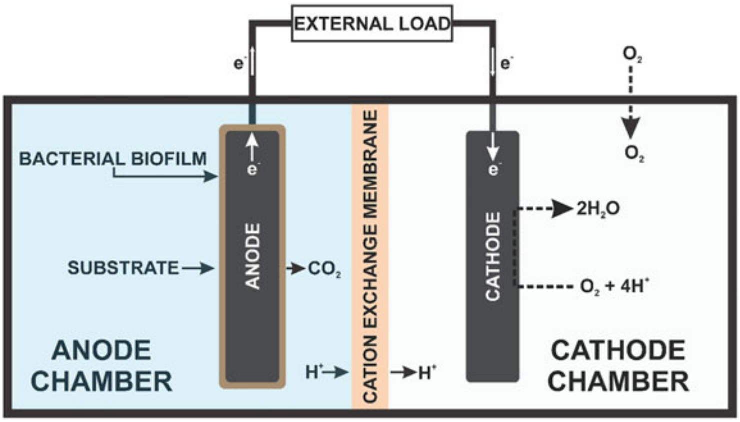

Fig. 9.2 Dual compartment microbial fuel cell design

et al. 2018), internal resistance (Ma et al. 2017), absence or presence of cation exchange membrane (Goel 2018), and overall system design (Bhargavi et al. 2018).

There are different designs of microbial fuel cells; however, the double-compartment design is the most common and recommended for testing on a laboratory scale (Fig. 9.2). Although convenient for testing new configurations and materials, this type of design is counterproductive in the long term by making it difficult to scale to larger dimensions (Flimban et al. 2019).

9.4.1.2 Microbial Electrolysis Cell (MEC)

Microbial electrolysis cells are an anaerobic biological process that converts organic substrates to renewable energy in hydrogen or methane (Lee et al. 2022; Rousseau et al. 2020). In this cell type, organic matter is transformed into CO_2, H^+ and electrons by electrochemically active microorganisms, similar to MFCs. The main difference between the two cells consists in the synthesis of by-products in a controlled manner in the cathode compartment of the cell, seeking, in most cases, to reach sufficient electrochemical potential for the production of hydrogen or methane. For the above to occur properly, it is necessary to overcome the cathodic overpotential present in this type of system, commonly achieving this objective by using an external power source that supplies the necessary electrical current to compensate for what is naturally generated by the microorganisms in the anode compartment during the biodegradation process the organic substrate fed to the cell (Katuri et al. 2019). Among the main applications of MECs is the production of hydrogen, methane, formic acid, and hydrogen peroxide, among others (Aiken et al. 2019; Carrillo-Peña et al. 2022; Hua et al. 2019; Sim et al. 2018). Similarly, due to the ability of this type of system to degrade complex organic matter and change the oxidation state of some inorganic substances, interest in this type of process has increased from an environmental preservation and remediation perspective (Chen et al. 2019).

9.4.1.3 Microbial Desalination Cell (MDC)

Microbial desalination cells are a promising technology for seawater purification and polluted water desalination (Zahid et al. 2022). The functioning and operation of these cells (Fig. 9.3) consist of the following: (1) from an MFC-DC, a third compartment is located that divides the anode and cathode compartments of the cell; (2) said compartment, called desalination chamber, which will be separated from the anodic compartment by an anion exchange membrane (AEM) and of the cathode compartment by a cation exchange membrane; (3) the potential differential that occurs during the oxidation process of organic matter and the consequent flow of electrons from the anodic compartment to the cathodic compartment will cause a migration of the anions through the AEM and toward the anionic compartment and of the cations from the desalination chamber toward the cationic compartment of the cell (Tawalbeh et al. 2020). The final product of this process will be treated water with a significantly lower content of soluble salts compared to the initial solution, and depending on the degree of pollution, a completely treated sample could be obtained (Salehmin et al. 2021). This technology is, therefore, relevant not only for its ability to purify saline water but also for the parallelism it presents when treating wastewater rich in organic matter (anodic compartment) and the consequent generation of electrical energy by oxidizing the substrate (Al-Mamun et al. 2018). Despite this, more in-depth studies are required to define the multiple variables that affect this process.

9.4.1.4 Plant Microbial Fuel Cell (PMFC)

PMFCs are a type of technology that allows a plant system to be coupled to a conventional MFC, seeking to take advantage of the root exudates secreted by plants during the photosynthetic process and convert these substances into electrical energy through microbial action (Kabutey et al. 2019). This technology also turns out to be interesting if it is considered that, indirectly, and using plants as intermediaries; it is

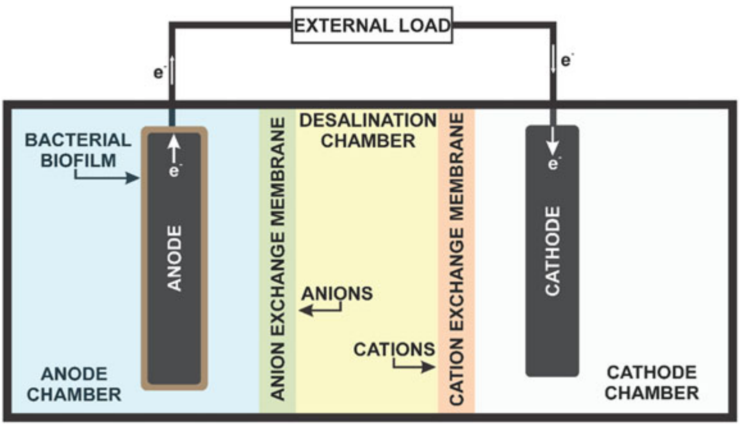

Fig. 9.3 Microbial desalination cell design

possible to convert solar energy (total irradiance) into electrical energy (Chiranjeevi et al. 2019). As this technology depends on multiple elements, there is a large number of factors that can affect the performance of this type of system, among which are the genus and species of the plant used, the source, intensity and spectral balance of the light irradiance applied to the plant, CO_2 concentration in the experimental area, operational parameters of the cell (pH, temperature, electrical conductivity, and electrode material), macro- and micronutrient content in the anodic compartment, type and source of the inoculum, and cell design, among others (Regmi et al. 2018b). One of the main advantages of this process is that it can be carried out indoors or outdoors depending on the site's needs (Osorio-de la Rosa et al. 2019). Similarly, being a technology that can be easily adapted to different environments, the PMFC can be placed on the ground without modifying the area's landscaping, thus reducing the environmental impact (Regmi et al. 2018a).

9.4.2 Recent Advances in BES

Recent research on BES has focused on three main axes: (1) selection and optimization of the materials that make up the electrodes, (2) design and configuration of the devices used, and (3) identification, isolation, and application of new electrochemical active microbial species (Chen et al. 2021).

9.4.2.1 Novel Electrode Materials

Graphene-Based Electrodes

Graphene is a substance with a laminar structure made up of a single layer of two-dimensional oriented carbon atoms. It has a high specific surface area and thermal and electrical conductivity (Zhang et al. 2022). These properties make it an extremely promising material for its application in bioelectrochemical systems by increasing the amount of electrical energy captured and porosity and enhancing substrate degradation (Cui et al. 2018; Tremblay et al. 2020). Anodes modified with graphene deposits have been used to increase the conductivity of these components and maximize the power density achieved in the systems, resulting in significantly higher results when compared to unmodified base materials (Camedda et al. 2019; Lescano et al. 2018; Tian et al. 2019). Graphene coatings have also been applied to obtain biocathodes to increase the porosity and therefore the surface area, of these materials, thus maximizing the mass transfer of the substrate toward the microbial community (Aryal et al. 2019; Fu et al. 2022; Yao et al. 2021). Some other examples of this type of application are shown in Table 9.1.

Conductive Polymer-Based Electrodes

Conducting polymers have generated much interest today due to their chemical stability, biocompatibility, and high conductivity, making these materials suitable for BES as electron acceptors (Narayanasamy and Jayaprakash 2020). Various authors have addressed the advantages of applying this type of materials to increase

Table 9.1 Comparison of application of graphene electrodes in various types of bioelectrochemical systems (BES)

Type of BES	Anode	Cathode	Cationic exchange membrane (CEM)	Inoculum	Substrate	References
MFC	Graphene/carbon brush	Carbon cloth	Nafion 117	Sewage wastewater	Sewage wastewater	Sayed et al. (2021)
MFC	PDDA, graphene, and carbon cloth	Carbon felt	Nafion 117	Anaerobic sludge	Phosphate buffer solution	Chen et al. (2020)
MFC	Graphene	Stainless steel mesh	Nafion 117	Anaerobic sludge	Phosphate buffer solution	Pareek et al. (2019)
MFC	Tungsten carbide/graphene/carbon felt	Carbon paper	Nafion 117	Sugar industrial wastewater	Sugar industrial wastewater	Mohamed et al. (2021)
MEC	Graphite plate	Graphene/nickel oxide nanocomposites	Nafion 117	Sugar industrial wastewater	Sugar industrial wastewater	Jayabalan et al. (2020)
MEC	Graphite fiber brush	Graphene/Pt nanoparticles	–	–	Sodium acetate buffer	Sánchez-Peña et al. (2022)
MEC	Graphite brush	Graphene/graphite plate	Ultrex CMI-7000	Domestic wastewater	Anaerobic sludge	Hu et al. (2019)

Table 9.2 Comparative application of conductive polymer electrodes in various types of bioelectrochemical systems (BES)

Type of BES	Anode	Cathode	Cationic exchange membrane (CEM)	Inoculum	Substrate	References
MFC	CNT/ PPy	Carbon cloth	–	Anaerobic slugde	Wastewater and nutrient solution	Zhao et al. (2019)
MFC	nPPy/ graphite felt	Carbon cloth	–	*Shewanella putrefaciens*	Glucose-based nutrient solution	Sumisha and Haribabu (2018)
MFC	nNIBP/ carbon felt	Carbon felt	–	*Cystobasidium slooffiae*	Xylose-based nutrient solution	Moradian et al. (2022)
MFC	NIBP/ CNT sponges	Graphite rod	Nafion 117	Anaerobic slugde	Sodium acetate-based nutrient solution	Xu et al. (2019a)
MFC	nPTh/ carbon cloth	Carbon cloth	Nafion 117	*Shewanella putrefaciens*	Glucose-based nutrient solution	Rajendran et al. (2021)

the performance of BES, emphasizing the low production cost, which would allow this type of system to be scaled more easily (Fischer et al. 2018; Sonawane et al. 2018). A bioanode composed of polypyrrole-carboxymethylcellulose-titanium nitride/carbon brush hydrogel (PPy-CMC-TiN/CB) was developed to increase the mechanical stability of the supports and increase the performance of the system, observing highly favorable results (Wang et al. 2020). In chromium (VI) reduction, an abiotic cathode made from carbon/manganese dioxide cloth coated with polypyrrole (PPy) was used to prevent corrosion of the composite, managing to remove 100% of the contaminant in less than 32 h. Therefore, this material is considered suitable for its use on larger scales (Liu et al. 2020a). The viability of PPy/MnO_2 nanocomposites focused on identifying their electrochemical behavior when applied in BES and evaluating their ability to generate electrical power has also been determined (Prakash et al. 2020). More examples of the above are mentioned in Table 9.2.

Electrode-Based Nanomaterials

In recent years, the development of nanomaterials (materials with a size between 1 and 100 nm) has experienced rapid growth due to its great application potential in a wide variety of technological areas such as electronics, data storage, and ceramics, among others (Liu et al. 2020b). These materials facilitate the extracellular transfer

Table 9.3 Application of nanomaterial electrodes in various bioelectrochemical systems (BES)

Type of BES	Anode	Cathode	Cationic exchange membrane (CEM)	Inoculum	Substrate	References
MFC	CNT/ carbon cloth	Pt/carbon cloth	Nafion 117	Municipal wastewater	Municipal wastewater	Iftimie and Dumitru (2019)
MFC	Carbon cloth	Co/N-CNT/ carbon cloth	–	Anaerobic digester sludge	Sodium acetate nutrient solution	Yang et al. (2019)
MFC	Carbon felt	CNt/MoS_2	Nafion 117	Originated on a stable operating MFC	Phosphate and acetate nutrient solution	Xu et al. (2019b)
MEC	Carbon cloth	nFe_3O_4 / carbon cloth	–	Dairy industry wastewater	Dairy industry wastewater	Rani et al. (2021)
MEC	Carbon felt	Several types of CNTs and metals/carbon felt	–	Originated on a stable operating MFC	Acetate nutrient solution	Choi et al. (2019)

of electrons from microorganisms toward the anode in BES (Kaur et al. 2020). Electrospun carbon nanotube and nanofiber nanocomposites (CNTs/CNFs) were evaluated in an MFC, observing an increase in electrical conductivity, excellent biocompatibility, good hydrophilicity, and high electrocatalytic activity (Cai et al. 2019). A cathode composed of nitrogen-doped carbon nanofibers was developed to maximize the catalytic capacity of the electrode in a single-compartment MFC, increasing cell performance and obtaining similar results to other works where Pt was used as a cathodic catalyst (Massaglia et al. 2019). Carbon cloth anodes coated with gold nanoparticles increased the generation of electrical energy in a double compartment MFC, reaching remarkable values due to the high affinity of these materials with the electrochemically active microorganisms of the cell (Wu et al. 2018). Table 9.3 shows other examples of this type of application in BES.

9.4.2.2 Systems Design and Configuration

Although most of the scientific research related to BES has indeed focused on developing new anodic and cathodic materials to favor the conductivity of this type of materials, in recent years, modifications have been promoted similarly regarding the architecture in this type of systems, seeking to obtain designs and configurations that are easier to scale, lower cost, and generally more efficient (Bakonyi et al. 2018). Different authors have focused on increasing the ionic conductivity of exchange membranes, this being one of the main limitations for obtaining good performance in BES. A cation exchange membrane based on sulfonated polyether ether ketone (SPEEK) and sulfonated titanium nanotubes

(S-TNT) was synthesized to increase the ionic conductivity of an MFC, thus reducing the resistivity of this component (Kugarajah and Dharmalingam 2020). Ceramic membranes with different pore diameters have been evaluated as separators in MFC, comparing their performance with a membrane more commonly used in this type of study (Nafion 117), verifying the feasibility of applying this type of materials in BES, and managing to increase the total conductivity of the cell (Daud et al. 2018). A sulfonated biocarbon composite membrane was applied as CEM in BES, with good results; additionally, this membrane is low-cost and easy to scale (Chakraborty et al. 2020). Membranes made with natural clays mixed with goethite were used as CEM in a laboratory-scale MFC, achieving efficiencies similar to conventional systems that use commercial CEM (Nafion 117) but at a fraction of the production cost, being this promising for the eventual scaling of this type of systems (Das et al. 2020).

9.4.2.3 Novel Electroactive Microorganisms

A wide variety of microorganisms can produce an electrical current and transfer it to external electron acceptors, such as the anodes in the BES. These exoelectrogens are present in the three taxonomic domains (superkingdoms) and can be used as long as they are provided with the appropriate conditions for their correct development (Logan et al. 2019). Currently, more attention has been paid to the identification, isolation, and use of this type of microorganisms to increase performance in BES. The role of microorganisms of the genus *Clostridium* in generating electrical energy by acting as adjuvants in anaerobic biofilms in BES has been reported (Rivalland et al. 2022). A biofilm of fruit peel leachate was formed onto carbon felt bioanode, probing the versatility and diversity of sources from which microorganisms can be isolated with the capacity of using external materials as final electron acceptors (Kebaili et al. 2020). Up to 17 species of 6 different genera of microorganisms within the rhizosphere and plant tissue of angelica and sweet potato were reported, such as *Bacillus* sp., *Pleomorphomonas* sp., *Rahnella* sp., *Shinella* sp., *Paenibacillus* sp., and *Staphylococcus* sp., (commonly known species), and there may still be other species that could be present that could present superior capacities for the generation and transport of electrons from inside the cell and toward an external acceptor (Ling et al. 2022).

9.5 Membrane Bioreactors

Membrane bioreactors (MBR) combine the activated sludge remediation process with a membrane filtration process. This technology can treat a wide range of organic substrates, generate a lower amount of sludge as a by-product, and have high degradation rates of pollutants (Pervez et al. 2020). These systems allow the elimination of secondary and tertiary treatment processes, reducing costs and increasing the volume of treated water (Al-Asheh et al. 2021). Recent studies have verified the wide versatility of this type of system when applying them for the remediation of water contaminated with microplastics (Bayo et al. 2020), pharmaceutical residues (Femina-Carolin et al. 2021), and hydrocarbons derived from petroleum (Zeirani et al. 2019), among others.

9.5.1 Types of MBR

MBRs are categorized based on their configuration or design, with two main types: (1) submerged membrane bioreactor and (2) lateral flow membrane bioreactor (Goswami et al. 2018).

9.5.1.1 Submerged Membrane Bioreactor (SMBR)

In submerged membrane bioreactors (as their name indicates), the filtration membrane is placed inside the bioreactor and in direct contact with both the substrate and the biomass (Fig. 9.4), thus reducing operating costs. Therefore, the substrate will be forced to interact with both the microorganisms in the system and the membrane, increasing the contaminant removal rate. Despite the above, as the membrane is located within the system and under constant adverse conditions, its care and maintenance will be complex, creating the opportunity for system failures to occur (Miyoshi et al. 2018). One of the main problems when developing and implementing this type of technology is the so-called biofouling, a phenomenon described as the accumulation of microorganisms on unwanted surfaces (Pichardo-Romero et al. 2020). In the case of SMBR, biofouling occurs primarily on the surface of the remediation membrane, as the biomass accumulates and occludes its pores, decreasing the flow of the substrate through the membrane and, therefore, affecting the performance of the system in the long term (Aslam et al. 2018). Therefore, most of the research on this type of system has focused on reducing this phenomenon by modifying the porosity and chemical composition of the filtration membranes (Nazmkhah et al. 2022; Sano et al. 2022; Xiao et al. 2021).

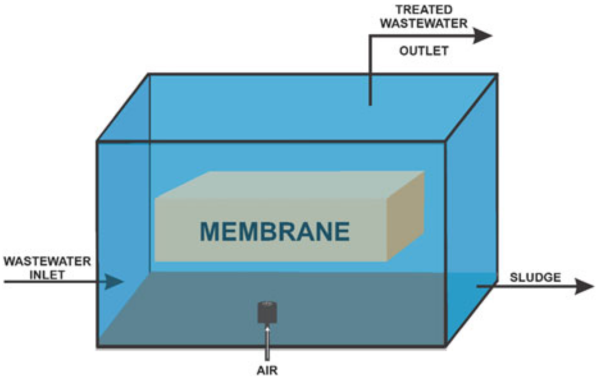

Fig. 9.4 Basic configuration of a submerged membrane bioreactor

9.5.1.2 Side Stream Membrane Bioreactor (SSMBR)

Lateral flow membrane bioreactors remove the filtration membrane from the system and convert it to a secondary treatment. Both the substrate and the biomass are recirculated from the bioreactor to the membrane (and vice versa), increasing the process efficiency and the quality of the treated water (Fig. 9.5). In this typeof design, the maintenance of the filtration membranes is facilitated, but considering that an extra step is added to the treatment process, the operational costs, labor, and complexity of this type of technology will increase (Lim et al. 2021). Another advantage of this type of system, when compared to SMBRs, is that biofouling can be avoided by increasing the feed rate of the substrate from the bioreactor and toward the membrane (inlet flow), using the hydrodynamics of the solution to detach the biomass or other contaminating particles that are occluding the superficial or internal pores of membrane (Martínez et al. 2021).

9.5.2 Recent Advances in MBR

In recent years, research related to MBR has focused on modifying the filtration membranes' manufacturing materials to prevent biofouling, one of the main limitations of scaling and implementing this type of system (Sun et al. 2019). The performance of a filtration membrane coated with a nanofibrous cellulose composite for the treatment of domestic wastewater has been reported, resulting in a great capacity of these materials to prevent biofouling, associating this phenomenon with the high hydrophilicity of these composites, considering these results as promising for the scaling of this type of membranes (Lotfikatouli et al. 2021). Modifications have been made to the surface of a polyvinylidene fluoride (PVDF) ultrafiltration membrane by applying coatings of tannic acid (TA) and copper (Cu^{2+}) in different proportions, evaluating the ability of these coatings to prevent biofouling of the membranes; the modified membranes presented greater permeability and ease of cleaning than their unmodified counterpart, but they highlight the need to continue carrying out complementary studies (Maneewan et al. 2021). Polysulfone

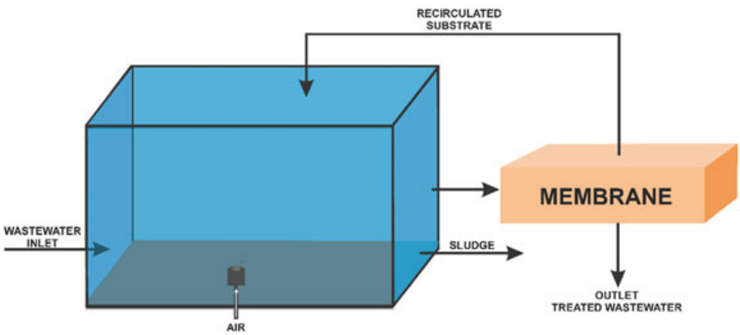

Fig. 9.5 Base configuration of a lateral flow membrane bioreactor

Table 9.4 Comparison of membrane bioreactors and their most notable advances by component

Type of MBR	Filtration membrane	Inoculum	Substrate	References
SMBR	Zinc oxide and sodium alginate on a polyacrylonitrile	Activated sludge	Urban wastewater sludge	Sokhandan et al. (2020)
EMBR	Conductive copper hollow fiber	Anoxic digest sludge	Acetate solution	Liu et al. (2018)
MaMBR	Fe_3O_4-coated ZrO_2 /PAN nanocomposite	Activated sludge	Urban wastewater	Noormohamadi et al. (2020)
MBR-like system	Electrospun cellulose acetate PVDF	–	Synthetic wastewater	Bakhsha et al. (2021)
exMBR	AgNPs on a zeolitic imidazolate framework-8	–	Phenol and NaCl solution	Wang et al. (2022)
mcs	Vanillin/CPE	Activated sludge	Activated sludge	Leda et al. (2018)
SSMBR	Lytic phage therapy on a modified SGO-PVDF	–	Municipal wastewater	Ayyaru et al. (2018)
MF-MBR	AgNPs on a sterapore polyethylene hollow fibers	Activated sludge	Glucose, NH_4Cl and KH_2PO_4 solution _	Le et al. (2019)
MBR-like	Quaternary ammonium compounds loaded on silica nanopollens on a PVDF base	*E. coli*	Simulated domestic wastewater	Zhai et al. (2020)
MBR-like	PES modified membrane with semiconductor diode laser	Activated sludge	BSA solution	Polat et al. (2020)
MBR-like	Nanocurcumin/PES	*E. coli*	Nutrient agar medium	Kumar and Arthanareeswaran (2019)

ultrafiltration membranes with metal organic frameworks (MOF) were used to evaluate their ability to treat wastewater from a dairy industry that generated cheese whey, obtaining positive results not only in the reduction of organic load but also in increasing the feeding flow of this substrate, improving the efficiency of the treatment (Bazrafshan et al. 2021). A combination of Ag-MOF was immobilized on the surface of a polydimethylsiloxane (PDMS) nanocomposite to mitigate biofouling in the membrane, resulting in a great anti-biofouling capacity of the synthesized materials, attributing this property to the slow release of Ag^+ ions, counting this type of ions with a high antimicrobial capacity (Yuan et al. 2022). Hydrophilic nylon fiber membranes modified with polyvinyl alcohol (PVA) have been used for the long-term operation of an ANAMMOX membrane bioreactor, verifying the ability of this type of material to reduce the biofouling generated in this type of bioreactor (Ni et al. 2021). More examples of the above are presented in Table 9.4.

9.6 Constructed Wetlands

Constructed wetlands are engineered systems designed and built to use natural processes involving vegetation, soil, and associated microorganisms, allowing wastewater treatment (Vymazal 2014). The UN has reported that wetlands have a high potential to address water problems and offer multiple advantages that lead to sustainable development (Kataki et al. 2021). Constructed wetlands are designed to take advantage of natural wetlands but with a more controlled environment; most research done on constructed wetlands has been designed to treat municipal and domestic wastewater, but lately, constructed wetlands have been employed in the treatment of other types of wastewater from different industries and slaughterhouses (Navarro-Frómeta et al. 2020; Pérez et al. 2022). Under the climate change scenario, constructed wetlands will face challenges, such as increased concentration of pathogens in wastewater due to increased global temperature; increased precipitation, which will cause an increase in water runoffs containing pathogens; and finally the reuse of treated wastewater, related to water scarcity (López et al. 2019; Vymazal 2014). These systems also function as water treatment plants, creation of new habitats, recreational and educational facilities, and ecological art areas (Stefanakis 2020). Constructed wetlands can remove various pollutants (organic, nutrients, microelements, among others) through a series of processes, which improve water quality (Pérez et al. 2022) and have been applied for the secondary and tertiary treatment of urban and industrial wastewater (Marín-Muñiz 2017). The main constructed wetlands used, both at an applied level and in research, are free surface flow, horizontal subsurface flow, and vertical subsurface flow, based on the path of water flow through the system (Asprilla et al. 2020; López et al. 2019; Stefanakis et al. 2014). Free surface flow systems design includes a 10–50 cm water column over a substrate layer (mainly soil); subsurface flow-constructed wetlands are gravel beds (Asprilla et al. 2020; Stefanakis 2020; Vymazal 2014). According to the vegetation type, a classification includes constructed wetlands of emergent macrophytes and submerged macrophytes, respectively, (López et al. 2019; Stefanakis 2020); common systems are those with rooted emergent macrophytes (López et al. 2019). When more than one type of constructed wetland is used at the same facility, it is known as a hybrid wetland system (López et al. 2019; Asprilla et al. 2020).

9.6.1 Free Water Surface-Constructed Wetland

A free water surface constructed wetland (FWSW) consists of a series of flooded channels whose objective is to mimic the natural processes of a natural wetland, marsh, or wetland (Fig. 9.6) (Asprilla et al. 2020). By flowing gently through the wetland, the particles settle, the pathogens are destroyed, and the organisms and plants use the nutrients, allowing the water to flow over the ground and be exposed to the atmosphere and sun radiation (Marín-Muñiz 2017). The channel or dam is lined with an impermeable barrier (clay or geotextile) covered with stones, gravel, and

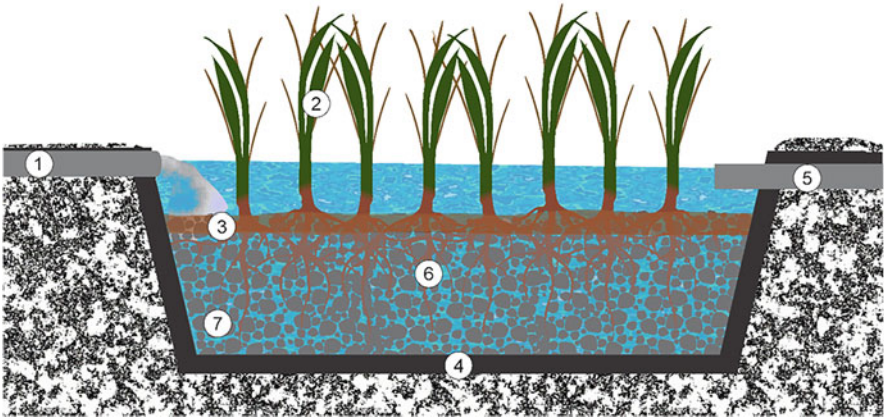

Fig. 9.6 Free water surface constructed wetland. (1) Inlet, (2) plants (macrophytes), (3) sludge, (4) liner, (5) rhizome network, (6) outlet, (7) gravel bed

earth and planted with vegetation from the region. The wetland is flooded with wastewater to a depth of 10–45 cm above ground level; wastewater flows gently through the wetland and goes through simultaneous physical, chemical, and biological processes; solids are filtered, organic matter is degraded, and nutrients are removed (Asprilla et al. 2020). Once in the wetland, the heavier particles settle out, thus removing the nutrients attached to them; plants and the communities of microorganisms (in stems and roots) take in nutrients such as nitrogen and phosphorus; chemical reactions can cause other elements to precipitate; pathogens are removed from water by natural decomposition, predation by higher organisms, sedimentation, and ultraviolet radiation (Asprilla et al. 2020; Marín-Muñiz 2017; Stefanakis 2020). Although the underwater layer of the wetland is anaerobic, the roots of the plants release oxygen in the area surrounding the root hairs, creating an environment conducive to complex chemical and biological activities (Marín-Muñiz 2017). The efficiency of the FWSW also depends on the good distribution of water at the inlet; wastewater can enter the wetland using dams or drilling holes in a distribution pipe to allow it to enter at regular intervals (Asprilla et al. 2020; Marín-Muñiz 2017). The advantages of using this type of wetland are: it is aesthetic; it performs a high reduction of the BOD, a moderate removal of pathogens, the construction, and repair with local materials; it does not require electricity; and it does not generate bad smells. Among the disadvantages are the high reproduction of mosquitoes, the startup of the wetland slowness, the requirement of a large area of land, the monitoring of an expert, and the moderate investment in land and materials (Marín-Muñiz 2017). Plant species commonly used in this type of wetland are *Phragmites australis*, *Typha* spp., *Scirpus* spp., and *Juncus* spp. (Stefanakis et al. 2014; Vymazal 2013). Other macrophytes have also been evaluated in this type of wetland by comparing the behavior of an FWSW and a horizontal subsurface flow (HSFW) wetland for wastewater treatment with other macrophytes *(Eichornia crassipes*, *Typha domingensis*, *Paspalum paniculatum*, *Cyperus articulatus*) and two HRTs to determine the removal of pollutants from the wastewater from the bathrooms and dining hall of a university campus; it was observed that the FWS wetland with *Typha domingensis* had the highest removal efficiency in terms of to turbidity, color, COD, BOD_5, TN, TP, and total suspended solids (TSS) (Solís-Silvan et al. 2016). Combinations of different macrophytes have also been made when evaluating the performance of an FWSW to restore the Jialu River in China, using a combination of emergent plants + floating plants + submerged plants for 2 years in 1 area of 7400 m^2 and a hydraulic loading rate (HLR) of 14 cm/day with a high viscosity silt loam substrate, finding that the average removal rate of total nitrogen, total phosphorus, ammoniacal nitrogen (NH_4^+-N), COD, and TSS was 75%, 78%, 85%, 40%, and 80%, respectively, in summer and autumn, and in winter, they were 30%, 73%, 45%, 25%, and 78%, respectively. The system demonstrated stability and great potential to improve water quality, in addition to being easy to maintain during its operation, which is why it was considered an effective treatment option to restore a polluted river and to be feasible to apply in a generalized way and any place (Wang et al. 2012).

9.6.2 Horizontal Subsurface Flow Constructed Wetland

The horizontal subsurface flow constructed wetland (HSFW) consists of a large concavity filled with gravel and sand planted with vegetation, where wastewater flows horizontally (Fig. 9.7). The advantages of this system include a high reduction of BOD, suspended solids, and pathogens, no mosquito problems that occur in FWSW, no electricity required, and low operating costs (Cisterna Osorio and Pérez Bustamante 2019; Zurita-Martínez et al. 2011). Among the disadvantages of this type of wetlands are that they require a large area of land, there is little nutrient removal, there are risks of obstructions, the initial adaptation period is long, and

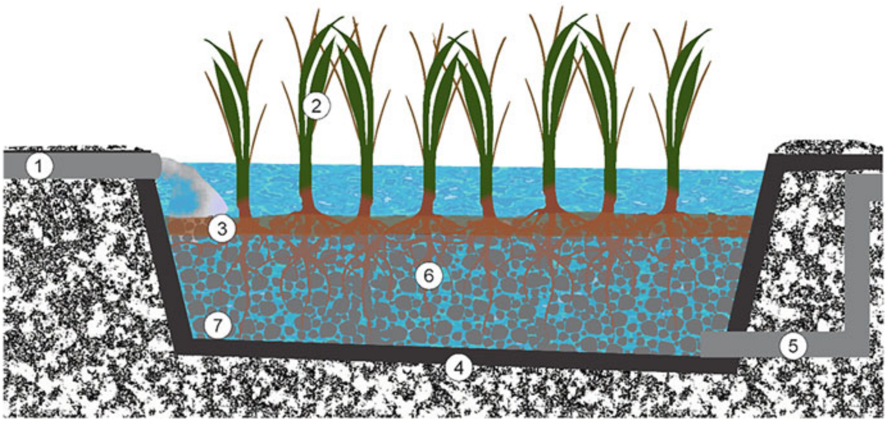

Fig. 9.7 Horizontal subsurface flow constructed wetland. (1) Inlet, (2) plants (macrophytes), (3) sludge, (4) liner, (5) rhizome network, (6) outlet, (7) gravel bed

experience in design and construction is required (Cisterna Osorio and Pérez Bustamante 2019; Vymazal 2014). In the HSFW, the circulation of water is carried out through a granular medium, with a depth that depends on the size and length of the roots of the plants (Asprilla et al. 2020; Vymazal 2014). The vegetation transfers a small amount of oxygen to the root, so aerobic bacteria can colonize the area and degrade organic materials (Cisterna Osorio and Pérez Bustamante 2019). The design of the HSFW depends on the treatment objective and on the quality and quantity of the effluent, including decisions on the number of parallel flow paths and compartmentalization as well as the efficiency of wetland removal, which depends on the surface area (length by width), while the cross-sectional area (width times depth) determines the maximum possible flow. Generally, an area of 5–10 m^2 per person is required; the bed should be lined with an impermeable liner (clay or geotextile) to prevent leaching, and it should be wide and shallow to maximize the flow path of water in contact with the roots of the vegetation, and a wide area should be used for entry, in order to distribute the flow evenly and avoid clogging, and the outlet must be variable so that the water surface can be adjusted and thus optimize the treatment performance (Asprilla et al. 2020). *Phragmites australis* was used in an HSFW fed with different HLR for domestic wastewater treatment; authors determined that the removal rates of the system were closely dependent on the applied HLR levels, and the highest removal rates detected were 64.9%, 62.5%, 86.3%, and 80.34% for BOD_5, COD, TSS, and oils and fats, respectively, with an HLR of 0.050 m^3 day^{-1} m^{-2}, and above this load, the efficiencies decreased (Çakir et al. 2015). Most antibiotics are not efficiently removed in conventional wastewater treatment plants, making implementation efficient and low-cost treatment alternatives necessary. *Phragmites australis* has also been used in HSFW to remove different concentrations of the antibiotic florfenicol (10, 15, 20, and 25 mg/L of florfenicol) at an HTR of 4.2 days, detecting a maximum removal of 77.9% of florfenicol and 85.2% of COD during the first days of exposure, and the results showed that florfenicol was not retained in the granular material of the wetlands, nor the macrophytes and that the removal mechanism was by biological degradation (Rodriguez et al. 2021). Four HSFWs planted with *Cyperus alternifolius* bioactivated with efficient microorganisms for treating slaughterhouse wastewater to assess the effect of HRT, microorganisms activation, and the type of substrate (combinations of brown soil, limestone gravel, zeolite, and river sand) on the efficiency in the removal of contaminants. As a biostimulant element of the autochthonous microbial flora in wetlands, the commercial bioproduct ME-50 (LABIOFAM) was used, consisting of a mixture of autochthonous microorganisms where filamentous fungi and yeasts, *Lactobacillus*, photosynthetic bacteria, and actinomycetes predominate. The commercial bioproduct is described as a brown aqueous suspension with a slight odor of wine or ferment and acidic pH (3.2–3.8). The maximum removal efficiency was achieved in bioactivated systems at 96 h, with a removal of 96% for COD, 96.4% for BOD_5, and 94% for fats and oils, so HSFW can be considered a method efficient for the treatment of wastewater generated in slaughterhouses, achieving high levels of pollutants removal (Esperanza-Pérez et al. 2022). The HSFW has been used as a tertiary treatment of wastewater from a dairy industry on a pilot scale using *Typha domingensis* and river gravel as a substrate, and 32 influent and effluent samples were collected over 7 months, finding significant differences in the removal of suspended solids (SS), NTK, NO_3^-, and TP of 78.4%, 25.7%, 47.8%, and 29.9%, respectively; the concentration of fecal coliform bacteria (FC) decreased in the order of magnitude, and the total biomass increased 4.6 times, recommending the use of this type of wetland for tertiary treatment of wastewater from dairy industries (Schierano et al. 2020).

9.6.3 Vertical Subsurface Flow Constructed Wetland

The vertical subsurface flow constructed wetland (VSFW) is a wastewater and sludge treatment process that is a relatively recent technology (Fig. 9.8) and is still under development. Still, it offers economic, environmental, and social advantages (Stefanakis 2020). The VSFW achieves a high oxygen transfer rate, unlike the horizontal ones, where the nitrification of the wastewater is difficult due to the limitation in the availability of oxygen (Almuktar et al. 2018; Pérez et al. 2022; Stefanakis 2020). This type of wetland allows relatively high volumes of water to be treated per square meter; it is beneficial for the agricultural sector since it is highly efficient in terms of treatment against different pollutants from wastewater, in reducing COD, BOD, and solid particles in wastewater (Almuktar et al. 2018; Pérez et al. 2022). However, they are not very efficient in removing phosphorus due to the insufficient interaction between the wastewater and the system media. Several studies have shown that HSFWs perform well in nitrification, while others indicate their inadequacies in denitrification (Almuktar et al. 2018). This operation can be improved by inserting aeration pipes to favor nitrification and removal of organic matter, compared to HSFW (Pérez et al. 2022; Stefanakis 2020). The VSFW has been used for the removal of ammonium when treating sewage with two

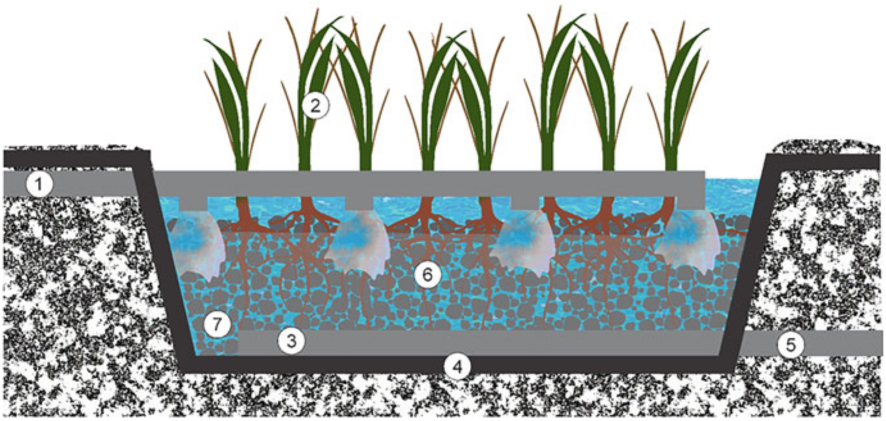

Fig. 9.8 Vertical subsurface flow constructed wetland. (1) Inlet, (2) plants (macrophytes), (3) drenage, (4) liner, (5) rhizome network, (6) outlet, (7) gravel bed

different substrates (gravel and a mixture of gravel and zeolite), finding that the HLR used (3 m day^{-1}) and zeolite produced the removal of a 96.69% of NH_4^+-N through a change in structure and microbial gene expression, promoting nitrification (Zhang et al. 2021). *Typha domingensis* was used to remove ammonium and other contaminants in domestic wastewater in a VSFW to further reuse the treated effluent in a hydroponic culture of *Lactuca sativa*. The wetland was operated for 3 months at a HLR of 83 g COD/m^3 day. The average removal percentages for each parameter were 91%, 64%, 89%, 81%, and 88% for COD, TKN, orthophosphates, NH_4^+, and FC, respectively. The results of the reuse of the treated effluent as a hydroponic culture medium indicate that the effluent from the system allowed the growth of *Lactuca sativa* but in a lower proportion compared to the growth obtained in the conventional hydroponic culture medium (Arias et al. 2021). *Phragmites australis* and *Typha latifolia* were tested in a VSFW as a tertiary treatment for removing emerging contaminants (caffeine, galaxolide, tonalide, parsol MCX, sunscreen UV-15, naproxen, methyl dihydro jasmonate, *alkylphenols, triclosan, alkylphenol mono*-, and diethoxylates); the results showed a high removal of caffeine, which is a compound with high biodegradability, and tonalide, triclosan, sunscreen, and naproxen also presented a high removal, being slightly higher with *Typha latifolia* (Navarro-Frómeta et al. 2020).

9.7 Conclusion

Various biological processes derived from nature and addressed in this work provide an overview of the recent advances in wastewater treatment. Nature can remove pollutants from water, but anthropogenic activities have exceeded their limits by generating enormous amounts of wastewater with high concentrations of biodegradable pollutants, wastewater with recalcitrant, and highly toxic or emerging pollutants. The biological process(es) necessary to remove the pollutants present in the wastewater must be established accordingly to the type of pollutants, the available area for wastewater facilities implementation, the economic resources, the technical support, and the by-products that can be generated (methane, nitrogen, electricity, biomass, etc.), during the process(es). A wide variety of processes, reactors, microorganisms, and plants can be combined to have a good removal percentage. In addition to the factors above, the climate, the landscape, the proportions such as C/N, and the need to meet a certain quality for reuse or discharge of treated water will be very important also.

Advanced processes offer more advantages than conventional processes in terms of saving space, time, and money; this goes hand to hand with the discovery of reactions and metabolic routes of microorganisms and plants since, with the understanding of these, the optimal operating conditions of the reactors will be proposed.

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Recent Developments in Wastewater Treatments

10

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Abstract

Water is a crucial resource for life and daily life activities. Its demand is rising due to rapid population growth, which, coupled with the misuse and contamination of freshwater, has put this resource under growing stress. Its use for daily human life, agricultural, industrial, and energy sector activities results in wastewater that contains different substances depending on each activity. Such substances include organic (sewage), heavy metals, medicinal drugs, colorants, pesticides, bacteria, viruses, etc. Therefore, wastewater sanitation is a crucial activity for the sustainable use of water. Sanitation treatments depend on the contaminant substance(s) and are divided into three main conventional groups named: physical, chemical, and biological treatments. Besides, in recent years, new developments

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have been made for water sanitation. This book chapter summarizes conventional and recent developments, aiming to have a broad perspective on wastewater treatments. A particular emphasis is made on recent developments such as filters based on polymeric membranes, plasma treatment, UV radiation, and electrochemical oxidation. Membranes of polymer nanocomposites increase the flow and prevent fouling; some nanoparticles can even have antibacterial properties. Organic compounds such as pharmaceutical and synthetic dyes and pathogenic bacteria can be removed using plasma treatment. UV radiation helps to reduce wastewater treatment times when photocatalytic materials are used. Finally, it has been reported that electrochemical oxidation removes residues from pharmaceutical industries that cannot be removed with conventional treatments.

Keywords

Wastewater · Pollutants · Treatments · Physical process · Chemical process

10.1 Introduction

Water pollution is a problem that continues to grow every day, causing many aquatic ecosystems around the world to be contaminated. This phenomenon has become an environmental problem reported in the literature. The different human activities of land use, mainly deforestation, urbanization, agricultural activities, etc., are the leading causes of contamination of surface water systems.

Why is it essential to treat wastewater, and in what scenario can this process be applied? Currently, drinking water is an essential resource. However, only 30% of existing water is suitable for human consumption. Therefore, the use of this element must be conscious and sustainable. In this sense, once used for the first time, drinking water automatically becomes wastewater. However, this does not mean it should be lost entirely because various treatments allow it to become a potential resource again. Therefore, residual water contains impurities, from human waste and has chemical and biological components. This waste can be categorized as domestic, industrial, and urban.

According to UNESCO, 80% of wastewater returns to the ecosystem without any treatment, increasing the risk of consuming contaminated water.

Why is it important to treat wastewater? Due to the scarcity of this particular element. Wastewater, previously treated, allows its reuse and favors the circulation of flows in various industry and agriculture sectors. This way, a sustainable water resource with a reduction in the water footprint is obtained. Furthermore, this wastewater treatment is an implementation opportunity for different organizations and brings significant benefits to the ecosystem, such as the following:

- The reuse of water for industrial purposes.
- It becomes an excellent resource for agricultural irrigation.
- Solid waste can be used as fertilizer.

Regarding the industry, they are significant consumers of water. On a global scale, they consume ~22% of the total water produced, while in high-income countries, it can be as much as 60%. It is estimated that by 2050, manufacturing industries could increase their water consumption by 400%. Aqueous discharges in various industrial steps, like cooling towers, boiler heating, purification, etc., can contain numerous suspended or dissolved contaminants, and these effluents are called industrial wastewater. Industries such as chemical and petrochemical, paper and pulp, food processing, tannery, and other manufacturing industries constitute the primary sources of industrial wastewater. These wastewaters usually have a high organic compound concentration, non-neutral pH, different temperatures, salinity, turbidity, and a high content of heavy metals (Dash et al. 2021; Sahu et al. 2022). Wastewater from leather manufacturing, food processing and preservation, textile processing, and petroleum refining can have a high salt concentration. The wastewater composition varies according to the chemical products used in the previous processes and the nature of the treatment they have been subjected to; therefore, classifying industrial wastewater into specific categories is challenging (Ahmed et al. 2022; Prakash et al. 2022; Ranjan et al. 2022).

Conventional management for wastewater treatment includes chemical precipitation, flotation, and ion exchange. However, these processes have several drawbacks, such as low removal efficiency, high energy consumption, and the generation of toxic sludge, which limit their widespread application. Recently, several alternative treatment methods have been investigated to improve the quality of the treated effluent. As a result, different treatment technologies have been developed, such as coagulation-flocculation, adsorption, membrane filtration, reverse osmosis, advanced oxidation, biological processes (activated sludge, anaerobic-aerobic treatment, and membrane bioreactors), electro-technologies, and photocatalytic processes (Singh et al. 2021; Debbarma et al. 2021; Suyal and Soni 2022). Therefore, knowledge of the processes and their effects is fundamental to making a proper plan to address this challenge (Ceretta et al. 2021; Ahmed et al. 2022).

The scientific community has developed various ecological and low-cost nanomaterials for wastewater treatment. These materials are designed with specific unique functional and surface characteristics to purify polluted water. Furthermore, these nanomaterials were also used in the degradation of industrial effluents. In recent decades, nanoparticles have been studied for their ability as adsorbents and tested on a broad spectrum of contaminants (Jayakaran et al. 2019).

10.2 Conventional Methods of Water Treatment

10.2.1 Physical Processes

10.2.1.1 Filtration

One of the most widely used processes for the treatment of contaminated water is filtration, which consists of passing a gas or a liquid through a filter to separate the matter in suspension, because it is a simple, economical, and with high percentages of contaminant removal. Water treatment technology, especially filtration, is one of the main processes of water treatment systems in the world; it has thousands of years of development history (Thuptimdang et al. 2021).

The bank filtration method involves locating pumping wells near a surface spring. The pumping action causes a hydraulic head differential between the surface spring and the groundwater table, further inducing water flow through the porous medium to the producing well. In addition, several physicochemical and biological processes occur due to percolation, which attenuates the pollutants in the body of water. Therefore, the interface between surface and groundwater plays a fundamental role in pollutant attenuation processes, presenting intense activities and functioning as a biogeochemical reactor. Finally, the water derived from bank filtration is a mixture of the infiltrated water from the surface spring and the groundwater in the aquifer (Freitas et al. 2017). As a result, the removal of diclofenac has been successfully achieved. However, complementary treatment techniques are required to achieve 100% removal of this compound (de Carvalho Filho et al. 2022).

Another method that has been used is constructing wetlands, which are resilient, cost-effective, and environmentally friendly eco-technology and are an alternative option for heavy metal removal (Kataki et al. 2021). They are significant for developing countries, especially those seeking green economic development. With extensive research on this technology in the last decade, the specific removal mechanisms, such as adsorption, sedimentation, filtration, precipitation, coprecipitation, plant uptake, and microbial-mediated processes responsible for heavy metal removal, have been gradually revealed. The removal performance is highly dependent on the type of substrate, plant species, and microbial activity, with extensive interactive effects (Yu et al. 2022).

Moreover, compared with traditional mechanical filtration, biological filtration can significantly improve the removal efficiency of pollutants due to biological effects. It is also essential for biological water treatment in many industrialized and developed countries. It is often used to treat municipal wastewater, aquaculture wastewater, leachate from landfills, and dyeing wastewater (Li et al. 2016; Xiao et al. 2019). Biological filtration can be optimized for the removal of various types of contaminants. The main types of biological filtration developed or applied include aerated biological filters, denitrification biofilters, constructed wetlands, etc. Although biofiltration systems are widely used, more than existing studies are needed to explain the intrinsic mechanism of biological filtration operation clearly and on the microorganisms involved (Jin et al. 2023).

Carbon and its derivatives have been successfully used to purify polluted water. Activated carbon (AC) is one of the most common commercial adsorbents, including a wide range of processed amorphous carbon-based materials with high porosity and surface area. The AC preparation process consists of carbonizing the carbonaceous raw material and activating the carbonized product. Activated carbons can be used in a few forms: powdered, granulated, and fibrous. Granular activated carbon (GAC) is an excellent adsorbent for various contaminants and has been used in abundant applications in water and wastewater treatment (Benstoem et al. 2017).

In addition to adsorption capacity, biological growth on GAC during water and wastewater treatment is also an expected consequence due to the favorable environment provided by this material. The biomass attached to the GAC can carry out the biodegradation of compounds. Biologically activated carbon (BAC) filtration has been used for drinking water; the removal of organic matter, especially the biodegradable fraction responsible for forming by-products of disinfection processes and microbiological regrowth within the systems water distribution; removal of micropollutants such as pesticides, algae toxins, and substances accountable for causing flavor and occurrence of nitrification-denitrification; and essential processes for the removal of ammonia that can cause environmental problems and risks to public health (dos Santos and Daniel 2020).

10.2.1.2 Adsorption

The adsorption process, also known as the surface phenomenon, has some advantages, such as simple design, lower capital cost, and lower operating cost. Recently, there has been a search for low-cost adsorbents that can be produced using agricultural waste, industrial waste, and natural materials. The adsorbent selection depends mainly on low production cost, adsorption capacity, regeneration properties, high surface area, and pore volumes. During the adsorption process, the solute will deposit on the surface of the adsorbent due to attractive forces. Eventually, it can form a monolayer or multilayer, depending on the experimental results. Recent investigations have shown that the adsorption process is spontaneous and requires a low temperature, and the adsorbate concentration will increase (on the adsorbent surface) over more extended periods (Yagub et al. 2014). Therefore, the adsorption process can be classified into two groups: physical and chemical adsorption.

The equilibrium adsorption capacity (q_e, mmol/g) can be determined based on the following equation (Eq. (10.1)).

$$q_e = \frac{V(C_0 - C_e)}{M} \tag{10.1}$$

where V, M, C_o, and C_e represent the volume (L), the mass of adsorbent (g), the initial adsorbate concentration (mmol/L), and the equilibrium adsorbate concentration (mmol/L), respectively.

The Langmuir model and the Freundlich model can be used to describe the adsorption data. The adsorption isotherm explains the amount of solute adsorbed on the surface of the adsorbent under constant temperature, the adsorption capacity of the monolayer, and the heterogeneous surface.

The adsorption process can be represented using the Langmuir isotherm (Eq. (10.2)) and Freundlich model (Eq. (10.3)), respectively.

$$\frac{C_e}{q_e} = \frac{1}{(b)X_m} + \frac{C_e}{X_m} \tag{10.2}$$

where Ce, X_m, and b represent the equilibrium adsorbate concentration (mmol/L), theoretical maximum adsorption capacity (mmol/g), and constant value (L/mmol), respectively.

$$\text{Log}q_e = \text{Log}k + \frac{1}{nLogC_e} \tag{10.3}$$

where Ce, k, and $1/n$ represent equilibrium adsorbate concentration (mmol/L), constant value (mmol/g), and constant value, respectively.

Activated carbon (AC) can be synthesized from any carbonaceous material (agricultural residues, different parts of plants, biomass) that contains high amounts of carbon and less inorganic content. The obtained activated carbon (powdered, granular, fiber, and extruded form) showed high surface area and porosity structure and can be used to remove contaminants from aqueous solutions. Granular activated carbon can be used in the food and beverage industry, wastewater treatment, and air and gas purification. There is great demand for the granular form due to its simple regeneration and reuse. On the other hand, powdered activated carbon showed a higher surface-volume ratio with a particle size of less than 0.177 mm. Therefore, it can eliminate unwanted odors, flavors, and colors. In general, activated carbon can be classified into three groups, namely, microporous (less than 2 nm), mesoporous (2–50 nm), and macroporous (greater than 50 nm), based on the pore size according to the IUPAC (Reza et al. 2020).

In addition to activated carbon and carbon derivatives, other particles have been used as adsorbents, among which are zeolites, nanoclays, and metal oxides, against a wide variety of contaminants, including heavy metals, due to their large surface area and pore volume, as described in Table 10.1.

10.2.2 Chemical Processes

10.2.2.1 Coagulation-Flocculation

Coagulation-flocculation is widely used in the post- and pretreatment of domestic or industrial water. This technique comprises two stages: in the first, the coagulant is added by dispersing it in the residual water with rapid agitation; in the second stage, the flocculant is added, whose function is the agglomeration of small particles that are dissolved in the residual water, and finally, the flocs settle creating a sludge that is removed, and the supernatant is transferred to the subsequent treatment; one of the disadvantages of this process is the production of sludge (Golob et al. 2005; Teh et al. 2016).

This process has been used to treat leachate from sanitary landfills considered highly contaminated water, studying the type and dose of coagulant, the effect of pH, and the optimal treatment conditions. Fresh leachates have shown low pH and high

Table 10.1 Adsorbents used in the removal of different contaminants

Adsorbent	Pollutant	Test conditions	Removal/ adsorption capacity	Reference
Zeolite	Brilliant blue	pH = 3–5	87%	Pinedo-Hernández et al. (2019)
Activated carbon	Congo red	pH = 2, T = 25 °C	0.47 mg/g	Litefti et al. (2019)
Activated carbon	Reactivate red 198	pH = 2, T = 25 °C	253 mg/g	Mahvi et al. (2014)
Zeolite	Disperse orange 25	–	125 mg/g	Markandeya et al. (2021)
Chitosan/multi-walled carbon nanotube	Direct blue 71	pH 6.25	29 mg/g	Abbasi and Habibi (2016)
Multi-walled carbon nanotube	Maxilon Blue 5G	–	260 mg/g	Alkaim et al. (2015)
Multi-walled carbon nanotube	Malachite green	–	142 mg/g	Shirmardi et al. (2013)
Magnetic zeolite	Crystal purple	pH = 10.3, T = 50 °C, time = 45 min	98%	Shirani et al. (2014)
Zeolite 4A	Methylene blue	Time 180 min	99%	Belachew and Hinsene (2022)
Activated carbon	Rhodamine B	pH = 3	5.6 mg/g	Mousavi et al. (2021)
Zeolite	Rhodamine B	T = 30 °C	7.9 mg/g	Cheng et al. (2016)
NaY zeolite	Phenol	Time 120 min, pH = 4.0	77%	Ba Mohammed et al. (2021)
$FeCl_3$ zeolite	Phenol	Time 100 min, pH = 3.0	65–95%	Ebrahimi et al. (2013)
Coconut activated carbon	Phenol	–	13–24 mg/g	Ma et al. (2013)
Natural montmorillonite	Ametryn	–	188 mg/g	Shattar et al. (2017)
Commercial activated carbon	Carbofuran	–	96 mg/g	Salman and Hameed (2010)
Clay	Carbaryl	Time 120 min, T = 20 °C, pH = 5.1	2.2 mg/g	Ouardi et al. (2013)
N-doped biochar	Pb^{2+}	pH = 2–7	214 mg/g	Jiang et al. (2019)
N-doped biochar	Cr(VI)	pH = 2	100 mg/g	Guo et al. (2019)
N-doped biochar	Ni (II)	–	44 mg/g	Yin et al. (2019)
N-doped biochar	Cd^{2+}	pH = 6	197 mg/g	Yu et al. (2018)
N-doped biochar	Cu^{2+}	pH = 6	104 mg/g	Yu et al. (2018)

(continued)

Table 10.1 (continued)

Adsorbent	Pollutant	Test conditions	Removal/ adsorption capacity	Reference
ZnO	Pb^{2+}	pH = 6.5, time = 30 min, T = 25 °C	434 mg/g	Yin et al. (2018)
ZnO	Cd^{2+}	pH = 7, time = 120 min, T = 55 °C	217 mg/g	Khezami et al. (2017)
ZnO	Hg^{2+}	pH = 7, time = 120 min, T = 55 °C	714 mg/g	Sheela et al. (2012)
ZnO	As(III)	pH = 7, time = 105 min, T = 50 °C	52 mg/g	Yuvaraja et al. (2018)
ZnO	Cr(VI)	pH = 2, time = 90 min, T = 50 °C	12.2 mg/g	Pandey and Tripathi (2017)
γ- MnOOH	Cu(II)	pH = 5, T = 40 °C, time = 120 min	11.9 mg/g	Cano-Salazar et al. (2020)
PO_4/CO_3 composite	Tetracycline	pH = 6.5, time = 360 min	118 mg/g	Yukhajon et al. (2023)
Carbon nanotubes	Ciprofloxacin/ Indigo carmine	pH = 4–6, T = 25–50 °C	95 mg/g, 93 mg/g	Elamin et al. (2023)
rGO / nZVI	Doxycycline	pH = 7, T = 25 °C	31.6 mg/g	Abdelfatah et al. (2022)
NH_2-MIL-101 (Al/Chitosan)	Azithromycin	pH = 7.9, time = 64 min	238 mg/g	Azari et al. (2022)

chemical oxygen demand (COD) content, while partially stabilized leachates were characterized by high pH and low COD (Tatsi et al. 2003). Because of this, coagulation-flocculation has been proposed as a pretreatment method for fresh leachates or as a post-treatment process for partially stabilized leachates. Previous studies report using coagulants and flocculants in leachate from landfills to reduce turbidity and membrane fouling (Marañón et al. 2008).

On the other hand, commonly used coagulants and flocculants can cause an increase in the concentration of metals in water, which can have implications for human health, and consequently, the production of large volumes of toxic sludge. As an alternative to this problem, in recent years, biopolymers have been used since they have a less environmental impact and are low cost (Renault et al. 2009). Chitosan is one of the promising biopolymers in the coagulation-flocculation area, and this polymer is used in different effluents from the food industry. Some of the properties that chitosan has that help its application as a coagulant and flocculant are its cationic charge and its ability to bind to certain solids specifically, and it also exhibits good solubility (Roussy et al. 2005a). Roussy et al. studied the effect of the coagulant dose, in this case, chitosan, and the effect of the pH. These authors found that at pH 5, the protonation of the amino groups led to the coagulation effect. For this study, low amounts of biopolymer were needed to achieve the decantation of colloids (Roussy et al. 2005b). Some naturally based flocculants include biopolymers such as starch, chitin, pectin, and cellulose derivatives. The latter has been little studied; one method to produce cellulose flocculants is introducing aldehyde functionalities by aqueous oxidation of periodate. Chemical modification of cellulose with functional groups of carboxylic acids, imines, and aldehydes gives good performance in water-soluble colloids (Suopajärvi et al. 2013).

Coagulation-flocculation is a process widely used in treatment plants as a pretreatment to eliminate contaminants from water. However, the new coagulants and flocculants must be developed with high sludge dehydration characteristics; as mentioned above, it is a by-product of the process and represents a disadvantage to the method; besides, it must be viable at an industrial level and be environmentally friendly (Wei et al. 2018).

10.2.2.2 Disinfection

Water is a primary means of spreading diseases since it contains various pathogenic microorganisms, including bacteria, viruses, intestinal protozoa, and other microorganisms. The disinfection process is the elimination of all microorganisms present in the water through different techniques such as the following:

- Physical treatment (the application of heat or physical agents)
- Radiation (ultraviolet)
- Metal ions (silver or copper)
- Chemicals (surfactants)
- Oxidants (chlorine, chlorine dioxide, ozone, potassium permanganate)

The most widely used technique is with oxidants, since it can be scaled to an industrial level in a treatment plant and eliminates most pathogens. However, the disadvantage of this technique is the production of by-products that can become potentially toxic. In this way, another compound is added to pre-treat the water sample and not generate by-products; Fe(VI) pre-oxidizes the organics in the water to control the formation of by-products when chlorine is added (Liu et al. 2020).

The recent development of new disinfection technologies focused on attacking a wide variety of pathogens is based on advanced oxidation processes characterized by the generation of highly reactive oxidants, particularly hydroxyl (OH) species. Among these advanced oxidation processes are the Fenton reaction, which uses a mixture of iron ions and hydrogen peroxide that generates OH species under mild acid conditions (García-Fernández et al. 2019; García-Espinoza et al. 2021).

10.2.3 Biological Processes

10.2.3.1 Anaerobe

In recent years, municipal wastewater has been considered a source of nutrients, energy, and water. Anaerobic digestion represents an alternative for managing activated sludge contained in sewage. It is characterized by converting organic matter to methane and CO_2 in the absence of oxygen and interacting with different bacterial populations that have to be anaerobic (Vinardell et al. 2020). It has different stages of hydrolysis, acidification, acetogenesis, and methanogenesis. The microorganisms present can convert the sludge into biogas and reduce its volume (Zhang et al. 2020). One of the limitations of this biological process is the low methane production in the methanogenesis stage.

Sun et al. studied the effect of incorporating conductive materials based on carbon and iron to improve the methanogenesis process; the total methane production increased by 9.38%. In addition, conductivity measurements and concentrations of conductive particles indicated that the particles accelerate the metabolism of microorganisms and promote methane production (Sun et al. 2021).

The anaerobic oxic sedimentation process reduces sludge and is dependent on biological enzymatic activity. Xu et al. conducted a study based on an anaerobic oxic sedimentation process for 168 days for the treatment of wastewater from the textile industry, and the results indicated that the process could increase the sludge reduction performance and improve the sludge discoloration rate, with an efficiency of 90% (Xu et al. 2021).

Anaerobic digestion for producing biomethane from anaerobic sludge from wastewater treatment presents some drawbacks, such as the difficulty of cell lysis of the sludge, which entails a long time. Due to this, pretreatments are carried out on the sludge. In recent years, cavitation has been proposed as an emerging technology that helps biological processes. Some of the advantages of this pretreatment are time, greater enzymatic digestibility, and removal of organic contaminants; cavitation can be hydrodynamic or acoustic with the use of ultrasound (Bhat and Gogate 2021).

10.2.3.2 Aerobic

Biological aerobic treatments are applied to convert organic and inorganic compounds in wastewater in the presence of oxygen. Aerobic treatment plants are classified into three generations:

- The first that is mechanical.
- The second consists of large tanks with several thousand cubic meters in which flow conditions are poorly controlled and oxygen transfer is poor.
- Third-generation plants contain bioreactors and sedimentation tanks and have several advantages such as small volumes, closed, emission-proof, high performance, flexibility, no scaling problems, and high utilization of oxygen content in the air (Gavrilescu and Macoveanu 1999).

Zeolite improves the aerobic process and helps nitrogen removal, sludge sedimentation, and biomass retention, capable of removing phosphorus. It is also used to immobilize microorganisms, such as the bacteria *Acinetobacter junii*, which allows a suitable microenvironment for the growth and survival of pure microorganisms. In addition, it improves membrane bioreactors to reduce membrane fouling (Montalvo et al. 2020).

On the other hand, aeration is a critical element in the aerobic treatment process. It provides microorganisms with the dissolved oxygen required, keeps solids in suspension, and controls the fouling of membrane bioreactors. Other advantages of aeration administration through pure oxygen are accelerating enzyme activity, producing less sediment, and minimizing foam formation (Skouteris et al. 2020).

Jiang et al. studied the effect of the concentration of Mg^{2+} in the residual sludge systems for treating residual waters with aniline. The experiment was in two batch reactors of parallel sequencing where Mg^{2+} was placed in only one reactor. They concluded that the high concentration of Mg^{2+} could inhibit the metabolism of aerobic bacteria, and it was also found that some aniline-degrading bacteria decreased, but denitrifying bacteria increased with the presence of Mg^{2+} (Jiang et al. 2020).

10.2.3.3 Enzymatic

Isolated enzymes produce by-products in less quantity and toxicity; the process is environmentally friendly. Enzymes can degrade organic compounds, such as dyes, and offer greater specificity and regulation under certain conditions. They belong to the family of oxidoreductases that catalyze oxidation and reduction reactions, finding applications in the diagnosis and treatment of wastewater (Routoula and Patwardhan 2020).

Enzymes can be administered directly to wastewater, immobilized in a material, or incorporated into a release and dosing device. In recent studies, the use of nanoparticles for enzyme immobilization is considered complex and often requires a surface modification of the enzymes before their integration into nanoparticles. Some disadvantages are the cost of manufacturing, the recovery of the nanoparticles, and the implementation on an industrial scale (Karthik et al. 2021).

The immobilization and the use of enzymatic bioreactors help solve the problems experienced with enzymatic treatment in a water treatment plant, such as enzymatic denaturation and effluent washing. In addition, the enzymatic reactor removes emerging contaminants, such as estrogen, since they facilitate the conversion of estrogens and the purification of the mixture. However, attention should be paid to the porosity of the reactor membrane for effective enzymatic binding and efficient component separation (Zdarta et al. 2022).

10.3 Recent Developments for Water Treatment

10.3.1 Filters Based on Polymeric Membranes

Membranes and filters act as a structure composed of pores that remove contaminants through affinity or size exclusion, allowing the separation of pollutants according to their size and the pore size of the filter or membrane. On the contrary, affinity membranes reject contaminants based on the electrostatic interaction of the functional groups of the pollutants.

Membranes can be classified based on the contaminant size that they can reject. Figure 10.1 shows the classification by size, as well as the contaminants retained in each membrane according to pore size; it is worth mentioning that a smaller pore size

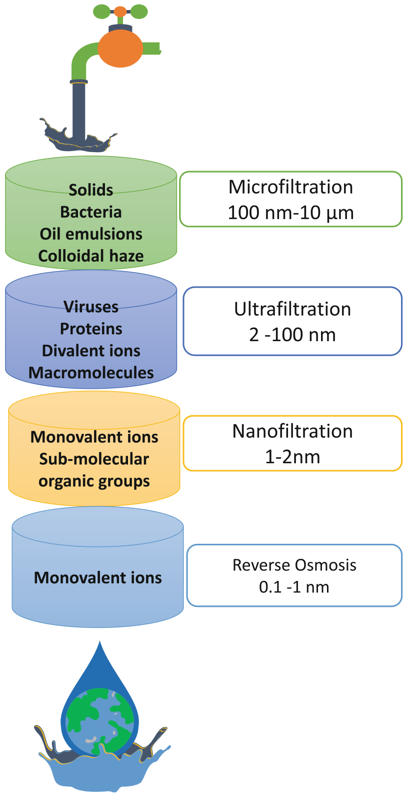

Fig. 10.1 Type of filtration membranes by size and type of rejected contaminants

requires a higher pressure needed to force the water through the filter or membrane (Mautner 2020).

Membranes based on natural polymers such as cellulose were among the first used to separate solids. Then in recent years, membranes based on nanocellulose have emerged that help to improve separation due to their size. One of the characteristics of these membranes is that they are made entirely from renewable resources and are biodegradable compared to composite membranes and synthetic membranes. The preparation of nanocellulose membranes has been done by the solvent evaporation method that results in films (Kontturi et al. 2006; Metreveli et al. 2014).

Membranes of synthetic origin have been used due to their excellent thermal and chemical stability, control of the pore formation mechanism, and low cost. Polymers used for the manufacture of membranes for wastewater treatment are polyamide, polyester, poly(acrylonitrile), polyurethane, poly(tetrafluoroethylene), and polysulfone (Hamid et al. 2011; Bhol et al. 2021).

Polysulfone is one of the polymers that stands out due to its mechanical and temperature resistance. Therefore, membranes based on polysulfone and nanoparticles have been developed to cover defects that the membranes may have,

Table 10.2 Polymer composite membranes and pollutants removed

Nanoparticle	Polymer	Method	Pollutant	Reference
γ-Al_2O_3	Polyamide	Interfacial polymerization	Polyvalent cations such as Mg^{2+}	Hassan et al. (2022)
Titania nanotube	Polyamide	Interfacial polymerization	Endocrine-disrupting compound	Ahmad et al. (2022)
Graphene oxide, Au	Polyamide 6	Nonsolvent-induced phase separation	Desalination and sewage treatment	Xu et al. (2022)
Silica	Polyether–polysulfone	Interfacial polymerization	Heavy metal	Al-Gamal et al. (2022)
Zeolite	Polysulfone Chitosan	Phase inversion	Dyes	Gowriboy et al. (2022)
TiO_2 –SiO_2	Polyether–Polysulfone	Vapor-ventilated in situ chemical deposition	–	Gan et al. (2022)
ZnO	Polysulfone–polyurethane	Phase inversion	Dyes	Kalaivizhi et al. (2022)

such as high hydrophobicity, pore blockages, and low resistance (Esfahani et al. 2015; Yadav et al. 2019).

Membranes composed of polymers and nanoparticles receive more and more attention for wastewater treatment; the inorganic nanostructures used are ZnO, TiO_2, SiO_2, and ZrO_2. These particles mainly increase the flow and prevent fouling; also, some nanoparticles have antibacterial properties for eliminating pathogenic bacteria in wastewater (Sheikh et al. 2020).

The incorporation of ZnO and SiO_2 into a polyethersulfone membrane to separate water and oil has been reported. The SiO_2 membrane had better organic resistance and 25% more permeate flux than the polyethersulfone membrane with ZnO (Ismail et al. 2020). Table 10.2 shows the composite membranes with nanoparticles and the pollutants they remove.

10.3.1.1 Plasma Treatment

The use of plasma has grown for the removal and mineralization of polluting organic compounds (pharmaceuticals and synthetic dyes) and pathogenic bacteria in wastewater in recent years. Plasma technology consists of an electric field source with highly charged particles that generate oxidizing species, reducing species, and electrons (Zeghioud et al. 2020). Plasma in contact with water is part of an advanced oxidation process since it generates OH ions from water molecules through the impact of dissociation electrons and radical reactions. There are different plasma treatments, serpentine-shaped discharge plasma on a solution with argon bubbling, plasma generated within gas bubbles, radiofrequency plasma, and plasma sliding arc, among others (Takeuchi and Yasuoka 2021).

Cold or non-thermal plasma is another type of plasma that is generated where the electrons have a higher temperature than the heavier species. With the passage of energy, the gas dissociates into several reactive species, followed by ionization. This method achieves a more energy-efficient decomposition compared to others. This technique has been used for the decontamination of various harmful elements such as medicinal products, organic dyes, pesticides, herbicides, biomolecules, phenolic compounds, and antibiotic substances. It also decreases chemical oxygen demand and pathogenic bacteria and viruses (Gururani et al. 2021).

Rashid et al. used an air discharge plasma at atmospheric pressure from multiple parallel tubes underwater to decompose residues of dyes from the textile industry, such as remazol blue, red, and yellow. They observed that as the incidence time increased, the percentage of discoloration increased, removing 70% of the dyes. Therefore, the plasma technique is considered an alternative ecological way of degrading dyes from the textile industry (Rashid et al. 2020).

Hafeez et al. fabricated a hybrid plasma and ozone reactor by connecting six hybrid plasma reactors in parallel to create a hybrid plasma microreactor system for ozone generation. The efficiency of this hybrid reactor was evaluated for its application in the degradation or elimination of the methyl orange dye. In addition, the parameters of the effect of pH, concentration, and solution volume were studied, achieving the elimination of 86% of the dye at pH = 3 with a concentration of 10 ppm and 900 mL of solution in 8 min in the reactors (Hafeez et al. 2021).

Moreover, non-thermal plasma treatments have been used to eliminate the growth of bacteria, with an antibacterial and sterilizing effect. They could surpass conventional methods because it is low cost, it is not necessary to add chemicals, and it is considered a clean energy (Ekanayake et al. 2021).

10.3.1.2 UV Radiation

Ultraviolet radiation has been used as a water disinfectant to eliminate pathogenic microorganisms from different water effluents. However, the UV irradiation process ranges from a wavelength of 315–400 nm, which causes oxidative damage to cells. This radiation can be direct or indirect using photocatalytic materials that absorb radiation and carry out a process of generation of reactive oxygen species (ROS) (Wang et al. 2020).

Using UV integrated with H_2, O_2, and TiO_2 helps to demobilize pathogenic microorganisms; when UV radiation is contrasted with photocatalytic materials, it helps to reduce wastewater treatment times. On the other hand, TiO_2 promotes the inactivation of the growth of microorganisms via photocatalysis, generating internal cell destruction generated by the actions of ROS species, causing physical destruction followed by an oxidative attack of hydroxyl radicals on cell membranes, inducing oxidative stress and loss of permeability (Ghernaout 2020). A disadvantage of this photocatalyst is that the recycling and recovery of these suspended particles become a costly problem; as an option, supports for these particles have been developed (Garrido-Cardenas et al. 2019).

Salazar et al. functionalized the TiO_2 surface with silver nanoparticles and prepared membranes of electrospun poly(vinylidene-co-hexafluoropropylene fluoride) as support with different concentrations of TiO_2 alone and functionalized with silver. The application of this membrane was in the degradation of norflaxin, an emerging pollutant present in wastewater and rivers polluted with pharmaceutical industries, and the degradation of this pollutant was carried out under UV and visible radiation. The nanocomposites showed a degradation efficiency of 64.2% under UV radiation, contrasting with the visible 80.7% during 90 and 300 min. In addition, the membranes demonstrated recyclability and application to remove an emerging contaminant (Salazar et al. 2020).

Andrade et al. developed a nonwoven fabric membrane with photocatalytic ZnO, nanoparticles manufactured by melt blowing, with different concentrations of nanoparticles. The methylene blue adsorption efficiency was 93% in a time of 60 min. It is worth mentioning that the nonwoven fabric presented antimicrobial activity against two strains, *Staphylococcus aureous* and *Candida albicans* (Andrade-Guel et al. 2021).

The photocatalysts are activated by UV radiation, producing highly reactive photoinduced charge carriers that react with contaminants. This technology would help solve problems of high energy consumption in traditional methods, such as high temperature and pressure required for water decontamination. There is a technique that has attracted attention in recent years. It is based on a photocatalytic process called Fenton and produces reactive oxygen species (ROS). It includes three reaction steps: initiation, propagation, and termination reactions. It effectively eliminates persistent organic compounds, bacteria, and viruses (Liu et al. 2020).

10.3.1.3 Electrochemical Oxidation

Electroxidation is one of the most researched techniques today because it can mineralize different polluting organic compounds. The most widely used anodes are mixed metal oxides based on SnO_2 and PbO_2 coatings, boron-doped diamond anodes, and titanium suboxide ceramic anodes (Radjenovic et al. 2020).

Oxidation processes are based on passing an electric current through wastewater with the help of electrodes, creating oxidizing agents such as hydroxyl radicals and chloride that can oxidize ammonia. The cost associated with this process lies in the electric current or energy consumed compared to other methods. However, the cost of operation is low, and it can destroy persistent organic pollutants (Almomani et al. 2020).

Wang et al. reported the electrochemical oxidation in pulsed current mode to treat wastewater with dyes such as indigo and methyl orange with a PbO_2/Ti anode. In addition, the parameters that influence the degradation of dyes were determined: the current density, pulse duty cycle, and flow rate for dye degradation (Wang et al. 2020).

Electrochemical oxidation has been used to treat wastewater containing residues from the pharmaceutical industries that cannot be removed with conventional methods. For example, the electrochemical oxidation of the antibiotic sulfadiazine has been investigated using a titanium suboxide mesh anode; the results revealed that the optimal time for electrolysis is 60 min with 100% degradation. Furthermore, the anode exhibited oxygen evolution potential for OH radical formation and long-term stability (Teng et al. 2020).

10.4 Conclusions

The growing increase in the world population, as well as the intensive use by the manufacturing industry and various agro-industrial activities, has generated, to a great extent, the contamination of fresh water and the bodies that contain it. The use of water for all the daily activities of human beings is unavoidable, for which the development of new technologies that reduce the water footprint and lead to more efficient treatments for its reuse is vital.

The different primary treatments have shown a trend toward the development of new and improved processes for wastewater treatment, emphasizing physical and biological processes. Furthermore, with the increasing progress in developing low-cost adsorbents from renewable and natural sources, viable alternatives have been proposed to remove pesticides, heavy metals, medicines, and pigments, which can be implemented in sewage treatment plants (Kumar et al. 2021). However, it is known that the persistence of these contaminants in water can cause various adverse health effects. Their bioaccumulation can lead to new and more aggressive forms of cancer, chronic renal failure, metabolic syndrome, and others, for which there is still no effective treatment.

Biological processes have shown significant advances, with increases in biogas production as a by-product; however, sludge production is still a considerable challenge. Possibly, the participation of consortia of microorganisms such as bacteria, fungi, and enzymes, which have shown to be effective for the elimination of different heavy metals, could be of great help for the generation of fertilizers.

For their part, advanced processes for wastewater treatment represent viable alternatives for their implementation in wastewater treatment plants. Moreover, since it has been possible to develop more functional materials, lower cost, and higher profitability, the joint use of some of these techniques would lead to obtaining fresh water suitable for human consumption.

Although the extraordinary efforts of the scientific community to develop new techniques for the treatment of wastewater, one of the main challenges to overcome is the awareness of the population to make a more rational use of the precious liquid. Without a behavioral change, the human being will continue contributing to water pollution, reducing its availability for future generations.

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Wastewater Treatment: Perspective and Advancements

11

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Abstract

Water is a basic necessity on which the human life dwells. Water pollution through human activities is making it unfit for its legitimate use. This makes a huge impact on the environment as well as public health bearing considerable economic and social costs. There are numerous ways of water pollution among which the industrial waste and agricultural pollution, e.g. chemical fertilizers and pesticides, are major contributors. The water pollution negatively affects the declining water table and levels in major water bodies. There is a pressing need for efficient as well as cost-effective wastewater treatment strategies and processes to make the clean water available for domestic and industrial use. In this chapter, we are going to discuss about the developments in wastewater treatment like microalgae, membrane-based nanofiltration, and photocatalysis-based treatment of wastewater. The principle, application, and their effectiveness toward remediation of wastewater to meet the reusable water standards will also be addressed. The processes though conventional are emerging as effective ways to industrialize wastewater treatment with certain modifications and combinations in reactors and the material used for treatment. The design of reactors for these wastewater treatment technologies has also been discussed. Wastewater treatment via microalgae bioremediation has proven to be very cost efficient as it generates biomass, which can be further used in different biological processes. Nanofiltration and photocatalysis have the advantage of minimum contamination,

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and they can be optimized for better removal of pollutants from wastewater. These emerging wastewater treatment technologies can form an outline for development of better processes for wastewater remediation and can play an important role in water conservation through reuse and recycle of treated wastewater.

Keywords

Water pollution · Wastewater treatment · Nanofiltration · Photocatalysis · Microalgae

11.1 Introduction

11.1.1 Water: Basic Source of Life Sustainability

Water is a basic need and resource for human life sustainability and survivability. There is a total of 1400 million km^3 of water on earth. However, only a fraction of this total water is available for human use. The world population doubles in almost every 30 years making the demand for fresh water stratospheric. The availability of water per annual capita is decreasing rapidly. It was about 3300 m^3 in 1960 and then decreased to 1250 m^3 in 1995. It has been estimated that it will further decline by about 50%, leaving only 650 m^3 by 2025. In most of the countries, 70–90% of the available water is used in agriculture (Abdel-Raouf et al. 2012; FAO 2014). It has been documented earlier that though the population of the world is increasing threefold, the water consumption has skyrocketed at sixfold creating an alarming situation. It has been predicted that by 2050 about 40% of the world population will be living in severe water-deficit areas (Leflaive et al. 2012). A normal person requires about 20 l of water per day for drinking, cooking, and washing. However, there are regions where people have less than a liter per person for these purposes. In India, only 20% of the population has access to tap water (Shagun 2019). This precious resource is being converted to waste through human activities including industrial technologies and domestic purposes. Other major factors such as population growth and density, industrialization with nonrenewable sources of water, dwindling economic situation, and institutionalization of majority of human activities also contribute toward water scarcity.

11.1.2 Water Pollution

Water pollution is one of the major reasons behind the declining levels of water resources and scarcity of water that humans are experiencing today. Pollution is one of the worst vices of man-made inventions and industries. All these human activities are releasing a lot of natural and nonnatural, organic, and inorganic compounds into the environment leading to pollution. Water pollution not only affects human health

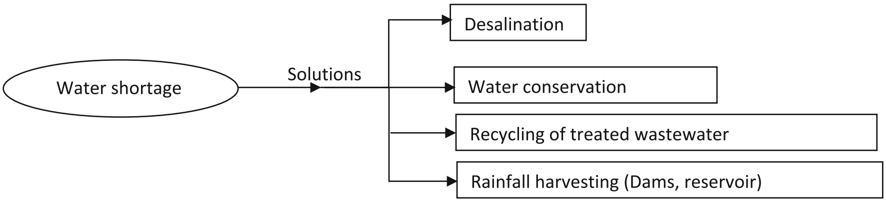

Fig. 11.1 Flowchart depicting solutions to problem of water shortage

and ecosystem negatively but also makes the freshwater resources unfit for use. This decreases the availability of water resources, thus creating a situation of water scarcity (Fig. 11.1). The problem of water shortage has few solutions like desalination, water conservation efforts, recycling and reuse of wastewater, and rainfall conservation projects (Chauhan and Kumar 2020; Vedavyasan 2007; Xie et al. 2009). The current desalination capacity across the globe is 97.4 million cubic meters per day (m^3/day). As of 30 June 2018, more than 20,000 desalination plants had been contracted around the world.

11.1.3 Clean Water and Sanitation: An Important Sustainable Development Goal

In India, lack of proper sanitation results in a lot of human wastewater to be directly released into the water bodies without treatment. In several towns and villages, which are being converted to towns, water supply networks are being setup without proper sanitation facilities like establishment of sewage systems and reforming the existing ones. Though this improves the water supply facility, there is an increase in water pollution and health hazards among the people of these civilizations. The contents that are being released into the water bodies include salts, solid suspension, and pathogens, which together create oxygen deficit in the water.

The wastage of water has a lot of economic as well as health implications. The world is recognizing the efforts that work towards a sustainable and economically reliable treatment of wastewater. Wastewater treatment is a tool, which has the capability to convert this waste to resource with the help of emerging technologies. Proper discharge of wastewater and treatment technologies entails a complicated process with health and economic consequences. Currently, India has the capacity to treat only 37% (approximately 22,693 million l/day) of the sewage waste generated, which is approximately 61,754 million l/day (Sugam et al. 2017). Ineffective wastewater treatment is an example of the inept use of water resources and is contributing negatively to water scarcity our country is facing today. Availability of clean water and proper sanitation is one of the "Sustainable Development Goals" as given by the UN. This goal can be achieved through effective and economic wastewater treatment strategies.

The contents of wastewater are a multifaceted mixture of natural and man-made materials. It consists of both organic and inorganic compounds. Carbohydrates, proteins, fats, and a number of acidic compounds outline the majority of carbon present in the wastewater. The inorganic compounds present in wastewater majorly consist of sodium, calcium, potassium, magnesium, chlorine, sulfur, phosphates, ammonium salts, and heavy metals (Horan 1990; Lim et al. 2010; Tebbutt 1983). Wastewater creates a perfect niche for growth of thousands of microorganisms, which include protozoan, viruses, and bacterial species. Though majority of these microorganisms are harmless, there are pathogenic organisms as well. The pathogenic species in wastewater can cause cholera, typhoid, dysentery, tuberculosis, hepatitis, and many other infections (Henery 1989).

There are numerous types of contaminants in wastewater that need to be removed for making it suitable for reuse. Based on the type of contaminants, the wastewater has been categorized into different classes: domestic wastewater (includes sewage), agricultural wastewater, food-processing wastewater, pharmaceutical wastewater, antibiotic-rich wastewater, academic laboratory wastewater, industrial wastewater (includes textile, packaging, paper, etc.), dairy wastewater, and petroleum sludge. According to the type of contaminants in the wastewater, different remediation and treatment technologies come into play for wastewater treatment.

11.1.4 Conventional Steps of Wastewater Treatment

The treatment of wastewater is a four-step process starting from preliminary treatment followed by primary, secondary, and tertiary treatment processes. Each step is meant to play an important role in removal of pollutants from wastewater. Preliminary treatment involves removal of large solid materials discharged into the water through sewers. These materials can be rags, plastics, wood, heavy stones, fecal discharge, etc., which can block the treatment plant or hamper the functioning of the equipment. These large-sized materials are stopped via bars with a space of about 20–60 mm between them. The stopped material is removed by raking. The grit materials, that is, sand, clay, and pebbles, are removed by decreasing the speed of the wastewater flow. At slow speed, the grit gets collected with large floating material and is removed along with it (Gray 2004; Tebbutt 1983).

The primary treatment of wastewater is meant to remove solids through sedimentation. It includes the passage of wastewater through sedimentation tanks where solids are removed after they settle down via gravity. Almost 70% of solids are removed through this process. However, during primary treatment, the microbiological species removal fluctuates as each microorganism has different sedimentation rate (Sivagurunathan et al. 2015).

The secondary treatment of wastewater includes the removal of biological oxygen demand (BOD). BOD refers to the oxygen needed by microorganisms to convert organic matter into CO_2 and H_2O. The higher BOD suggests that more oxygen is needed by the microorganisms indicating that there is high level of organic matter in the water. BOD is referred to as water quality indicator or water pollution indicator.

BOD is removed by using certain heterotrophic bacteria that metabolize the organic matter for their own growth (Horan 1990). Biological units are used for operation in treatment plants for removal of BOD. Depending upon the mode in which the biological units are utilized, there are majorly two types of reactors:

1. **Fixed film reactors** wherein the microorganisms are used as a biofilm attached to a fixed surface. The organic matter is absorbed onto the surface of the biofilm and gets aerobically metabolized (Sivagurunathan et al. 2015).
2. **Suspension reactors** wherein the microorganisms are suspended or mixed in the wastewater via motorized stirring or air circulation channels. The suspended microorganisms metabolize the organic matter in suspension (Rao and Subrahmanyam 2004).

The tertiary treatment of wastewater helps in the removal of all organic material. It can be achieved by both biological and chemical methods. Tertiary treatment removes excess ammonium, nitrogen, and phosphorous in wastewater (Horan 1990). Though primary and secondary treatment result in a clear effluent, the inorganic and organic content of the effluent can result in eutrophication and degrade the water quality when released into the environment. Nitrogen and phosphorus are generally removed from the wastewater through a number of processes including reverse osmosis, adsorption, ultrafiltration, and biological methods (Oswald 1988).

The primary, secondary, and tertiary treatment of wastewater cannot ensure 100% removal of the contaminant load. There are multiple quaternary treatment processes as well, which are meant to disinfect the water, that is, to eliminate harmful disease-causing microorganisms and remove any heavy metals and other inorganic compounds present in the treated water. Quaternary treatment can also be done via both chemical and biological processes.

Numerous processes are employed in tertiary and quaternary treatment of wastewater. Here are some efficient and economically sustainable wastewater treatment technologies:

1. Reverse osmosis-based membrane filtration (Ben Aim et al. 1993)
2. Nanofiltration (nanoparticles in membrane bioreactor) (Barakat 2011; Choi et al. 2002)
3. Electrodialysis (Caprarescu et al. 2012; Habib et al. 2018; Hell and Lahnsteiner 2002; Lee et al. 2002)
4. Absorption through hydrogels (Bekiari et al. 2008; Ju et al. 2009; Peng et al. 2012)
5. Nanocomposites (Badruddoza et al. 2011; Hasan et al. 2016; Horst et al. 2016; Rajendran et al. 2016; Saravanan et al. 2013)
6. Iron-based nanoparticles (Adegoke and Stenström 2019; Aragaw et al. 2021)
7. Microalgae for bioremediation (Phang 1991; Shi et al. 2007)
8. Ozone treatment (Glaze 1987; Hernández-Leal et al. 2011; Perkowski et al. 1996; Rice 1996)

9. Microbial fuel cells for bioremediation (Logan 2004; Zhang et al. 2016)
10. Photocatalysis (Ren et al. 2021)

In this chapter, we are going to discuss three advanced strategies toward wastewater treatment: (1) microalgae-based bioremediation, (2) nanofiltration, and (3) photocatalysis. We will confer about their principles, reactor design, pollutant removal, and other aspects.

11.2 Microalgae-Based Bioremediation

Microalgae-based treatment is primarily used as a tertiary treatment procedure and has been proposed as a potential secondary treatment process as well. The conventional wastewater treatment methods have certain drawbacks such as large area requirement, huge amount of energy consumption, and heavy monetary burden of operation and maintenance. The microalgae offer an efficient alternative with a lot of advantages.

Microalgae are the micro form of mainly water-based photosynthetic organisms, which cannot be seen with naked eye. Microalgae are found in freshwater resources as well as in saline water sources. They can be categorized as both prokaryotic cyanobacteria (blue green algae) and eukaryotic microorganisms. Microalgae are primarily differentiated through their colors into green algae, red algae, golden algae, and diatoms (Stengel et al. 2011).

Algae are ecologically important organisms as they are major producers of oxygen and food suppliers for the aquatic animals. Biomass obtained through algae production can be used as source of multiple industrial and pharmaceutical products. The principle behind the use of microalgae for wastewater treatment is the use of sunlight and organic matter, inorganic nutrients, and heavy metals from industrial wastewater for algal growth, which in turn produces biomass to be collected and used in further processes (Fig. 11.2) (Oswald et al. 1957).

There are certain factors that affect the nutrient removal from the wastewater and algal growth during the treatment process. These factors include microalgae species and properties of wastewater, that is, N/P ratio, pH, light, and temperature. These factors are optimized for maximum nutrient removal and supreme algal growth via pre-treatment of wastewater, algal acclimatization with wastewater, and blend of

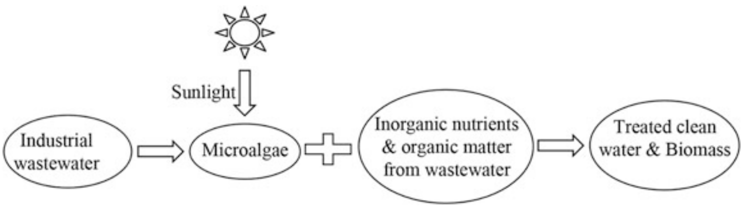

Fig. 11.2 Process of wastewater treatment via microalgae

different wastewater (Cai et al. 2013; Li et al. 2019). There are certain limitations that are posed by the composition of wastewater. High ammonium concentration is one of the major factors that affect the pH of the effluent and the microalgae growth as well. It can have detrimental effects on microalgae diversity in the bioreactor giving rise to resistant species, which have high nitrogen-metabolism-related enzymes. This helps in better detoxification of ammonia in the wastewater (Zhao et al. 2019).

11.2.1 Mechanism of Carbon, Nitrogen, Phosphorus, and Heavy Metal Utilization by Microalgae

Carbon: Microalgae can use both organic carbon, that is, sugars, alcohol, acids, and inorganic carbon, that is, CO_2 and $COOH^-$, which in turn is almost equal to 50% of the biomass that is generated via microalgal growth. Organic carbon is majorly utilized for generation of energy and mass accumulation via glycolysis. Inorganic carbon is mostly consumed through Calvin cycle wherein CO_2 is converted to ATP and NADPH (Singh and Mishra 2019; Uggetti et al. 2018).

Nitrogen: Microalgae requires nitrogen for a number of processes in their growth. It can exploit inorganic nitrogen in the form of ammonia (NH_4), nitrate (NO_3^-), and nitrite (NO_2^-) and organic nitrogen in the form of urea and amino acids. Microalgae produces the basic building blocks of growth, that is, proteins, nucleic acids, and phospholipids via utilization of organic and inorganic nitrogen. Inorganic nitrogen is absorbed through the cell membrane of algae and organic nitrogen is digested through metabolic pathways (Sniffen et al. 2018; Zhu et al. 2013).

Phosphorous: Microalgae consumes both organic and inorganic phosphorus for the production of ATP, nucleic acids, phospholipids, and proteins. It utilizes the inorganic phosphorus in the form of PO_4^{3-}, HPO_3^{2-}, and $H_2PO_4^-$, which is the preferred form of uptake by microalgae. In case of organic phosphorus, it converts it into inorganic form via phosphatase enzyme present on the cell surface and then further utilizes it (Dyhrman et al. 2012; Su 2021).

Heavy metals: Microalgae use certain heavy metals like boron, copper, iron, and zinc for incorporation into enzymatic reactions and cell metabolism processes. Cell wall and membrane of algae have numerous binding sites for heavy metal ions facilitated by large surface area. Primary way of heavy metal removal is biosorption by microalgae wherein the heavy metal ions are transported through cell membrane into the cytoplasm and adhere to the internal binding sites present on the proteins and peptides (Leong and Chang 2020; Monteiro et al. 2012; Singh et al. 2021).

11.2.2 Microalgae Growth and Cultivation System for Wastewater Treatment

There are three modes for microalgae growth and cultivation that can be optimized for maximum biomass production, which in turn gives us treated clean water.

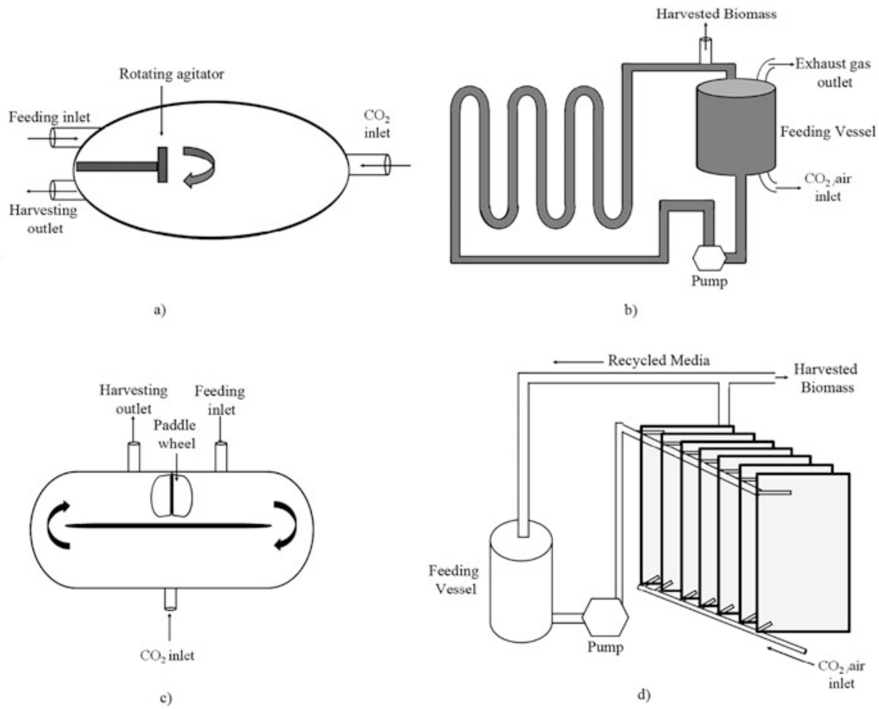

Fig. 11.3 Cultivation systems for microalgae production and wastewater treatment: open cultivation systems, (**a**) circular pond and (**b**) tubular photobioreactor, (**c**) raceway pond; closed cultivation systems, and (**d**) flat plate photobioreactor (Image reference: Faried et al. (2017))

1. Autotrophic: It generally takes place in open ponds where algae utilize natural light sources and inorganic carbon for biomass accumulation.
2. Heterotrophic: Algae cultivation is done in closed chambers where algae utilize organic source of carbon and does not require light for biomass production.
3. Mixotrophic: This mode includes utilization of both organic and inorganic form of carbon by algae in light conditions for maximum biomass production.

For large-scale production of algal biomass and treatment of wastewater, open cultivation system and closed cultivation systems have been designed (Fig. 11.3). Open cultivation system consists of circular ponds and raceway ponds. Both of these systems implement stirring and agitation mechanisms for continuous aeration, suspension of algae, and optimum flow of water. They both are autotrophic cultivation mode with advantage of low-cost construction and operation along with great production area and easy to clean after cultivation. There are certain limitations of open cultivation system, which includes water evaporation, influence of environmental factors, contamination of other microorganisms, and requirement of large area (Lee 2001; Pulz 2001). Requirement of large open area and high evaporation rate through the same is another cause of concern during the microalgae wastewater treatment. An action plan to overcome high evaporation rates includes the usage of deeper ponds and short hydraulic retention time with the limitation of light penetration (Young et al. 2017). Obstruction of light by solids present in the wastewater also impedes the microalgae growth in the reactors. The usage of broad filters as primary treatment to stop the solid material from entering the reactor helps to control the situation and minimize the light obstruction in the reactor. Closed cultivation system consists of vertical tubular photobioreactors and flat plate horizontal photobioreactors. In case of closed bioreactors, the variable conditions can be maintained accordingly, and better results can be obtained with stable parameters. Moreover, there is no contamination through external microorganisms. Though high capital requirement for assembling of a closed photobioreactor remains a concern, the use of wastewater as nutrient and water source for growth of microalgae and recovery of biomass and clean water after the process makes it cost-effective (Gupta et al. 2019; Posten 2009; Ugwu et al. 2008; Wen et al. 2016).

11.3 Nanofiltration

Nanofiltration refers to the process in which membranes, with a pore size of 0.5–2 nm and corresponding molecular weight of 100–1000 Da, are used for wastewater treatment. This process assists in improving the quality of drinking water as it helps in removal of suspended solids, bacteria and viruses, metal ions, and oil particles as well (Guo et al. 2022; Zhao et al. 2021). It is one of the applications of membrane filtration technology with some advanced modifications to achieve better efficiency and lower energy consumption. Certain modes of membrane filtration are size exclusion, ion exchange, and adsorption.

11.3.1 The Design and Specifications of Nanofiltration Unit

In size exclusion mode, the contaminants from wastewater are excluded via the membrane with diameter larger than the pore size of the membrane. The micro-/ ultrafiltration techniques are unable to remove heavy metal ions and microorganisms such as viruses. Nanofiltration provided a solution with a smaller pore size, thereby helping in removal of heavy metal ions, viruses, and other pollutants from wastewater. The ion exchange mode of nanofiltration is where nanofiltration is combined with ion-exchange technology. In this process, firstly the water is subjected to ion exchange wherein exchange of equivalent of ions takes place and the osmotic potential of water is governed by the concentration of ions in the effluent. This effluent is then subjected to nanomembranes wherein the maximum of the ions, pollutants, and pathogens are removed via filtration. Nanomembranes display a greater efflux potential even with low osmotic pressure (Sarkar and SenGupta 2009). Adsorption nanofiltration is the process where adsorption is combined with nanofiltration. Adsorption of heavy metals, biological molecules, organic matter, and inorganic ions is followed by nanofiltration via membrane separation

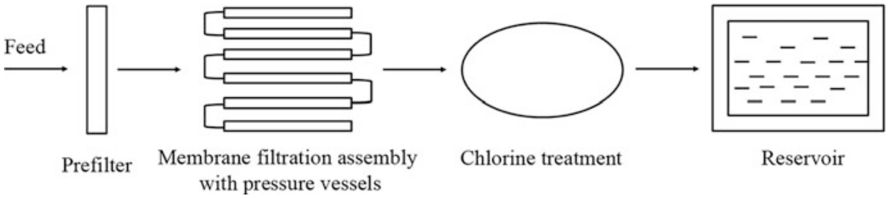

Fig. 11.4 A flow diagram of nanofiltration for wastewater treatment

technology. This increases the efficiency of contaminant removal from wastewater and aids in improving the drinking water quality that can be obtained after this course of action (Hanif et al. 2021). A commonly used nanofiltration unit is composed of a prefilter with a pore size of around 50 μm. This prefilter helps in the removal of the sediments and other pollutants of large size. The effluent is then fed into the membrane filtration unit, which consists of nanomembranes, and the pressure of circulation flow is increased. This results in a clean water stream. This clean water is then subjected to chlorine treatment and the treated water flows to the reservoir (Fig. 11.4) (Aliverti et al. 2011). The membrane filtration unit can be modified according to the quality and properties of the wastewater feed, and it can also be combined with other energy efficient techniques as discussed above.

Recently, Higgins et al. suggested that pH of feedwater affects the adsorption as well as the removal of ibuprofen (IBU) from the wastewater. They illustrated that removal of IBU is directly proportional to the increase in feed pH, whereas adsorption of IBU is indirectly related to the increase in feed pH. It was also conveyed that stainless steel equipment of flat-sheet reactor also affects the adsorption of the IBU up to 27.3% (Higgins and Duranceau 2020). Another development in nanofiltration is the preparation of membrane with ionic liquids (ILs). Ionic liquids are basically molten organic salts with high polarity as well as thermal and chemical stability. ILs are involatile and are known to dissolve both organic and inorganic materials (Zheng et al. 2020).

11.3.2 Thin-Film Composite Structure

Nanomembranes have been optimized for better separation performance via change of material and structure. Recent nanomembranes consist of thin-film composite structure (TFC). Thin-film composite membranes are basically molecular semipermeable membranes, which are custom prepared for water purification systems as a multilayered film and acts as a molecular filter for removal of impurities from water. A composite nanofiltration membrane can be described as a bilayer film, which is constructed via two-step process. Firstly, a thick, porous, and nonselective layer is formed followed by overcoating with an ultrathin barrier layer. The nonselective layer is normally composed of woven fabric, and the ultrathin barrier layer is made up of chemical polymers. Both these layers are always different in their chemical composition (Petersen 1993). Usually, in a thin-film composite membrane, the

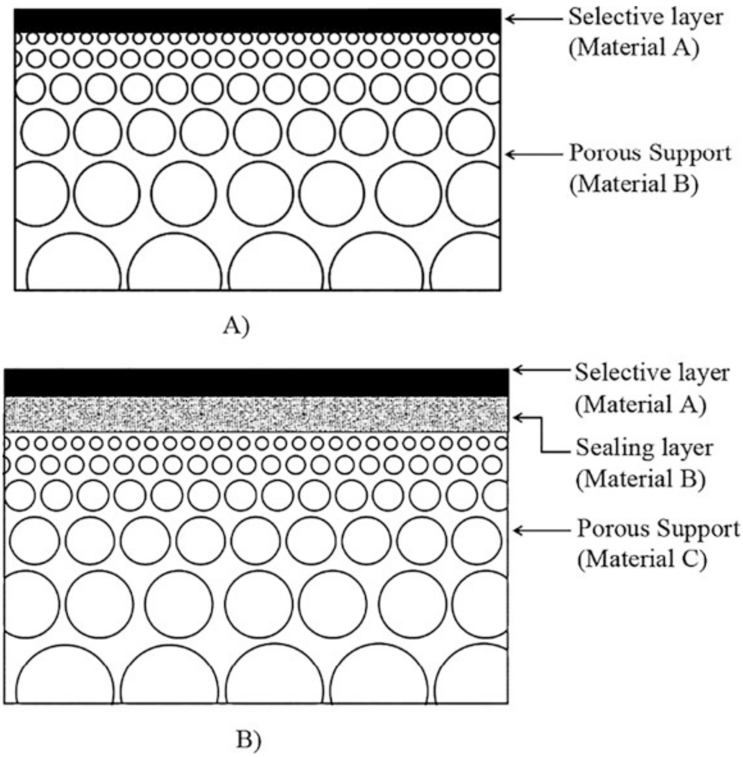

Fig. 11.5 Schematic representation of thin film composite membrane: (**a**) single-layer composite and (**b**) multilayer composite (Pinnau 2000)

selective layer is constructed through a process called as interfacial polymerization (IP). IP takes place at the interface of two immiscible solutions, aqueous and organic, containing different monomers. The most common TFC membrane is made up of polyamide, which is formed by IP between trimesoyl chloride (TMC) and a diamine. Desired membrane structure and separation performance can be obtained by optimization of membrane properties such as pore size, thickness, roughness, and hydrophilicity and understanding its chemical properties (Bui et al. 2011; Hermans et al. 2015; Ismail et al. 2015; Verbeke et al. 2019).

There can be two forms of composite membranes: (1) single-layer and (2) multilayer (Fig. 11.5). The single-layer composite membrane is assembled with two components only. It consists of a porous material over a woven or unwoven fabric support, and on this porous layer, a thin selective layer is formed through solution coating method. During the solution coating method, a dilute polymer solution is poured over the porous layer to form a thin deposition with a thickness of less than 1 μm. However, it is very difficult to form a selective layer of such thickness without loopholes. These defects are formed as the polymer solution is sucked into the porous layer and a uniform coating of selective layer is not formed (Pinnau 2000).

To overcome this difficulty, a multilayer composite membrane is assembled. The suction by porous membrane is avoided by fabricating a sealing layer between porous support and ultrathin selective layer. This sealing layer helps in blocking the suction pores of the porous support and helps in the formation of a uniform and disruption-free selective layer. The sealing layer should be composed of a highly permeable material than the selective layer, so that it does not interfere with the mass transport during the separation process (Pinnau 2000). Another method has also been developed by Malakian et al. wherein the colloidal fouling is minimized by using line and groove patterns on NF membranes by thermal embossing. Colloidal fouling refers to the accumulation of silica, iron, aluminum, and other inorganic compounds, which decrease the permeability of the membrane (Malakian et al. 2020). Ozonation before the nanofiltration has been shown to be beneficial for the wastewater treatment process. Pre-ozonation results in mineralization of organic matter, thus decreasing the membrane fouling and maintaining the membrane permeability. The flux rate can be sustained through pre-ozonation (Amadou-Yacouba et al. 2022).

11.3.3 Water Purification by Nanofiltration Process

Nanofiltration as a technique has been applied on many fronts of water purification process, namely, groundwater treatment, surface water treatment, and brackish water treatment. Nanomembranes are also used at the point of use and water reuse. Nanofiltration excels in removal of most pollutants, pathogens, and natural organic matter from water. The process involves pretreatment, nanofiltration, and post treatment of wastewater. Pretreatment majorly includes coagulation, sedimentation, and micro-/ultrafiltration to largely remove particles, organic matter, and bacteria, thus reducing the membrane damage during nanofiltration. Nanofiltration through tailored separation potential then removes the pollutants up to a significant level. The nanomembranes can be coupled with other techniques for better separation performance and efficient wastewater treatment. Ion exchange, coagulation, electrical filtration, plant-derived nanoparticles, etc. can be combined with nanomembranes for enhancing the efficiency of the treatment (Ang et al. 2016; Kumar and Chauhan 2022; Sarkar and SenGupta 2008). During the post treatment process, disinfecting agents are added to prevent the regrowth of bacteria and other microorganisms. Since nanofiltration is able to remove majority of the infectious pathogens, the concentration of disinfecting agent required is quite low during the post treatment process (Ohkouchi et al. 2013).

11.4 Photocatalytic Wastewater Treatment

Photocatalysis is emerging as a striking solution for degradation of natural organic matter and heavy metal ions in wastewater. Photocatalyst is a combination of word “photo” and “catalyst.” Photo means light, and catalyst means accelerator of chemical reaction. A substance that absorbs light and in turn catalyzes a chemical reaction is known as a photocatalyst. The most common photocatalysts that are used for wastewater treatment are metal oxides, metal sulfides, oxysulfides, and oxynitrides. Some of the metal oxides that are used as photocatalysts are iron (III) oxide (Fe_2O_3), zinc oxide (ZnO), niobium pentoxide (Nb_2O_5), titanium oxide (TiO_2), tin (II) oxide (SnO_2), tungsten trioxide (WO_3), bismuth oxide (Bi_2O_3), vanadium oxide (V_2O_5), and zirconia (ZrO_2) (Raizada et al. 2017).

11.4.1 The Concept of Photocatalysis

The principle behind photocatalysis involves the activation of photocatalyst via light source, which instigates the reaction. The photocatalyst produces electron and hole pairs, which take the reaction forward. The electrons (e^-) present in the valence band are excited, and they are transferred to the conduction band leaving holes (h^+) behind in the valence band. The smaller the bandgap between valence band and conduction band, more visible light photons can be captured, and more electrons can be excited. The separation of electron and holes is necessary as electrons and holes present on the surface can directly be involved in the chemical reactions (Fig 11.6). The electrons can reduce the heavy metal ions, while holes can interact with hydroxyl ions OH^- or water molecules H_2O to form·OH hydroxyl radicals. The excited electrons can also react with dissolved oxygen in water to form $\cdot O_2^-$ superoxide radicals. The pollutants firstly get adsorbed on the surface of the catalyst and increase the redox capability of the catalyst. The radicals generated by the catalyst then participate in a sequence of chemical reactions wherein organic matter, heavy metal ions, and other contaminants present in water are degraded (Ren et al. 2021). However, the catalysis is slowed down by the recombination of electrons and holes. The large number of charge carriers results in recombination, which produces photons or heat, thereby decreasing the rate of photocatalysis. There has been development of different strategies to overcome the problem of recombination. Advances in the assembly of semiconductors with heterojunctions or external circuits with photocatalytic effects have provided an alternative with a solution to

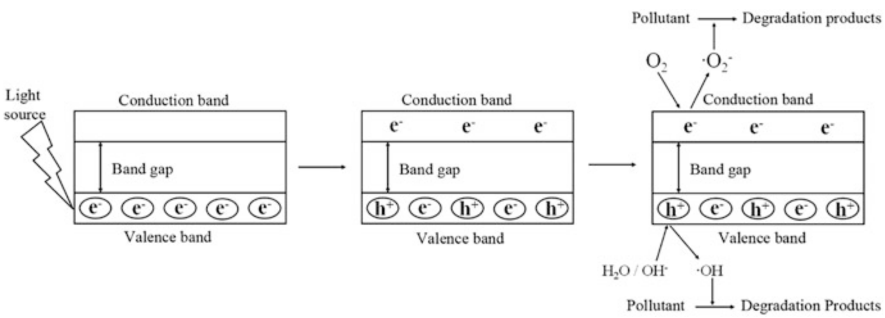

Fig. 11.6 Simplified working principle of photocatalytic unit

recombination and enhance the photocatalytic ability of the semiconductor (Wang et al. 2014; Zhang et al. 2013).

11.4.2 Semiconductor Photocatalysts Used for Degradation of Different Pollutants

Different photocatalytic reactors have been studied to degrade a variety of pollutants in wastewater. The organic matter or organic compounds like dyes, phenolic compounds, and hydrocarbons are decomposed into biodegradable molecules, which are further broken down into harmless CO_2 and H_2O. A cluster of photocatalytic semiconductors such as TiO_2, ZnO, Fe_2O_3, and C_3N_4 have been studied for degrading a variety of organic pollutants (Cao and Zhu 2008; Horikoshi and Serpone 2020; Lee et al. 2016; Moreira et al. 2019). Dyes have been shown to be degraded via TiO_2, ZnO, $BiFeWO_6$, SnO_2, and MoS_2. Similarly, phenolic compounds and petroleum hydrocarbons have been photocatalytically degraded by CuO-TiO_2, Fe_3O_4, or C_3N_4 (Ghasemi et al. 2016; Li and Lin 2020; Yang et al. 2020). The heavy metal ions with high valency are degraded or reduced to low-valence or zero-valence ions via photocatalytic process. The set of photocatalytic compounds that are used for degradation of different heavy metal ions include TiO_2-ZrO_2, Mn_3O_4-ZnO, $NiFeO_4$, Fe_3O_4-TiO_2, Fe_2O_3-C_3N_4, and nanoFe_3O_4 (Bi et al. 2019; Kadi et al. 2020; Li et al. 2017; Liu et al. 2019; Thomas and Alexander 2020; Yan et al. 2020). Pharmaceutical compounds such as antibiotics, anti-inflammatory drugs, lipid regulators, etc. are also degraded into harmless compounds with the help of photocatalysts. The semiconductor photocatalysts used for degradation of pharmaceutical compounds include TiO_2, CuO, Fe_3O_4, ZrO_2, and $NiFe_2O_4$ (Carbuloni et al. 2020; Khanmohammadi et al. 2021; Liu et al. 2020; Wang et al. 2019). The semiconductor photocatalysts that are used for removal or degradation of pesticides are TiO_2, ZnO, and Ag/$LaTiO_3$ (Shawky et al. 2020; Taghizade Firozjaee et al. 2018). The elimination of microorganisms from wastewater is a very crucial step for water to be drinkable. The photocatalysts majorly help in inactivation of microorganisms by production of reactive oxygen species (ROS) such as OH· (hydroxyl free radical) and $\cdot O_2^-$ (superoxide free radical). ROS helps in oxidation of nucleic acids, cell membrane, and other macromolecules leading to inhibition of respiratory cycle of the cell and leakage of important cell ingredients, thereby causing cell death. The photocatalysts that are employed for removal of microorganisms include TiO_2, $BiVO_4$, and Fe_2O_3 (Ng et al. 2016; Regmi et al. 2017; Song et al. 2019).

11.4.3 Structure and Design of Photocatalytic Reactor

Any process for wastewater treatment can be optimized and analyzed for better efficacy and low energy consumption. Similarly, photocatalytic reactors for wastewater treatment can be designed and developed according to the wastewater quality,

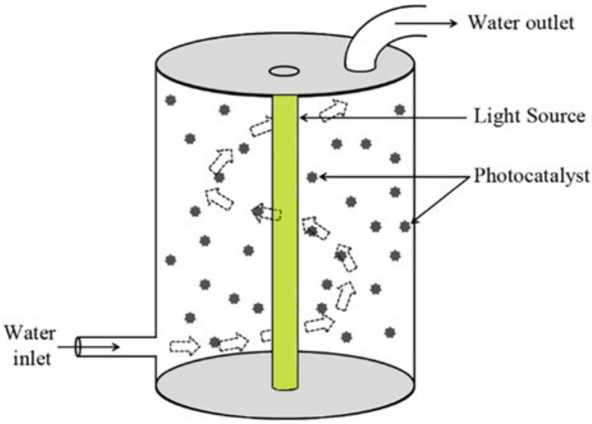

Fig. 11.7 Fluidized bed photoreactor (Image reference: Brame (2017))

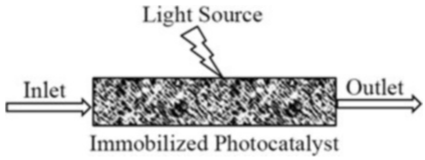

Fig. 11.8 Fixed bed photoreactor (Image reference: Ghuman et al. (2015))

photocatalytic material, and light source. Mainly photoreactors can be classified into (1) fluidized bed reactors and (2) fixed bed reactors. This classification is based on the form of the photocatalyst to be used.

Fluidized bed photoreactors (FBPR) are designed on the principle that the liquid influent is passed over the stationary bed of solid particles (i.e., photocatalyst semiconductor material) with sufficient velocity to suspend the solid particles into the liquid influent. When the velocity of liquid influent is low, it doesn't disturb the stationary bed and simply passes through the holes and gaps in the bed. However, when the velocity of the fluid is increased, there is an expansion of the bed until the solid particles are totally suspended in the liquid and behave like a fluid. In the fluid chamber, there is a light source, which activates the photocatalyst. In FBPR, the catalysts are loaded on the granulated carrier from which they are suspended in the influent wastewater supply. The light source present in the chamber excites the catalyst particles, which then give rise to hydroxyl radicals that degrade the contaminants present in the water. The effluent, that is, treated water, then comes out of the filter (Fig. 11.7). FBPR are advantageous as they have large surface area, rapid reaction rate, and minimum transfer constraint. The only drawback is the recovery of catalyst semiconductor material, which is quite difficult resulting in blockage of the reactor, and reuse of the catalyst is almost impossible, thus making it tricky to scale up in the form of large capacity industry reactors (Dong et al. 2015; Enesca 2021).

Fixed bed photoreactors are based on fixed membrane-type reactors (Fig. 11.8). In this type of reactor, the photocatalytic semiconductor material is immobilized on a surface like glass, silica, clay, polymers, etc. The influent wastewater in low velocity is passed through the fixed bed of catalyst. The light source activates/excites the catalyst, which generates the hydroxyl free radicals in the influent and removes the pollutants from the wastewater. The effluent/clean water then comes out of the filter. Though the efficacy of fixed bed photoreactor is less than the FBPR, it is advantageous in terms of scaling up the reactor system as the photocatalyst is easy to recover and can be recycled and reused for a prolonged period of time (Ren et al. 2021; Vaiano et al. 2015).

Both fluidized bed photoreactor and fixed bed photoreactor are designed in two geometrical shapes: cylindrical and rectangular. In cylindrical photoreactors, the light is source is situated in the center of the cylinder or arranged in a circle inside the cylinder. The photocatalyst can be dispersed in the liquid inside or can be static on a substrate medium. The cylindrical reactors have advantage of circular motion aeration and proper mixing of the mobile catalyst as well as proper circulation of wastewater in case of immobilized catalyst. In rectangular photoreactors, the light sources can be adjusted according to the requirements, for example, on lateral sides of the chamber, evenly placed in the middle of the chamber, corners of the chamber, etc. The immobilized catalyst can also be arranged appropriately so as to obtain maximum reaction rate, for example, on the sides of the chamber, on the cover of the light source, on the roof of the chamber, or floor of the chamber. In case of liquid or fluidized catalyst, the flow rate and direction have to be optimized according to the shape of the chamber so as to obtain a homogeneous distribution of the catalyst (Enesca 2021).

11.5 Conclusions

Water is equivalent to life in all forms. People and governments, all over the world, have been warned about the colossal problem of water scarcity by the scientists and water management since the start of the twenty-first century. These alarm bells have been met with partial solutions and half-cooked attempts. It is now time to put in cohesive efforts and extraordinary steps to save this essential resource. In words of Jamie Linton "Water is what we make of it." Water is an indispensable element, which makes life sustainable on earth. This chapter sheds light on the wastewater treatment processes that can help not only save water but also recycle and reuse water. According to the World Water Council, major investments in wastewater treatment technologies have been able to help many developing countries to impede the water scarcity and improve the surface water quality. The microbial bioremediation, nanofiltration, and photocatalysis have been the most efficacious, cost-effective, low energy consuming, and conventional as well as modern wastewater treatment processes. These methods can be optimized for enhanced efficiency and performance with the proper knowledge of their working principle, which have been discussed in the chapter. Our aim is to develop a superior understanding of pros and cons of different wastewater treatment technologies and how they can be best developed for making water a resource rather than a waste through reuse and recycling of wastewater.

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Overview of Methods and Processes Used in Wastewater Treatment

12

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Abstract

Water itself is plagued by wastewater, the enormity of which has become a global environmental threat. Continuous and sustained efforts taken for treatment of wastewater have led a paradigm shift from conventional to contemporary methods, which are more convenient to tackle indelible pollutants by faster oxidation and sludge formation. This chapter highlights recent developments in wastewater treatment such as advanced oxidation processes, membrane processes, and nanomaterials.

Keywords

Waste water · Pollution · Adsorption · Electrocoagulation

12.1 Introduction

Water is existential to life, and it plays a pivotal role in sustaining communities, economies, and societies. Water is life, and clean water means health. The quality and quantity of water has now become a matter of our sustainability. Less than 3% of all the water resources available on earth is usable as freshwater in the form of ground or surface water, rainwater, or gray water and is meant for utilization in agricultural, domestic or municipal, and industrial sector.

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12.2 Water Pollution

Pollution is a threat to this most precious resource. Water pollution occurs when harmful substances, often chemicals or microorganisms, contaminate a stream, river, lake, ocean, aquifer, or other body of water, degrading water quality and rendering it toxic to humans or the environment (https://www.nrdc.org/stories/water-pollution-everything-you-need-know). Water pollution, by definition, is the contamination of available water by pollutants or alien materials that lead to disease and death of livestock, aquatic life, and humans (Abdulrazzak et al. 2020). Collateral damages of wastewater are soil and water pollution and water resources shortage, which ultimately affects the water cycle and food chain. Water pollution has become a life-threatening issue at global level as growth of industries at fast-paced and rapidly increasing manufacturing units is producing billions of tons of toxic and hazardous waste materials that are entering natural water bodies without any treatment. Some 80% of the world's wastewater is dumped, largely untreated, back into the environment, polluting rivers, lakes, and oceans.

12.3 Wastewater

12.3.1 Sources

The impetus of our societal development has become the root cause of water pollution. Among many sources of water pollution, two general categories may be clearly identified as direct or point and indirect or nonpoint contaminant sources. Direct sources include effluent outfalls from industries, refineries, waste treatment plants, etc. Indirect sources include contaminants that enter the water supply from soils/groundwater systems and from the atmosphere via rainwater. Industrial wastes such as dyes, heavy metals, surfactants, mining activities, sewage and wastewater, pesticides and chemical fertilizers, energy use, radioactive waste, urban development, etc. (Crini and Lichtfouse 2019) are different contaminants, which are broadly divided into three main categories on the basis of its nature: contamination by organic compounds, inorganic compounds (e.g., heavy metals), and microorganisms (Yadav et al. 2021). A distinction in municipal or domestic waste water and industrial waste water is presented in Fig. 12.1.

12.3.2 Wastewater Treatment

The scarcity of freshwater, changing climate, rapidly growing population, and environmental hazard associated with water push the horizon of wastewater treatment. To date, several interventions have been developed to minimize the wastewater discharges and mitigate the hazards of pollutants, using conventional and modern methods (Xu et al. 2018). Corominas et al. (2018) mention that wastewater treatment

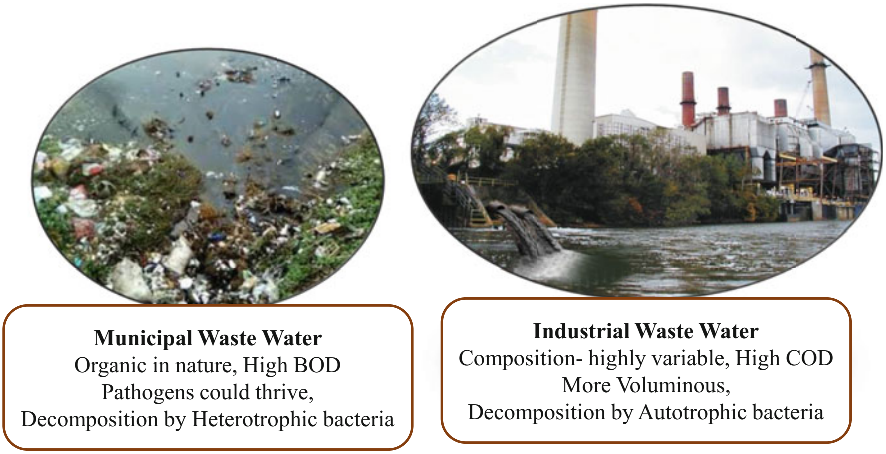

Fig. 12.1 Features of municipal and industrial wastewater

plants are complex systems, which have to maintain high performance at all times, despite suffering from hourly, daily, monthly, and seasonal dynamics. Life cycle assessment (LCA) has been employed to assess the environmental impacts of wastewater treatment, as a tool of sustainability.

12.4 Wastewater Treatment Process

Water treatment technologies are used for three purposes, that is, water source reduction, wastewater treatment, and recycling. Primary treatment includes preliminary purification processes of a physical and chemical nature, while secondary treatment deals with the biological treatment of wastewater. In tertiary treatment processes, wastewater (treated by primary and secondary processes) is converted into good quality water that can be used for different types of purpose, that is, drinking, industrial, or medicinal supplies. An overview of different waste water treatments is given in Table 12.1.

12.4.1 Conventional Methods

Physical Methods Mass transfer strategy is the base of the physical pollutant removal methods (Samsami et al. 2020). Physical methods include sedimentation, adsorption, filtration, and coagulation. The main convenience of physical methods lies in simplicity and flexibility for wide range of contaminants, and the drawback of these methods is generation of heap of sludge. Physical methods such as screening

Table 12.1 Different types of wastewater treatment

S. No.	WWT	Objective	Methods	BOD removal
1.	Preliminary treatment	Removal of coarse solids	Screening Skimming	–
2.	Primary treatment	Removal of suspended solids	Sedimentation flotation	35–40%
3.	Secondary treatment	Additional removal of organics and inorganic	Aerobic biological methods, advanced oxidation process	85–90%
4.	Advanced treatment	Removal of nitrogenous oxygen demand (NOD), nutrient removal, removal of toxic materials	Membrane technology, RO, desalination technologies, electro dialysis, ion exchange, freeze crystallization	95% or more

ensure efficient functioning of advanced methods by prohibiting potential damage, blockage, and process interruption.

Chemical Methods Chemical-based reaction due to specialized chemicals such as chlorine, hydrogen peroxide, sodium chlorite, and sodium hypochlorite assists in removing dissolved metals from wastewater and expedite disinfection. Among chemical methods, chemical precipitation, chemisorption, disinfection, ion exchange, and advanced oxidation process (AOP) are considered noteworthy techniques for wastewater treatment.

Biological Method Biological methods are based on microbial decomposition of organic pollutants via aerobic or anaerobic cycle (Saxena and Bharagava 2017). Aerobic treatment involves algal and bacteria-based oxidation ponds and aeration lagoons. Examples of anaerobic processes are anaerobic filter reactor, anaerobic contact process, fluidized-bed reactor, upflow anaerobic sludge blanket, ADI-BVF process, and expanded granular sludge bed process (Englande Jr et al. 2015). The efficiency of biological processes for degradation depends on the selected microbes' adaptability and enzymes' activity (Rashid et al. 2021). Apart from this, the availability of oxygen, retention time, temperature, and the biological activities of the bacteria judge the extent of biological oxidation (Gupta et al. 2012). Generally, heterotrophic bacteria play an important role in removing organic matters in wastewater treatment system (Dadrasnia et al. 2017). The distinctive features of biological method are relatively cheaper cost, minimal, or no secondary excretion of pollutants and most importantly lower damaging effects on the environment (Mingjun et al. 2009). The drawbacks of biological methods are slow process and low biodegradability and require an optimally favorable environment and proper maintenance of microorganisms.

12.4.2 Recent Advances

12.4.2.1 Adsorption

Adsorption is considered simple to operate and cost-effective wastewater treatment technology (Grassi et al. 2012; Liu et al. 2004) with pollutant removal efficiency of 99%. It is the surface phenomena of adherence of adsorbate on adsorbent via chemical or physical interaction. Chemical interaction involves ionic and covalent bonds, whereas physical interaction is based on van der Waals force between adsorbate and adsorbent. The constituents, porosity, and specific surface area (SSA) of adsorbents are responsible for elimination of pollutants from wastewater (Khattri and Singh 2009). There are a range of natural and synthetic adsorbents for the removal of organic pollutants, heavy metals, and dyes. Among them, the most commonly used adsorbents are activated carbon, silica gel, alumina, clay, metal oxides, polyacrylamide, adsorbent resin, and zeolite (Wadhawan et al. 2020; Prajapati et al. 2020). Adsorption onto activated carbons is often cited as the procedure of choice for wastewater pollutants because it gives the best results in terms of efficiency and excellent regeneration capabilities at industrial level (Crini and Lichtfouse 2019; Rathi and Kumar 2021). However, commercial activated carbon is relatively expensive (Abdullah et al. 2009) and has difficulties in separation from water bodies and generates additional pollution. The promising alternates are biochar; biosorbents; nanosorbents, namely, carbon nanotubes (CNT) and nanochitosan (Tan et al. 2015); and magnetic adsorbents (Moosavi et al. 2020) with adsorption capacity in the range of 800 and 5000 mg/g.

12.4.2.2 Chemical Precipitation

Chemical precipitation is a complex procedure with three distinct stages of nucleation, crystal growth, and flocculation (Zueva 2018). The process targets the removal of ionic constituents from water or wastewater by reducing their solubility with the aid of counter ions. The process relies on the use of an alkaline agent, for example, lime or caustic soda to raise the pH of the water that causes the solubility of metal ions to decrease and thus precipitate out of the solvent. It is usually followed by a solid separation operation that may include coagulation and/or sedimentation, or filtration to remove the precipitates. The process is efficient in water softening and stabilization, metallic cations, heavy metals, phosphate removal, and organic molecules (Wang et al. 2005). Significant reduction in chemical oxygen demand (COD), simplicity, and rapidity feature of this method are advantageous, whereas its operating costs from the chemical expense and the cost of disposing of the precipitated sludge that is produced are major limitations of chemical precipitation (Biver and Degols 1982).

12.4.2.3 Coagulation/Flocculation

Coagulation is defined as the process through which very fine solid suspensions are destabilized so that they can begin to agglomerate if conditions allow, while flocculation is defined as the process by which destabilized particles actually conglomerate into larger aggregates that can be separated from wastewater. The inorganic, organic, and biomaterials that promote aggregation and sedimentation of suspended particles in solution are called coagulants and flocculants (Armenante 2014). The working action of coagulation/flocculation is based on charge neutralization, charge patching, bridging, and sweeping (Yang et al. 2016).

Coagulation and flocculation occur in successive steps and followed by sedimentation. If coagulation is incomplete, flocculation step will be unsuccessful, and if flocculation is incomplete, sedimentation will be unsuccessful. Coagulation and flocculation have been reported to be widely used technologies in textile wastewater treatment (Uzal 2015) and paper and cardboard recycling industry wastewater (Gholami et al. 2020). The crucial factors influencing coagulation–flocculation are temperature, pH, effluent quality, coagulant type and its dosage (Nnaji 2012), mixing speed, and settling time of the floc formed. The most commonly used coagulants in wastewater treatment are aluminum sulfate (alum), ferric chloride, ferric sulfate, ferrous sulfate (copperas), sodium aluminate, polyaluminum chloride, and organic polymers (Shammas 2005).

12.4.2.4 Electrocoagulation (EC)

EC is an electrochemical process that uses a low electrical current in situ to eliminate pollutants, namely, heavy metals, dissolved metals, dyes, and suspended solids from wastewater (Nidheesh et al. 2022). When current passes through a metal (sacrificial) anode (M), metal oxidizes to its cation (M^{n+}), and water is reduced to hydrogen gas and the hydroxyl ion (OH^{-}) simultaneously. These charged metal cations destabilize any colloidal particles by the formation of polyvalent polyhydroxide complexes. These complexes have high adsorption properties, forming aggregates with pollutants. Evolution of hydrogen gas aids in mixing and hence flocculation. Once the floc is generated, the electrolytic gas creates a flotation effect removing the pollutants to the floc—foam layer at the liquid surface (Holt et al. 1999). Thus, this technique is an amalgam of electrochemistry, coagulation, and flotation (Boinpally et al. 2023). Parameters affecting EC process are operating current density (10–150 A/m^2), electrode material and arrangement, interelectrode distance, pH and conductivity, reaction time and temperature, and design of reactor (Tahreen et al. 2020). The anode materials play a significant role in the oxidation reactions that occur on the sacrificial electrodes (Shahedi et al. 2020).

EC is an effective and safe technology for elimination of contaminants of dairy industry (Bazrafshan et al. 2013), textile industry (Afriani and Tiandho 2020), etc. The cost-effective, easily operable low installation cost, and high treatment efficiency are perquisite benefits of EC technology. While on the contrary, the need to replace the "sacrificial anode" periodically, high cost of electricity, and toxic or harmful sludge produced are the major constraints of EC technology.

12.4.2.5 Ion Exchange Technology

Ion exchange is swapping of ions between two electrolytes or between an electrolyte solution and a complex. Ion exchange systems generally contain either cationic or anionic exchange resins, which are high molecular weight polyacids or polybases and are known for insolubility in aqueous and nonaqueous media. The most commonly used ion exchangers are sodium silicates, zeolites, polystyrene sulfonic acid, and acrylic and metha-acrylic resins (Dorfner 1991). The ion exchange technology has seen evolution from resins to membrane resulting in mass scale utilization for desalination of sea and brackish water and for treating industrial effluents (Xu 2005). The advantage of ion exchange as a water remediation technique is that it is very cost-effective in withdrawal of heavy metals and organic contaminants. Very little amount of energy is required, and regeneration of resins is very economical. Still limitations of fouling, adsorption of organic matter, and bacterial contaminations hamper its prolong usage (Kansara et al. 2016).

12.4.2.6 Membrane Filtration

Membrane technology has been widely used for water treatment. Pressure-driven membrane processes for wastewater are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), and membrane bioreactor (MBR) (Al Mahri et al. 2020). MF is more often chosen as a pretreatment for tighter downstream membrane systems, such as nanofiltration or RO units, to avoid particles that may cause fouling of the tighter membrane units (Paul 2002). UF membranes retain macromolecules and colloids from a solution on the basis of molecular weight cutoff (MWCO) and are often chosen for hybrid applications. RO and NF are highly efficient in separating small particles including bacteria and monovalent ions like sodium ions and chloride ions up to 99.5% (Muro et al. 2012). However, NF is water-softening technology, whereas RO is desalination technology (Fig. 12.2).

Feedwater composition, membrane material and type, operating conditions especially transmembrane pressure (TMP), and membrane fouling influence the success of membrane filtration technique. Fouling declines the membrane's permeability as it causes membrane pore blocking by forming a layer of organic compounds. In spite

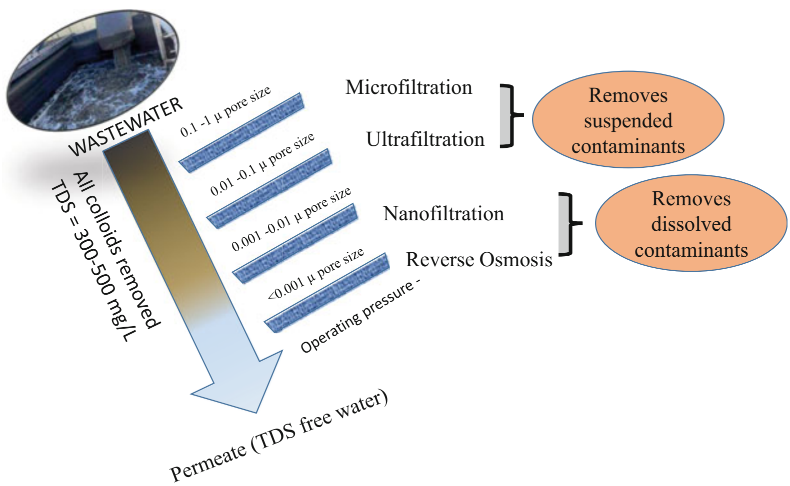

Fig. 12.2 Waste water treatment through different types of membranes

of fouling limitations, membrane technology is beneficial in terms of financial sustainability, low use of chemicals, eco-friendliness, and ease of access (Obotey Ezugbe and Rathilal 2020). Further application in textile, dairy, landfill, and composting plants establishes the coup of membrane technology in wastewater treatment. The waste-intensive nature of membrane fabrication (Razali et al. 2015) and escalating cost of fouling control push the demand for hybrid or integrated membrane technology.

12.4.2.7 Membrane Bioreactor (MBR)

MBR technology was developed in 1969 to overcome the drawbacks of conventional activated sludge process with the aid of membrane. The technology is bestowed with advantages like controlled biomass retention, improved effluent quality, and decreased footprint (Kraume and Drews 2010). WW to be treated by a membrane bioreactor flows into an aeration tank, where biodegradable organic matter and reduced nitrogen compounds are oxidized. Then sludge flow is channeled through a membrane filtration unit where sludge and water are separated. Filtrate is drained of as effluent, and the concentrate is recirculated into the aeration tank. Surplus sludge is discharged via a sludge valve (Van Dijk and Roncken 1997).

12.4.2.8 Advanced Oxidation Processes (AOPs)

Various AOPs are based on the addition or creation of a highly oxidizing species to degrade the organic matter as mentioned in Table 12.2. They have commercial potential for eliminating highly toxic and nonbiodegradable wastes.

12.4.2.9 Microbial Fuel Cell

A microbial fuel cell (MFC) is a galvanic cell in which the biochemical energy contained in the organic matter is directly converted into electricity under anaerobic conditions (Du et al. 2007). According to Munjal et al. (2020), the components of MFC are microbial anodic chamber and a cathodic chamber separated by a proton exchange membrane (PEM). At anode, anaerobic respiring bacteria, particularly *Geobacter* and *Shewanella*, efficiently degrade organic matter of wastewater into carbon dioxide as end product, while generating electrons and protons concomitantly. Electrons pass from the bacteria to the electrode (anode) in the same chamber and then via a circuit to the cathode where they combine with protons and oxygen to form water (Liu et al. 2004). At cathode, oxygen, ferricyanide, and hydrogen peroxide are primarily served as the terminal electron acceptor (TEA). The difference in the potential coupled to electron flow produces electricity in this fuel cell. The long-term stability and performance of MFC is affected by several parameters such as substrate, electrodes' material and distance, effect of cathode material, aeration rate, pH effect, electrolyte, and temperature effect (Jatoi et al. 2021).

Although the current applications of MFCs are still at lab-level, they have been proved to be of great potential industrial applications especially food processing, dairy (Mohan et al. 2010), and brewery industries. MFCs are environmentally friendly technologies (Gude 2016) with energy-saving features resulting from reduced aeration, less sludge production, and ambient temperature requirement,

Table 12.2 Description of various AOPs

Type of AOP	Principle	Suitability	Limitation	Reference
Fenton oxidation	Fe^{2+} is used as the catalyst and hydrogen peroxide (H_2O_2) as the oxidant	Organic pollutants	Large volume of iron sludge produced and limited optimum pH range	Palmer and Hatley (2018)
Photo-Fenton	Fenton reaction occurring between H_2O_2 and Fe^{2+}/Fe^{3+} with UV radiations	Emerging contaminants	Requires narrow range of pH	Legrini et al. (1993) Klamerth et al. (2010)
UV-radiation based AOP	Excitation of electrons	Destroys bacteria and degrade aquatic organic substances	High-energy costs or insufficient reductions	Ibrahim et al. (2019) Glaze et al. (1987)
Ozonation	O_3, a potent oxidant and a strong disinfectant	Decolorization of textile wastewaters	high ozone generation cost, short lifetime, pH dependence	
Sonolysis or hydrodynamic cavitation	Uses ultrasonic sound having 20 kHz to 10 MHz frequency range causing cavitation	Degrades volatile organic matter responsible for turbidity	More consumption of energy and less efficiency of mineralization	
Wet air oxidation	Under high temperature (200–325 °C) and pressure (50–150 bar)	Aqueous wastes from the chemical industry and domestic sludge	Corrosion issues	Debellefontaine and Foussard (2000)

which prove MFCs to be exceptional among the existing technologies for wastewater treatment. It promises the removal of heavy metals, ammonia, and COD depletion and recovery of valuable products like silver (Ag) and chromium (Cr) as reviewed by Munoz-Cupa et al. (2021). However, the low power density, concentration polarization, and the high capital cost of MFCs have been debatable for tackling large volumes of wastewater (He et al. 2017). Future scope lies in synergies between MFC and other treatment technologies along with scaling up challenges.

12.5 Nanotechnology

Nano, being the billionth of a meter, has high absorbing, interacting, and reacting capabilities due to its small size and high proportion of atoms at surface (Madhuri et al. 2018). At the nanoscale (1–100 nm), materials demonstrate significantly different physical and chemical properties from their bulk counterparts (Ahmed and Haider 2018).

According to Li et al. (2008), recent nanotechnology-based water treatment targets four areas:

(a) Adsorptive elimination of micro pollutants
(b) Filtration through membranes
(c) Microbial decontamination
(d) Catalytic degradation or photocatalysis

Carbon Nanotube (CNT) Treatment CNT treatment seems possible in wastewater treatment owing to the technology's large adsorption surface area and ability to adsorb a range of challenging materials that are of interest to the wastewater treatment industry (Qu et al. 2013; Lu and Chiu 2006).

Crystallization It is a solid-liquid separation technique, in which the solute crystallizes from the liquid solution and turns into a pure solid crystalline phase (Lu et al. 2017). This technique is effective for desalination and recovery of valuable resources. The panorama of crystallization ranges from ancient evaporation crystallization method to modern hybrid membrane distillation crystallization. Generally, crystallization is used for the wastewater released by cooling towers, coal and gas fired boilers, and the paper and dying industries.

12.6 Conclusion

Wastewater treatment will continue to be a dynamic engineering science necessary for our ecological balance. Wastewater characterization is an important factor in setting up a relevant effective management strategy or treatment process. Various methods highlighted for wastewater treatment have challenges associated with cost and engineering designs along with consideration of BOD. The future prospects lie in energy auditing, computer-aided design of wastewater treatments, and artificial intelligence approach for nearly 99% waste-free discharge.

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Role of Microorganisms in Polluted Water Treatment

13

Inoka C. Perera, K. A. G. de Alwis, and P. I. T. Liyanage

Abstract

Access to clean water is a problem for over 3 billion people in the world. Annually, around 300,000 children lose their lives due to ailments related to the consumption of polluted water. Therefore, neglecting this crisis may lead to severe consequences for the use of clean water to meet basic human needs. Dissolved, suspended, and deposited organic and inorganic substances and microorganisms can pollute water. This hinders the services provided by a pristine aquatic ecosystem. As the neglect of water security is expected to cost five times more than it is being addressed, the number of studies carried out in this field has been enhanced over time. In this context, the concept of bioremediation to address water pollution has become the cynosure, as it is a sustainable and less costly method in comparison to many other alternatives. Bioremediation is a treatment measure for contaminated water. Here, favorable environmental factors are facilitated to stimulate the growth of selected microorganisms that are capable of metabolizing intended pollutants. This way the quality of water can be improved by either removing or reducing the number of harmful substances in water. There are both in situ and ex situ bioremediation techniques, where the former is less costly while the latter is costly. A variety of microorganisms such as bacteria, fungi, and algae are used in this process. It is a vast subject area that is difficult to summarize to a single chapter. However, we have summarized existing knowledge on various aspects of bioremediation to treat polluted water, including the fundamentals, recent advances, and applications, and critically discuss their pros and cons emphasizing future perspectives.

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Bioremediation · Ecosystem · BOD · COD

13.1 Introduction

Removal of contaminants from the environment has become a global issue. The excessive cost associated with most of the conventional cleanup processes may cause neglection of this process entirely or partially paving the way for many adversities. However, the existence of microorganisms has shed some bright light on addressing this matter. This way environmental pollutants are successfully eliminated by incorporating the diverse metabolic reactions of the microorganisms in a technique called bioremediation. In general, bioremediation implies the eradication of pollutants by biological processes, with the use of microorganisms such as fungi and bacteria.

Here, they use enzymes to catalyze the degradation of environmental pollutants. Some of these digest organic matter and give out substances such as carbon dioxide and water. This way microbes consume these pollutants either to gain energy or to enhance their growth and survival denoting mutual benefits. Biodegradation may depend on either indigenous or exogeneous microbes where the former refers to the microbes at the local site while the latter refers to the foreign organisms introduced externally to the site. This could be conducted either at the contaminated site itself or by transporting them to a separate location away from the initial contaminated site, where the former is called in situ bioremediation and the latter is called ex situ bioremediation. Either way, bioremediation facilitates the growth of suitable microorganisms to eliminate a massive amount of pollutants in contaminated water.

13.2 Global Impact of Water Pollution

Water pollution can be considered a critical environmental issue faced worldwide, and people all over the world face circumstances caused by this issue. This statement can be further explained by analyzing the impacts of water pollution on several sectors.

Both surface water and underground water sources can get contaminated by pollutants (Hasan et al. 2019). Water pollution can be divided into three main categories: contamination by organic compounds, inorganic compounds, and microorganisms (Coelho et al. 2015). Several fields are directly connected to water pollution. Agriculture, industries, and oil spills are some. Agriculture is a field where many pollutants can be emitted to water sources if not handled correctly according to the guidelines. When discussing this matter, chemical fertilizers, insecticides, and other organic and inorganic pollutants would come into the topic. Various types of harmful dyes and other chemicals used in manufacturing clothes might be emitted into water resources in textile industries. Oil spills, automobile, and stationery industries are some other sources that can emit water pollutants. Heavy metals such as Cu (II) and Cr (VI) (Breida et al. 2019), dyes, and bleaching chemicals are some examples of the water pollutants that can be released to water bodies by industries.

13.2.1 Effect on the Human Health

Human health is seriously affected by water pollution. According to the WHO, around 829,000 people are estimated to die each year from diarrhea. This condition results from unsafe drinking water, sanitation, and hand hygiene. Considering "unsafe drinking water," it is directly linked to water pollution.

According to a study conducted in the Turag river in Dhaka, Bangladesh, the maximum concentration of turbidity, hardness, BOD, TDS, and COD are much higher than the standard limits. Moreover, it has been revealed that the local communities suffer from various health conditions, including diarrhea and yellow fever (Hasan et al. 2019). The following are some of the diseases that the consumption of polluted water can cause.

Cholera, which is caused by the infection of toxicogenic *Vibrio cholerae*, can be considered a severe diarrheal disease that can rise to epidemic and pandemic levels (Mandal et al. 2011). Cholera is also considered a reflection of social inequalities since it is primarily found in underprivileged populations worldwide (Somboonwit et al. 2017). Therefore, to a considerable extent, cholera is an indicator that shows the socioeconomic and health facilities of a particular country. Diarrhea is a term that is commonly associated with water pollution. Diarrhea can be considered more a symptom than a disease and can be described as loose and watery bowel movements. Diarrhea usually lasts for a few days. However, if it lasts longer, it could be a symptom of more severe conditions such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Consumption of polluted water can be a significant cause of diarrhea. In a study done in Shenzen, China, stool samples were collected from 412 patients having diarrhea. Nineteen pathogens had been detected in those samples. Some detected bacterial pathogens are *Salmonella*, *Campylobacter jejuni*, *Shigella*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, and *Vibrio cholera*. According to the study, bacterial infections are the dominant cause of diarrhea (Shen et al. 2016). Typhoid fever is another condition that can be caused by water pollution. Considering its epidemiology, *Salmonella enterica* subspecies *enterica serovar Typhi* (*Salmonella typhi*) is the cause of typhoid fever (Crump 2019). Feces is considered the major source of exit of *Salmonella typhi*, and scientific studies show the availability of this microorganism in urine as well (Sears et al. 1924). People can get infected with *S. typhi* due to the consumption of unsafe food and water contaminated with *S. typhi* through fecal matter.

Other than the diarrheal conditions described above, the conditions such as respiratory diseases, cancers, cardiovascular diseases, and neurological disorders are also associated with polluted water (Ullah et al. 2014). Reproductive failures can also be caused by the consumption of polluted water (Currie et al. 2013). It is said that there are many water contaminants (e.g., disinfected byproducts, fluorinated compounds), which are also endocrine-disrupting chemicals (EDCs). These EDCs can potentially cause detrimental effects on the endocrine system, which would finally lead to the impairment of the fertility and growth of humans and other animals (Gonsioroski et al. 2020).

Another important fact that should be considered is that, for a human to get affected by polluted water, he/she does not need to be in direct contact with polluted water. Pollutants get accumulated in human bodies via food chains as well. When the contaminants are passed through each trophic level, the accumulating concentration of the contaminant in the body of the organism is increased. The scientific term that is used to describe this term is biomagnification. Biomagnification can be defined as the condition when the chemical concentration in an organism exceeds the concentration of its food when the main route of exposure takes place via the diet of the organism (Svanback and Bolnick 2019). Since human is at the last trophic level of many urban food chains, we are in grave danger.

The above facts show that humans are severely affected by the infections associated with water pollution while shedding light on the necessity to implement urgent measures.

13.2.2 Mortality Caused All Over the World Due to Polluted Water and Lack of Hygiene

One of the most tragic aspects of this man-made water pollution is when it costs the lives of the people. So far, infections associated with polluted water and lack of hygiene have cost thousands of lives worldwide. According to the World Bank, the below map indicates the mortality rates caused due to unsafe water, unsafe drinking, and lack of hygiene (per 100,000 population), considering 2016 as the most recent year. By going through this map, it can be realized that many countries in the African region have mortality rates of more than 51.20 (per 100,000 population), which is a concerning issue. According to WHO, around 829,000 people die yearly from diarrhea caused by polluted and unsafe drinking water, lack of sanitation, and inadequate hand hygiene. WHO mentions that since diarrhea is highly preventable, higher mortality rates of children can be avoided if the necessary measures are implemented (Fig. 13.1).

13.2.3 Effect on Ecosystems

The polluted water would also deteriorate the life of aquatic ecosystems, which would ultimately result in the disruption of food chains and food webs. This condition would finally create an imbalance in environmental equilibrium and ecological balance. When considering the impacts of water pollutants on fish species, it is reported that high amounts of suspended water pollutants can interrupt the usual behaviors of fish species. Fish species that rely mainly on sight to catch

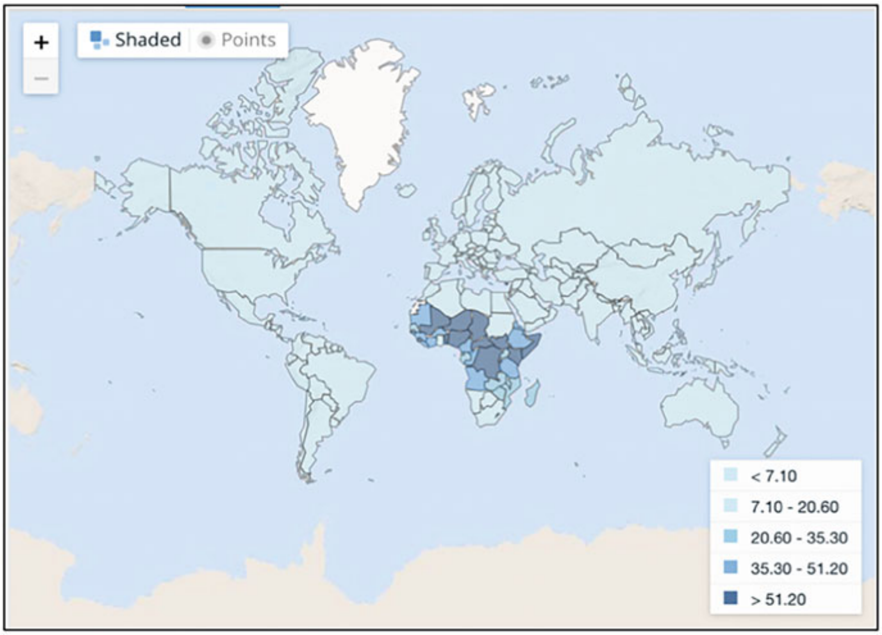

Fig. 13.1 Mortality rate attributed to unsafe water, unsafe drinking, and lack of hygiene (per 100,000 population). Source: https://data.worldbank.org/indicator/SH.STA.WASH.P5

their prey tend to show prominent avoidance behavior since they are highly susceptible to the high amounts of suspended solids in the water bodies (Malik et al. 2020).

On the other hand, aquatic ecosystems can be considered the ultimate sinks of pollutants. As a result of industrialization and urbanization, many environmental pollutants are emitted into water resources. It is known that water has a natural capacity to neutralize the effect of contaminants. However, once this natural capacity is exceeded, the self-generating capacity of the water will be lost. That can be mentioned as one of the main reasons to turn toward other methods to remediate the polluted water (Bashir et al. 2020).

13.3 What Is Water Pollution?

Water pollution can be simply defined as the alterations of biological, chemical, and physical properties of water in a way it would hazardously affect public health and the environment. Water pollution is one of the most critical issues in the twenty-first century that must be addressed immediately. Urbanization and industrialization caused by the increasing population are the main reasons for the emission of various pollutants to water resources. When considering the causes and means of water pollution, oil spills, medical waste, less-organized sewage disposal systems, and fertilizer runoff are noteworthy.

It is known that nearly 9 million barrels of oil are released into the ocean every year (Cohen 2013). DeBofsky et al. (2020) reported that in July 2016, a Husky Energy pipeline spilled, causing a portion of oil to enter the North Saskatchewan River, Canada. Then muscle, bile, and intestinal samples were collected from several fish species from the affected area. It had been revealed that the concentrations of polycyclic aromatic hydrocarbons (PAHs, a crude oil constituent) in dorsal muscles significantly correlated with the gut microbial composition of walleyes (*Sander vitreus*).

The authorities have implemented general rules and regulations for the proper disposal of garbage and other waste materials. Utilizing an efficient method to reduce water pollution is a timely need. Hence, bioremediation comes into the topic.

13.4 Why Opt for Bioremediation?

Several methods (physicochemical and biological) are available to remove the heavy metals from the water before releasing them into the environment (Coelho et al. 2015). Isolation and minimizing the mobility of metal ions using physical barriers made of materials such as steel and cement, solidification and stabilization of the contaminants, size selection processes to remove the large and cleaner particles from the small and more polluted particles, and electrokinetic processes are some of the examples for the physiochemical methods that are being used to treat contaminated water (Mulligan et al. 2001). However, these methods might not be cost-effective and environmentally friendly. Therefore, bioremediation can be suggested as a better method for the same purpose.

13.5 Bioremediation as an Answer to Mitigate Water Pollution

Bioremediation is the process of utilizing specific biomolecules or various biomasses to get bound and concentrate ions or molecules present in an aqueous solution (Coelho et al. 2015). In simpler words, it provides an option to minimize the detrimental impacts of contaminants using biological methods.

Considering the basic principles behind bioremediation, living organisms (primarily microorganisms) are utilized to treat waste materials to convert them into less toxic forms (Vidali 2001). As described under “1. Global Impact of Water Pollution,” it is evident that adequate measures must be taken to minimize water pollution. Otherwise, it would result in irreversible damage to the world in many sectors such as health and the environment. In that light, bioremediation comes into the topic mainly due to its advantages over many other methods.

13.6 Balance of Pollutants in a Natural Ecosystem

There are threshold levels for the nutrients in natural aquatic ecosystems. Once the threshold level is exceeded, a nutrient may be considered a pollutant. When the nutrient ratios are altered, the aquatic ecosystems might respond to them in various ways. Phosphorus is considered the limiting nutrient factor for phytoplankton growth in freshwater ecosystems. Atmospheric nitrogen also affects the acidification of freshwater ecosystems, resulting in detrimental effects. It could be challenging to isolate the effects of a particular nutrient (e.g., nitrogen) from the effects of the other nutrients. It is said that higher amounts of nutrients cannot be isolated and examined in the presence of other nutrients that are essential for the growth of plants, such as phosphorus and silicon. Altered stoichiometric values in nutrients would cause changes in the aquatic ecosystem (Rabalais 2002).

Nutrient cycling is important when considering the balance of nutrients in aquatic ecosystems. Water plays a prominent role in nutrient cycling processes (Malik et al. 2020). Animals are also involved in the nutrient cycling in freshwater ecosystems. Animals can supply nutrients (e.g., nitrogen and phosphorus) via excretory processes. It is known that the nutrient cycling by animals fulfills a substantial amount of the nutrient demand of the primary producers in ecosystems (Vanni 2003). According to a study by Pennifold and Davis (2001), in the presence of macrofauna, the release of phosphate and ammonium into the water column has been increased, while the release of nitrate has been decreased, which means nitrate has been uptaken by the sediments. Climatic conditions might also affect the nutrient cycling in freshwater ecosystems to a considerable level. According to Pennifold and Davis (2001), the season and the dissolved oxygen levels influence the nutrient concentrations. According to their study, ammonium has been released to the overlying water column at considerably higher rates in winter, and nitrogen has been released at higher rates in summer than in the winter.

Anthropogenic activities hugely affect the nutrient/pollutant balance in freshwater ecosystems. The disposal of domestic garbage in freshwater ecosystems can be commonly observed in many developing countries. Other than that, the emission of wastewater and chemicals from industries has also become a severe issue that directly affects the nutrient balance in freshwater ecosystems. Eutrophication is an important mechanism when it comes to nutrient imbalance in freshwater ecosystems. Eutrophication can be defined as the occurrence of an excessive accumulation of nutrients in a water body, which can finally result in higher growth of plants. This can be considered a growing issue in many freshwater bodies, which causes adverse effects on their ecosystem (Rathore et al. 2016). While causing detrimental effects on the water quality, it also hugely affects the biotic components. In eutrophication, extensive algal blooms are caused due to the increment of suspended particles in the water bodies, and the water clarity is also decreased. The rapidly increasing rate of precipitation leads to the destruction of benthic habitats (Dorgham 2014).

According to the above facts, it is clear that the balance of nutrients/pollutants plays a vital role in natural ecosystems.

13.7 Examples of the Microorganisms Used in Bioremediation

Microorganisms play a vital role in the biological degradation of materials. Hence, they are important in treating polluted water. Bacteria, protozoa, metazoa, filamentous bacteria, algae, and fungi are more prominent groups of microorganisms that are identified in bioremediation (Rani et al. 2019) (Table 13.1).

13.8 Mechanism in the Use of Microorganisms in Bioremediation of Polluted Water

Microbes have an innate capability to metabolize and remove toxic xenobiotics from their surrounding. The mechanism involved in this process breakdown substances with higher complexity into simpler forms such as CO_2 and H_2O. This way harmful substances in polluted water are converted into eco-friendly, safer forms by bioremediation. Apart from irradicating the toxins, this also aids to monitor the optimal levels of inorganic substances in water at equilibrium (Misal et al. 2011). The process of bioremediation relies on the temperature, nutrition, pH, concentration of various substances, time, and the types of microbes used (Rani et al. 2019).

The treatment process incorporates three key stages, that is, primary treatment, secondary treatment, and tertiary treatment. Initially, the undissolved substances that are suspended in polluted water are eliminated in the primary treatment. Wastewater has a comparatively higher biological oxygen demand compared to pure water. Thus, in the secondary stage of the treatment, process microbes are used to mitigate this issue. Here, the dissolved water content in water may be increased by degrading organic matter using both aerobic and anaerobic microbes. Also at this level, the turbidity of water is increased due to the purification process. In the final stage, a diverse set of approaches such as ion exchange and reverse osmosis are used to purify the effluent (Rani et al. 2019).

Problems such as toxicity to humans and scaling in pipes/containers arise due to the presence of some ions in water. Here, the former is caused by heavy metal pollution, whereas the latter is due to the high levels of minerals in the water. Therefore, resins made out of artificial or organic substances with ionic functional groups are used to eliminate these ions from water. Above ionic groups hold ions that facilitate ion exchange in contaminated water. Followed by several rounds of ion exchange, the resin can be treated and reused (Crist et al. 2010).

Reverse osmosis is a technique that uses a membrane to separate contaminants from wastewater. This is used to remove both organic and inorganic pollutants from water (Jamil et al. 2019; Madsen and Søgaard 2014; Nikbakht Fini et al. 2019; Urtiaga et al. 2013). The incorporation of sand filtration techniques together with membrane separation methods has reduced the amount of water that needs to undergo bioremediation, as the longer retention time in the sand filter is permitting the degradation of the contaminants in water (Schostag et al. 2022).

Table 13.1 Selected microorganims used in bioremediation

Microorganism group	Species	Property investigated	Reference
Bacteria	*Cronobacter sakazakii*, *Enterobacter asburiae*, *Leclercia adecarboxylata*, *Klebsiella oxytoca*, *K. pneumoniae*, *Bacillus* sp., *Enterococcus thailandicus*, *Chromobacterium vaccinii*, *Serratia* sp., *Kosakonia oryzae*, and *Escherichia coli*	• Removal of organic materials • Heterotrophs consume and use the energy of organic matter for cellular regeneration. This accelerates their growth in the medium • Concentration used: 10^8 to 10^9/ml	Lefebvre et al. (2006), Silva-Bedoya et al. (2016)
Protozoa	*Trachelophyllum pusillum*, *Vorticella microstoma*, *Carchesium polypinum*, *Chilodonella uncinata*, *Opercularia coarctata*, and *Aspidisca costata*	• Feed on pathogenic bacterial strains and suspended matter • Some depend on oxygen for survival, whereas others need very little to no oxygen • Flagellates and ciliates are prominent groups of protozoa that are employed in the bioremediation process where the former feeds on organic material and the latter feeds on the free-floating bacteria • Concentration used : 5×10^3 to 2×10^4	Amaral et al. (2004), Cacciò et al. (2003)
Metazoa		• Act as indicators • Rotifers and nematodes are metazoan groups that play dynamic roles in activated sludge • They can feed on other microbes • Higher concentrations of toxic materials are known to be detrimental to their survival	Rani et al. (2019)
Filamentous bacteria	*Microthrix parvicella*, *Thiothrix* sp., *Alcanivorax borkumensis*, *Sphaerotilus natans*, and *Beggiatoa* sp.	• Important in forming floc, which represents a loose clump of granules • The abundance of filamentous bacteria depends on factors such as the dissolved oxygen content, temperature, nutrition, etc.	Rani et al. (2019)
Algae	*Oscillatoria*, *Chlamydomonas*, *Phormidium autumnale*,	• Extract harmful substances (heavy metals and pesticides) from polluted water	Hoeger et al. (2005), Martins et al. (2010)

(continued)

Table 13.1 (continued)

Microorganism group	Species	Property investigated	Reference
	Limnothrix, *Synechocystis*, *Microcystis*, and *Lyngbya*	• Reduce the nutrient overload in wastewater by using it to produce biomass	
Fungi	*Sphaerotilus natans*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Absidia*	• Break down organic compounds at lower pH values • Convert ammonia into nitrite via an oxidation reaction that hinders the development of bacteria • Hyphae, which is a characteristic feature of fungi adsorb suspended matter in the wastewater • Secrete certain types of enzymes that aid in the degradation process	Hossain Molla et al. (2004), Rani et al. (2019)

13.9 Microbial Water Treatment Systems

The use of microorganisms is a promising approach for the purification of contaminated water. These microbial water treatment systems can be classified into two main types based on the oxygen demand. They are aerobic, anaerobic, and facultative methods (Shah and Roguez-Couto n.d.). Microorganisms break down substances to harvest energy via respiration. Microbes that require and do not require oxygen for this process can be incorporated into aerobic and anaerobic bioremediation, respectively. Furthermore, in the presence of oxygen, the former will take place, while the latter will not as oxygen may halt the process by interfering with the metabolic reactions of the anaerobes.

13.9.1 Aerobic Systems

These systems have a higher capability of limiting both pathogens and the biological oxygen demand. Aerobic systems can eliminate a diversity of organic materials. Also, this is a convenient method that is easy to establish and maintain. The sludge production in aerobic systems is minimal. The “membrane bioreactor system” is an example of an aerobic system in which the treated water can straightly be used in the recycling process.

Oxygen is considered to be one of the key growth retardation factors for the bacteria that degrade hydrocarbons. The excessive release of hydrocarbons to waterways depletes the dissolved oxygen levels in wastewater attenuating the

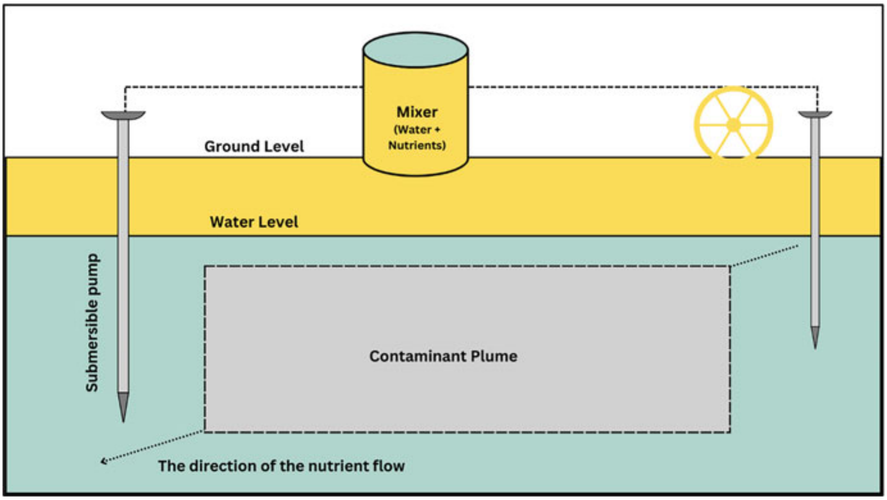

Fig. 13.2 Conceptual diagram: aerobic bioremediation

aerobic biodegradation process. Thus, measures are taken to aerate the wastewater to accelerate biodegradation (Fig. 13.2). This principle is applied in the technique called "enhanced aerobic biodegradation," which is typically used for water contaminated with high levels of petroleum hydrocarbons (Epa and of Underground Storage Tanks n.d.; Mikkonen et al. 2018).

However, the contamination of the membranes and the hindrance to aeration are plausible drawbacks of membrane bioreactors. Furthermore, another approach called "the conventional Activated Sludge Plants" demands higher levels of energy, and the resulting sludge is needed to follow complex disposal protocols. Also, some aerobic systems need a huge space, which is a limiting factor in many scenarios. "The rotating biological contactor" is an aerobic system that retains high levels of opaqueness in the treated water (Rani et al. 2019).

13.9.2 Anaerobic Systems

This is normally used to treat wastewater enriched with organic matter as signified with high biological oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS). In anaerobic systems, microbes degrade organic substances without using oxygen. This is a cyclic process that starts with the acceptance of wastewater into a bioreactor receptacle. This bioreactor has a gelatinous layer of sludge, which is the home of a variety of microbes. The anaerobes in sludge cause the biological degradation of substances in wastewater. This results in the formation of effluent with a low biological and chemical oxygen demand. Also, this accounts for low levels of the total suspended solids in the treated water. Hence, this approach is used in the treatment of wastewater generated from various

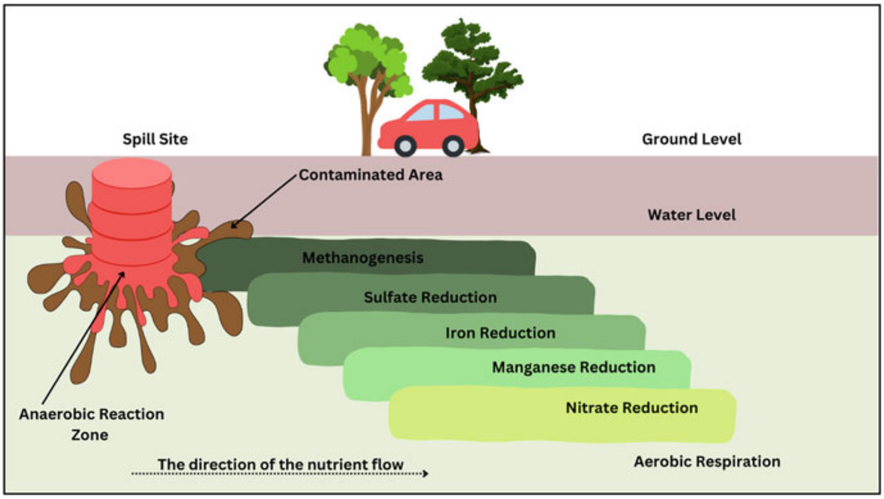

Fig. 13.3 Conceptual diagram: anaerobic bioremediation

industries such as agriculture, food, milk, paper, and clothing. Furthermore, an anaerobic system can also be used in the treatment of sewage (Cyprowski et al. 2018; Qadri et al. 2021; Scott 1995; Samcotech n.d.).

In anaerobic systems, the amount of biological waste formed in the process is less. The demand for nutrition is also minimum in this approach. Thus, anaerobic systems are user-friendly methods that require minimum management (Fig. 13.3). Furthermore, the production of methane as a terminal product is an added advantage. The growth retardation of microorganisms and the pungent smell are drawbacks of anaerobic systems (Rani et al. 2019).

13.9.3 Facultative Systems

Some microorganisms can survive both in oxygen-rich and poor environments. These are called facultative microorganisms. Thus, in the absence of dissolved oxygen, they may rely on other substances such as sulfates, nitrates, or other mechanisms to conduct biological degradation.

Hydrocarbons can be degraded in both aerobic and anaerobic environments. However, the rate of degradation is greater in the former compared to that of the latter (Coates et al. 1997; Holliger and Zehnder 1996). Facultative anaerobes, those that reduce nitrates, iron, and manganese, can degrade hydrocarbons (Coates et al. 1997; Fries et al. 1994). Interestingly, the ability of facultative anaerobes to survive and thrive under aerobic as well as anaerobic conditions has paved the way to maintain a balanced population status aiding the catabolism of pollutants in water. Therefore, studies are conducted to study the features of such bacterial strains that are used in facultative bioremediation (Grishchenkov et al. 2000). Facultative systems need less energy and are convenient to manage. On the contrary, the sludge deposits should be cleaned from time to time (Rani et al. 2019).

13.10 Concerns Regarding Bioremediation

Despite being a versatile technique, bioremediation is facing some limitations. For instance, substances saturated with chorine and polycyclic aromatic hydrocarbons are not successfully eliminated via microorganisms. Furthermore, it is plausible for microbial degradation to form more harmful byproducts upon metabolizing the xenobiotics in the medium. The formation of vinyl chloride after reacting with trichloroethylene exemplifies this. Here, the resultant product, vinyl chloride, is a harmful carcinogen (Megharaj et al. 2014). Thus, in some scenarios, the products generated following biodegradation could be more harmful than the initial contaminant.

Furthermore, the applicability of bioremediation to eliminate only biodegradable compounds limits the use of this approach. Therefore, some contaminants will not undergo partial degradation or will not be degradable at all. Compared to other conventional approaches of treatment, bioremediation is a time-consuming method. In the case of ex situ bioremediation, actions such as excavating and pumping are required, making the process lengthier than usual. Also, the control of the release of organic matter that is volatile is not feasible in an ex situ setting (Hlihor et al. 2017; Sharma 2021). Being a biological process, biodegradation demands specific requirements to yield optimal results. Therefore, the success of the process may depend on factors such as the availability of microorganisms with the optimum metabolic capacity, appropriate growth conditions with nutrients for the enrichment of their population, and so on. Also, contradictions may arise when extrapolating the outcomes of both laboratory and pilot studies into absolute sites. Thus, further studies are needed to formulate novel or modified bioremediation techniques to approach sites with a diversity of pollutants in the form of solids, liquids, and gases. Also, the reliability of the process has to be confirmed by establishing performance specifications including endpoints for bioremediation treatment. This signifies bioremediation as a field of study that urges intense research on the outcomes of the processes governed by microorganisms (Megharaj et al. 2014).

13.11 Costs Associated with Bioremidiation

Many factors such as the type of contaminant, location where the purification is carried out (ex situ/in situ), degree of contamination, and the type of the bioremediation technique used may influence the cost associated with bioremediation.

Since bioremediation depends on the ability of microorganisms to digest waste, the digestion of some contaminants might be difficult for them over others. To address this matter, research should be carried out to select a better species to be used in the process. This significantly increases the cost associated with the bioremediation project. In the case of ex situ bioremediation, wastewater should be transported from the site of origin to the treatment plant, making the process more complex and costly. Furthermore, depending on the level of contamination, the intensity of the purification will have to change. Thus, the approach for bioremediation may have to be advanced or intensified in the case of treating highly contaminated waster. In addition, it might be necessary to repeat some steps or the entire purification process to ensure that the desired purity is acquired. Hence, when using heavily contaminated water, the ingredient cost, labor cost, energy consumption, and many more will account for the expenses. Furthermore, the type of bioremediation technique used will also have an impact on the cost. Therefore, appropriacy, viability, and sustainability should be under scrutiny before implementing a bioremediation project.

13.12 Future Advances in the Use of Bioremediation on Controlling Water Pollution

Bioremediation is used as a treatment measure for industrial effluents, marine oil spills, etc. This is an economic method to mitigate many environmental issues. One measure that can be used to increase the efficiency of this method is by enhancing the interaction between the microbes and the pollutants. This is important, especially when dealing with hydrophobic contaminants. Among the many toxins present in wastewater, heavy metals cause various repercussions. They are environmental pollutants with detrimental impacts on human health. Heavy metals account for cancer, neurotoxicities, and much more, leading to the malfunction of the organ systems. Thus, advances in co-remediation techniques such as microbial electrochemical systems could be used to enhance the efficiency of the removal of heavy metals. Not only for heavy metals but also for many contaminants the combination of diverse technologies alongside the conventional bioremediation process has shed promising insights. However, the identification of suitable technologies to be coupled is a critical process. Also, there is an urge to search for novel microbes with higher capacity and efficiency in the removal of noxious heavy metals and other pollutants that are not currently eradicated efficiently (Rosanti et al. 2020). Hence, studies have been conducted to identify different approaches in which enzymes can be engineered to address a particular need. Methene monooxygenase and hydroxylating dioxygenase are some instances of this where the former can be used to clean up oil and the latter for polycyclic aromatic hydrocarbons in water. Such enzyme production can be efficiently regulated with bioengineered microorganisms with the capability of identifying and responding to the pollutant levels in the environment (Hemamali et al. 2022). In the future with the advanced application of in silico methods and synthetic biology, enzyme-mediated bioremediation could be novel approachs to mitigate water pollution (Dutta and Shityakov 2021).

13.13 Conclusion

In conclusion, many anthropogenic activities such as industrial effluents, urbanization, and many more have risked the balance of the ecosystems by causing environmental pollution. To date, many waterways are polluted with various chemicals such as hydrocarbons, pesticides, dioxanes, and many other xenobiotics that are toxic to flora and fauna. Hence, proper remediation methods are crucial to re-establish and maintain the sustainability of the environment. In that light bioremediation, the use of a variety of microorganisms serves as a solution in treating polluted water over other conventional remediation methods. Currently, most bioremediation approaches are centered on the innate capabilities of the existing microorganisms to metabolize organic matter. Therefore, in the future, a synthetic biology approach to redesigning enzymes will outsmart the capabilities of the existing microbes in the remediation of polluted water. Thus, there is a vacuum of knowledge that needs to be filled by novel studies on catalysts that can be used in bioremediation to ameliorate the ecosystem.

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Bioremediation of Polluted Water

14

U. M. Aruna Kumara, N. V. T. Jayaprada, and N. Thiruchchelvan

Abstract

Most human involvements such as domestic, agricultural, and industrial have an impact toward water and the ecosystem. Current situation water pollution has become a global crisis and affect serious threat to sustainable development for the whole world. Agriculture is one of the backbones of the economy for many developed countries and developing countries. Crucial agricultural activities are causing surface and groundwater pollution. Therefore, they are rendered unsafe, causing issues for human consumption, soil erosion including irrigation, industrial needs, change in nitrogen cycle, carbon sequestration, and relevant ecological patterns. Normally, water pollution happens due to excessive use of pesticides and chemical fertilizers, causing changes in the physiochemical activities of the water. Eutrophication is the main drawback that happens in the aquatic bodies near the agricultural yards due to the runoff of nutrients. As a result, algal blooms can grow on the water body's surface, causing very low dissolved oxygen levels , which can be problematic for aquatic animals. The current approach of this topic is to reduce the environment and ecological impact of using chemical and pesticides in agriculture and promote the necessity of organic farming. Bioremediation is a biological process that helps to recycle waste into a form of a valuable product or non-harm product. Microorganisms directly participate in converting waste into another form that can be recycled or reused. Due to the ability of microorganisms to survive in all places in the world, they are the most suitable

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ones for use as a biotic agent for bioremediation. Temperature and pH like abiotic factors also influence bioremediation. Degradation, eradication, immobilization, and detoxification are the main processes in bioremediation. With the improvement of biotechnological aspects in the world, different methods and strategies like biostimulation, bioaugmentation, bioventing, bio-piles, and bio-attenuation are used. Microorganisms play a major role in bioremediation.

Keywords

Pollution · Waste · Bioremediation · Phytoplankton

14.1 Introduction

Water bodies in the world are facing severe pollution due to rapid population growth and accelerated industrialization. It is reported that agricultural, industrial, and urban emissions are the main sources of pollutants released into the coastal marine and freshwater environment (Chen et al. 2022; Faroque and South 2022). Since the effective controls of industrial and urban domestic sewage are available, agricultural nonpoint source pollution from agricultural production has become the main source of water pollution (Chervier et al. 2022; Xiao et al. 2021). Aquatic ecosystem pollution causes severe repercussions in all living organisms along with human health (Zamora-Ledezma et al. 2021; Datta et al. 2022). This pollution can be due to organic compounds (e.g., hydrocarbons) and inorganic compounds (e.g., heavy metals). Sustainable removal of these pollutants is a greater concern. Organic contaminants like hydrocarbons and textile dyes are a substantial threat to ecosystems with detrimental effects on the biosphere. Water contamination by heavy metals especially through industrial activities is also one of the major causes of pollution (Zamora-Ledezma et al. 2021).

The conventional pollutant removal methods are considered to be expensive and also reported to cause secondary contamination. There is a need to shift from the conventional ways to new approaches requiring biological resources, which are eco-friendly (Noubactep 2019; Ahmed et al. 2022). Bioremediation is such a phenomena, which involves the use of living microorganisms to degrade environmental pollutants into harmless end products (Khalid et al. 2021). A diverse range of microorganisms, including algae, fungi, yeasts, and bacteria, can function as biologically active methylators, capable of modifying toxic species. Technical aspects of bioremediation involve various mechanisms such as biosequestration, biodegradation, phytohydraulics, biological extraction, and volatilization by which microbes immobilize or transform the complex groups of the pollutants remediating the water (More et al. 2022). Microorganisms play a crucial role in heavy metal bioremediation. The bioremediation strategy is based on the high metal binding ability of biological agents, which helps extract heavy metals highly and efficiently from polluted areas (Husain et al. 2022). Although microorganisms cannot destroy metals, they can alter their chemical properties through a surprising array of mechanisms.

Several works have been carried out in this direction using microorganisms for the uptake of heavy metal from solutions. In addition, large-scale treatment of petroleum hydrocarbon contamination in oceans has been reported to be successfully done through microbial-based bioremediation (Jimoh et al. 2022; Dell'Anno et al. 2021).

Apart from using natural microorganisms for bioremediation, microorganisms can be created specifically for the bioremediation process using genetic engineering techniques (Tran et al. 2021; Janssen and Stucki 2020). The studies so far have been reported of inserting two types of genes into the microorganism in this aspect. One is the degradative gene, which will be encoding proteins required for degradation of the pollutant, and the second is the reporter genes that will help in monitoring pollution levels. Another aspect of integrating novel technology into bioremediation is the use of nanotechnology (Thangavelu and Veeraragavan 2022; Tripathi et al. 2022). It reported the possibility of producing environmentally friendly nanomaterials to alleviate these contaminants. The use of microorganisms in nanoparticle synthesis gives green biotechnology a positive impetus to cost reduction and sustainable production as a developing nanotechnology sector. Furthermore, there are reports of using other advanced bioremediation techniques like microencapsulation technology for in situ bioremediation of polluted groundwater applications, particularly with respect to bio-augmentation and bio-stimulation (Ethica et al. 2021).

There are certain limitations associated with bioremediation. Especially with respect to genetically engineered microorganisms, microbial population should have metabolic capacity to degrade contaminants (Liu et al. 2019). In addition to that generally, environmental conditions should be proper for their growth and activity such as temperature, pH, etc. Also, there should be the right amount of contaminants and nutrients. If the process is not controlled, then there are chances that those organic contaminants may not be degraded completely and may result in toxic by-products (Sharma 2020). Bioremediation has a great potential with notable achievements, but still, this eco-friendly low-input biotechnology has been underutilized. The global market scenario of bioremediation technology and services is showing an elevation with compound annual growth rate of 8.3% from 2017 to 2025 (Basak et al. 2020). The main purpose of this chapter is to provide an update concerning the current status of bioremediation of polluted fresh and marine water bodies.

14.2 Importance of Water for Life

The embryonic development up to the last minutes of the floral and faunal organisms needs water as an essential element. Most of the biological processors are mediated by water as a universal solvent. Discarded chemical waste from industries and agricultural setup, surface water runoff, poorly maintained septic systems, maritime logistics, and many land disturbing activates create qualitative and quantitative damages to water and water-based systems such as streams, rivers, lakes, and ocean. The hydrology has given immense support to understand complex nature of water-based systems of the earth. We must understand all of the physical, chemical, and biological processes involving water as it travels through the water cycle if we are to learn how to protect it (USGS 2006; Winter et al. 1998).

Water withdrawal also impacts how a watershed functions and interacts with the water cycle. Use might range from a few homeowners or businesses pumping small amounts of water to irrigate lawns. It could also include large municipalities, industries, mining operations, and agricultural producers pumping large amounts of water to support water demands in the region (USGS 2005). Either way, withdrawing water will affect the rate of evaporation, transpiration, and infiltration in a watershed.

14.3 Potential Means of Water Pollution

Water pollution is considered to be a greater concern all around the world, which requires significant attention from international level to the individual. Agricultural runoffs, chemical spills from industries, and sewage leaks are the main sources for the pollution of surface and groundwater bodies. The agricultural runoffs consist of pesticides, herbicides, and fertilizers (Yang et al. 2022). Irrigation in agriculture is considered to be a carrier of excessive salt from fertilizers to water bodies (Cui et al. 2020). When animal husbandry farms are considered chemical oxygen demand (COD) and biological oxygen demand (BOD), ammonia nitrogen (NH3-N), total phosphorus (TP), total nitrogen (TN), and metals are considered to be the main water-polluting concerns (Muratoglu 2020). Following proper management practices in agriculture plays a significant role in controlling this ways of water pollution.

Industries are a major source of water contamination. There are evidences of releasing heavy metals and other toxic chemicals by industries due to the activities like mining and refining metals and other manufacturing processes (Arif et al. 2020; Yuan et al. 2019). When chemical industries are considered, most of the chemicals that are being released are considered to be toxic, carcinogenic, and mutagenic (Issakhov et al. 2021; Aldalbahi et al. 2021). When industrial and municipal wastes are not properly treated and finally are being released into the environment, it becomes a huge concern for the survival of clean water bodies (Liu et al. 2016). In addition, sewage leakage from domestic waste is also another source of contamination because most of the houses are not connected to the main sewage system in cities (Ringo 2016; Naveen et al. 2018). Adding to that, urbanization also becomes intense day by day.

Other than the abovementioned sources of contamination, deforestation, marine dumping, radioactive waste, and atmospheric deposition are considered to be other means of water body pollution. When water bodies become contaminated, they are not further safe for human consumption because of the presence of certain toxic chemicals, heavy metals, and some pathogenic bacterial spp.

14.4 Agricultural Wastes and Its Contribution for Water Pollution

Most human activities have an impact on water and the ecosystem, including domestic, agricultural, and industrial activities. Water pollution has now become a global crisis, posing a serious threat to the entire world's sustainable development (Mali et al. 2016).

Agriculture is one of the most important economic sectors in both developed and developing countries. Surface and groundwater are polluted by significant agricultural activities (Naveen et al. 2021). As a result of that, they are unsafe for human consumption; soil erosion, including irrigation; industrial needs; nitrogen cycle changes; carbon sequestration; and relevant ecological patterns. Water pollution is typically caused by the excessive use of pesticides and chemical fertilizers, which cause changes in the physiochemical activities of the water (Naveen et al. 2021). This is the primary disadvantage that occurs in aquatic bodies nearby (Mali et al. 2016).

The current population's depletion of freshwater resources, as well as the degradation of water quality, is becoming a major issue. Biological, organic, toxic, and inorganic pollutants commonly contaminate surface water resources and a growing percentage of groundwater reserves as a result of improper disposal of industrial effluents, domestic wastes, and agricultural pollutants (Mali et al. 2016). According to previous research, when harmful substances are released in large quantities into water bodies, they cause damage to people, wildlife, or habitats and naturally result in phenomena such as volcanoes, algal blooms, storms, and earthquakes (Mali et al. 2016). These changes have a significant impact on both the water quality and the water's ecological status. As a result, water is life and plays an important role in all types of life (Naveen et al. 2021).

14.4.1 Water Pollution

Water pollution, in essence, highlights contamination in bodies of water (lakes, seas, groundwater, surface water) (Naveen et al. 2021). Water contamination has a large impact on plants and organic habitats, and it is influenced by the harming of specific species or populations in natural networks. Water contamination can occur when toxins are released directly into bodies of water without adequate treatment to remove harmful mixtures. Nitrates, phosphorus, pesticides, soil sediments, salt, pathogens, toxins, agrochemicals, and some organic substances are examples of water pollutants (Norman 2016). In other words, they can also be referred to as agricultural wastes that pollute the water (Naveen et al. 2021).

14.4.1.1 Wellspring of Water Contamination

Water contamination is visible as surface water or groundwater contamination. It is further subdivided into marine contamination and supplemental contamination. The contamination of wellspring water occurs as follows (Naveen et al. 2021).

14.4.1.2 Point Source

Release into surface water from a single point using a pipeline, outfall, or discard. Surface water discharges from feedlots, food processing plants, and agrochemical handling plants, and chemical spills pollute groundwater (Naveen et al. 2021).

14.4.1.3 Nonpoint Source

Pollutants enter receiving water resources through a variety of human activities that do not have a clear entry point. Large agricultural practices and land use typically pollute water (Naveen et al. 2021).

14.4.2 The Relation Between Agricultural Wastes and Water Pollution

Several agricultural activities, including increased use of fertilizers and pesticides, as well as allied livestock activities, have had a negative impact on water quality (Mali et al. 2016). Nitrates, phosphorus, and some pesticides are the most significant water pollutants (Naveen et al. 2021). Rising nitrate concentrations have a significant impact on drinking water quality, while pesticide use contributes to indirect emissions of toxic substances into the water. Increased nitrate and phosphorus levels in surface water bodies reduce their ability to support plant and animal life (Wubetu 2016).

As a result, agricultural pollution is currently difficult to control because it occurs over a large area and has diffuse and difficult-to-identify sources. It is affected by the following environmental factors: patterns of rainfall, land slope, and soil characteristics (Mali et al. 2016).

14.4.3 Public Health Effect

A variety of human illnesses are caused by contaminated water. According to WHO reports, 4 million people die each year as a result of diarrhea, which is common in waterborne saline (Naveen et al. 2021). Furthermore, minor rural contamination can have a virtuous circle effect on human health. According to WHO, as cultivation practices have expanded, nitrogen in groundwater has increased and has been impacted globally (World Health Organization 1993). Human tumors and childhood disorders such as methemoglobinemia can develop as a result of this nitrogen contamination. And newborn children are even more vulnerable to nitrate contamination (World Health Organization 1993). The following are some of the health consequences of developing countries: many vectors, such as mosquitos, thrive in environments that are hostile to their reproduction.

14.4.4 Technologies to Control Agricultural Water Pollution

14.4.4.1 Reducing Leaching and Erosion of Fertilizer

Several technologies have been developed to reduce fertilizer losses and increase nutrient use efficiency, such as optimizing time, methods, and required doses in fertilizer application (NUE) (Mali et al. 2016). Fertilizer application practices that are well designed to match crop needs and soil fertility significantly reduce fertilizer pollution and leach into groundwater (Jeong et al. 2016).

14.4.4.2 Optimal Pesticide Management Practices

Pesticides enter bodies of water via sediments caused by soil erosion. To control pests, a system should be developed to collect surface runoff and leach to reduce the unmethodical use of pesticides. These activities can help to reduce pesticide levels in bodies of water. Adoption of appropriate soil and water conservation measures, as well as the prohibition of certain activities, is timely important (Mali et al. 2016).

There is an urgent need to prevent water resource pollution and promote environmentally friendly reuse of a large amount of wastewater in the agricultural sector. It is possible to reduce agricultural waste contamination of water through appropriate practices such as improved nutrients, pesticides, crops, soil, and other wastewater management (Mali et al. 2016). The primary focus of this review is the impact of agricultural action on water pollution (Naveen et al. 2021). These numerous exercises can alter the water quality boundaries, affecting physicochemical properties. This demonstrates yet another benefit of farmers adopting integrated aquaculture-agriculture, forestry in fish crops, and livestock, which can collectively grow in a cultivating procedure (Naveen et al. 2021). This is because it can reduce the amount of pollution produced. Farmers should also avoid using synthetic fertilizers (India Economic Survey 2018).

14.5 The Toxic Effect of Pollutants on Marine Phytoplankton

14.5.1 Marine Phytoplankton

Marine phytoplankton, or the autotroph portion of the plankton, exists in very well topmost layers of the ocean and obtains its energy from photosynthesis in the clean oceans down to a level of 200 m. The majority of phytoplankton types are minuscule, unicellular creatures that range in size from 0.4 to 200 μm. Less than 1% of the planet's photosynthetic biomass is constituted of marine phytoplankton. Furthermore, this compartment is where more than 45% of the planet's yearly net primary production is formed. The biomass of this extremely productive compartment is maintained low in comparison to the biomass of terrestrial photosynthetic creatures by constant grazing and recycling (Simon et al. 2009). Initiating the emergence of photosynthesis in the Archaean epoch, marine photoautotrophs began to evolve (Katz et al. 2007). The diversified photosynthetic biota, which is the foundation of all complex life, originated with these primordial organisms. Along with significantly altering the biological chemistry of the oceans and atmosphere, they are also accountable for the oxygenation of the atmosphere. With the combination of information from the domains of biology, ocean bio-geochemistry, and atmosphere interactions, comprehension of the origin of phytoplankton has substantially evolved over the last several decades, and awareness of the diversity of this category has been strengthened (Simon et al. 2009).

14.5.2 Marine Pollutants

The maritime ecosystem may be exposed to chemical pollution from both land- and ocean-based human activities. This can come from both discrete sources, such as discharges, dumping, or unintentional spills, and diffuse sources, like runoff from the atmosphere or rivers, which can travel great distances. Pollutants contain nutrients, which may promote undesired algae production, as well as a variety of potentially dangerous compounds, some of which are tenacious, toxic to living creatures, or bio-accumulative. International and national priorities are used to identify the most hazardous materials, as well as quantities in water, biota, and sediment, which are evaluated to make sure they are within justifiable standards. Trace metals, flame retardants, hydrocarbons, pesticides, dioxins and PCBs, and compounds that could interfere with the endocrine system are some of the pollutants the Marine Institute has identified (Marine Pollutants 2022).

14.5.3 Pollutants' Toxic Effects on Marine Phytoplankton

Throughout the previous two centuries of social and economic development, various synthesized organic compounds have been discharged into the environment as a consequence of agricultural, commercial, and household processes. Persistent organic pollutants (POPs), which are resistant to decomposition and semi-volatile, can withstand long-distance atmospheric transmission before being dumped in remote places, like the global oceans. Certain POPs have an impact on marine phytoplankton by altering the quantity of chlorophyll, the acquisition of carbon and silicate, or by impeding photo-system I and the downward transport of electrons from photo-system II. The toxicity of organic impurities on marine phytoplankton has been studied in the literature through laboratory and field testing processes, as well as investigations of particular contaminants and basic compositions of pollutants (Echeveste et al. 2016).

Specifically in the situation of rising sea surface temperature and stratification, atmospheric deposition plays a major influence in supplying nourishment and toxins to the ocean ecosystem (Yang et al. 2019). Research on the impact of natural aerosols, such as volcanic ash and dust, on marine phytoplankton, has received the most attention (Chien et al. 2016). As anthropological activity increases, more chemical constituents are released and transferred to the oceans, altering the chemistry of the water and impacting phytoplankton production (Mahowald et al. 2018).

Aerosols comprising metals have significant influences on climate and ocean bio-geochemistry. Mainly due to their iron content, dust transferred to oceans with high nutrients, low chlorophyll could encourage phytoplankton development (Yoon et al. 2018). Cu, which is essential for living things in contrast to Fe, has favorable and harmful effects on marine phytoplankton depending on the concentration. Cu-rich aerosols may restrict the growth of phytoplankton, and the effects varied among the various phytoplankton taxa.

The sustainability of an entire ecosystem can be damaged by chemical effects on these primary producers. By being exposed to the pollutants of newer generations and chemicals, phytoplankton populations have less ecological efficiency (Romero et al. 2020). Antibiotics, personal care aromas, and plastic nanoparticles are a few of the new generation substances that have an effect on aquatic ecosystems all over the world. They are a pervasive danger to ecosystem conservation due to the outflow they cause from urban wastewater treatment plants (Broccoli et al. 2021). Ecosystems' functioning may be significantly impacted by changes in the diversity and function of phytoplankton and bacterioplankton caused by chemical contamination. Research revealed that sediment resuspension and chemical contamination had a major impact on phytoplankton-bacterioplankton interactions, which can change how anthropogenic coastal ecosystems function (Pringault et al. 2021).

14.6 Technologies for Refine Water Bodies

14.6.1 Chemical Precipitation

Heavy metals are considered to be a serious concern in the context of water pollution due to the development of many industries and due to their nondegradable quality. They need to be removed as much as possible from water bodies to prevent the harm on ecosystems. Chemical precipitation is such one method that is being used for heavy metal removal (Zhang and Duan 2020). In chemical precipitation, changes in the form of heavy metals into solid particles have been made. In chemical precipitation of heavy metals, the ionic forms have been reduced by the addition of counterions (Chen et al. 2018). By doing so, solubility becomes reduced, and removal becomes easy. In conventional heavy metal removal in wastewater treatment, reagents such as sodium sulfide (Na_2S), lime ($Ca(OH)_2$), and soda ash (Na_2CO_3) are being used. Other novel methods are also in the experimented stage in the purpose of value adding this technique (Fu et al. 2012).

14.6.2 Reverse Osmosis

Reverse osmosis has been considered as an efficient technology for separation of pollutants from industrial and municipal wastewater (Li et al. 2020; Lan et al. 2019; Porré et al. 2022, Mejía et al. 2022). It is being highly employed in wastewater treatment plants due to its fine pores in filtration system along with low energy consumption. Several conventional reverse osmosis setups are being in use like tubular, spiral wound, plate, and frame and hollow fiber techniques. Other novel methods, such as developing high-throughput membrane systems, are also in the experimental stage, with the purpose of adding value to this technique (Jiang et al. 2021). But pressure-driven nature of reverse osmosis is considered to be a limitation of this technique.

14.6.3 Electro Fenton's Dialysis

Hydrogen peroxide and ferrous salt are considered to be Fenton's reagents and are being used for a long time for the purpose of oxidizing organics. This concept has been advanced by integrating electrogeneration of H_2O_2 and Fenton's catalyst to produce OH radicals (Hu et al. 2021). This process facilitates degradation of any organic pollutant (Mousset and Hatton 2022). Other sophisticated techniques have also been integrated to increase the efficiency of electro-Fenton dialysis technique (Khalifa et al. 2021).

14.6.4 Adsorption Electrocoagulation

The adsorption and coagulation processes are used for the persistent organic pollutants, which are difficult to remove from wastewater (Titchou et al. 2021). In the process of coagulation flocculation, the solids that are being in suspended state are transformed into more observable and removable flocs by the addition of chemical flocculants. In electrocoagulation, the coagulation flocculation and adsorption techniques are being integrated together. Here, colloidal particles have been destabilized and have been converted into aggregates by electrical double-layer compression. This novel technique facilitates removing a wide range of pollutants from different types of wastewater sources (GilPavas et al. 2019; Nigri et al. 2020).

14.6.5 Chemical Oxidation

In chemical oxidation, hydrogen peroxide (H_2O_2) is used to oxidize pollutants in wastewater. Usually, H_2O_2 is used independently or in some instances is used with available other physical and biological treatment processes. Usually, in chemical oxidation of pollutants in wastewater, catalysts are added. Examples for such catalysts are ion (Fe^{2+} or Fe^{3+}), UV light, and ozone (O_3) (Han et al. 2022). In H_2O_2-based chemical oxidation, in order to accelerate the OH radical formation process, several techniques are used (Dombrowski et al. 2018). Addition of peroxydisulfate and peroxymonosulfate is such highly employed technique. Apart from this, several other techniques like electrochemical oxidation are integrated into chemical oxidation to increase its process efficiency (Umadevi et al. 2022).

In addition to the above described technologies, chemical oxidation, microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) are several techniques that are being used for refining wastewater in different wastewater plants.

14.7 The Role of Microorganisms in Bioremediation

Bioremediation is a biological process that helps recycle waste into a form of a valuable product or non-harm product. Microorganisms directly participate in converting waste into another form that can be recycled or reused. Due to the ability of microorganisms to survive in all places in the world, they are the most suitable ones for use as a biotic agent for bioremediation. Temperature and pH like abiotic factors also influence bioremediation. Degradation, eradication, immobilization, and detoxification are the main processes in bioremediation. With the improvement of biotechnological aspects in the world, different methods and strategies like biostimulation, bioaugmentation, bioventing, bio-piles, and bio-attenuation are used. Microorganisms play a major role in bioremediation.

Microorganisms can be used as bioremediation agents to remove and reduce the harm of pollutants in soil, water, and sediments. Microorganisms live in a wide range of biosphere using their impressive metabolic activities, the capability of using a wide range of nutrients, and the ability to establish or grow in different environmental conditions quickly (Abatenh et al. 2017). Using bioremediation, pollutants like heavy metals, metalloids, hydrocarbons, phenolic compounds, and municipal waste like inorganic pollutants can be remediated (Buckner et al. 2018; Al-Mailem et al. 2017). Bioremediation techniques can be classified based on the applied strategies. These are ex situ bioremediation and in situ bioremediation. In the ex situ bioremediation, polluted or contaminated material is removed from the site and transported into the offsite treating place and treated them with particular microorganisms for remediation. In the in situ bioremediation, treating process is done at the contamination site. This method is more preferred than ex situ bioremediation due to less risk of spill out of contaminant while transportation and no need to excavate and pump out contaminant (Buckner et al. 2018). Bioremediation properties having microorganisms can be found in their native environment like oil spilled lake and discharged industrial effluent. But those native microbial can't be used in a foreign environment. Due to that, to select an effective agent microorganisms should be genetically engineered. This is a good approach for biodegradation of pollutants everywhere like surface water, groundwater, marine environments, and soil and sediments (Brar et al. 2017). For the bioremediation of complex pollutants, mixed microbial populations are needed and effective rather than a single population due to the broad enzymatic capabilities of mixed populations. The novel approach is using a consortium of genetically engineered microorganisms rather than using single species. Bacteria, fungi, algae, and yeast are the microorganisms used in bioremediation. Microorganisms react on inorganic pollutants, heavy metal contaminations, antibiotic contamination, organic pollutants, aquaculture waste, and petroleum contamination by acting as bio-catalysts using their enzymatic pathways and enhancing the biochemical reactions of degrading the pollutants (Pal et al. 2020; Abatenh et al. 2017).

14.7.1 Factors Affecting Microbial Remediation

All the factors that affect microbial growth are directly controlling the efficiency of microbial bioremediation. Those are the chemical nature of the pollutant, available nutrients, environmental temperature, pH, salinity, available oxygen amount, and moisture. Microorganisms should have access to the ample amount of compounds that are required to generate energy and nutrients to grow. If the requirements are fulfilled, the growth of the microorganism population happens optimally. It will lead to efficient biochemical reactions of degrading pollutants (Abatenh et al. 2017).

14.7.2 The Role of Microorganisms in Different Bioremediation Methods

When using fungus as a bioremediator, it is called a mycoremediation. Fungi release enzymes in the polluted sites, degrade pollutants, and remove contaminants (Buckner et al. 2018). Also, fungi act as a biocatalyst for heavy metal due to their ability on absorbing heavy metals into the mycelium and spores. After that, heavy metals accumulate intracellularly and precipitate extracellular. Fungi have the ability to decompose lignin, de-colorize dyes, detoxify contaminated water, and degrade hydrocarbons and polychlorinated biphenyls like complex chemicals into nontoxic chemicals (Pal et al. 2020).

Pollutant	Bioremediator
Heavy metals	
Cr	• *Aspergillus* sp.
	• *Rhizopus* sp.
Heavy metals	
Zn	• *Rhizopus arrhizus*
	• *Penicillium spinulosum*
Pentachlorophenol	• *Rhizopus oryzae* CDBB-H-1877
Polychlorinated biphenyls (PCBs)	• *Penicillium chrysogenum*
	• *Scedosporium apiospermum*
Oil	• *Fusarium* sp.

León-Santiesteban et al. (2016), Pal et al. (2020), and Abatenh et al. (2017)

Bacteria can be used as bioremediator to degrade hydrocarbons, pesticides in the soil, and different textile dyes; utilize heavy metals; and purify crude oil-contaminated water. The abilities of bacteria like sorption, hydrolysis, photolysis, oxidation, and reduction reactions, are directly involving the degradation of pollutants by bacteria. Bacteria act as an absorbent by supplying the proteins and lipids contained in the peptidoglycan layers as active binding sites for heavy metals (Pal et al. 2020). Some bacteria like *Nitrosomonas* and *Nitrococcus* involve nitrification process of sediments in ponds. It helps to clean the toxic compound like ammonia and nitrate, which reduce the quality of the water (Pal et al. 2020). The bioremediation process done by aerobic bacteria can be enhanced by supplying oxygen.

Pollutant	Bioremediator
Oil	• *Pseudomonas aeruginosa*
	• *Pseudomonas putida*
	• Arthobacter sp.
	• *Bacillus* sp.
Hydrocarbons (benzene, xylene)	• *Pseudomonas putida*
Textile dyes	• *Bacillus* spp. ETL-2012
	• *Pseudomonas aeruginosa*
Heavy metals like Cu, Ni, Cr	• *Pseudomonas aeruginosa*
Endosulfan contained in pesticides	• *Bacillus*
	• *Staphylococcus*

Abatenh et al. (2017) and Pal et al. (2020)

Algae and cyanobacteria are also effective and efficient bioremediators. The method that uses algae and cyanobacteria for bioremediation is called phytoremediation. Their ability on growing at a faster rate and can be cultivated on nonarable land caused the popularity of phytoremediation. Algae uptake nutrients from pollutants and use atmospheric CO_2. Due to that, pollutants are degrading. After the process, algal biomass can be used to produce biofuel, can be used as fertilizer, or can be used as feed (Brar et al. 2017).

Pollutant	Bioremediator
Dairy manure effluent	• Nostoc sp.
Textile effluent	• *Chlorella pyrenoidosa*
Agro-industrial waste water	• *Chlorella sorokiniana*
Effluent of leather processing	• *Chlorella vulgaris*
Benzene, bisphenol, toluene	• *Selenastrum capricornutum*
	• *Monoraphidium braunii*

Pal et al. (2020) and Brar et al. (2017)

Yeast species like *Saccharomyces cerevisiae*, *Candida tropicalis,* and *Candida utilis* have the ability to bio-absorb heavy metals like Cu, Ni, and Cd and to remove the pollutant from the contaminant sites (Pal et al. 2020).

Microorganisms play a major role in bioremediation. Using microorganisms in bioremediation is very cost-effective and efficient method rather than doing it with different mechanical and chemical methods. The efficiency of microorganisms can be improved using genetic engineering methods.

14.7.3 Microbial Contribution for Eutrophication and Their Metabolic Activities

The term eutrophication refers to the increase of the production of organic materials leading to a progressive rise in the biomass of primary producers in aquatic ecosystems such as lakes, ponds, rivers, oceans and streams, wetlands, swamp, estuaries, etc. Eutrophication is a natural phenomenon, which accompanies the evolution of an aquatic ecosystem over geologic time; however, it has been induced by anthropogenic activities in recent past (Le Moal et al. 2019). The main drivers of anthropogenic eutrophication are diffuse nitrogen and phosphorus pollution due to application of agricultural inputs (Beusen et al. 2016). This article reviews different ways of how microorganisms contribute and response to eutrophication.

14.7.3.1 The Contribution of Microorganisms for Eutrophication of Freshwater Ecosystem and Their Metabolic Activities

The increase of phytoplankton biomass including frequently dominant cyanobacteria is the main effects of lake eutrophication. Therefore, it creates two water layers such as upper layer, the warmer and lighted epilimnion, where primary production occurs, and a colder deeper layer, the hypolimnion. The epilimnion was rich in oxygen, and hypolimnion was deoxygenated. The hypoxia condition leads to increase the internal phosphorus load released by sediment. Further, increase of cyanobacteria blooms is directly linked to ongoing climate change, and diastrophic species of cyanobacteria has the ability to fix atmospheric nitrogen. Moreover, cyanobacteria are altering the food web in lake due to low nutrient levels for zooplanktons (Vinçon-Leite and Casenave 2019).

The characterization of bacterial and microbial eukaryotic communities associated with an ephemeral hypoxia event in Taihu Lake, a shallow eutrophic Chinese lake, was studied by Cai et al. (2018). The hypoxia is defined as a dissolved oxygen level below than 1.42 ml/L and hypoxia zone inhospitable to macrobiota; however, these zones allow to thrive unique bacterial communities, which they can cycling of bulk nutrients and trace elements. The results revealed that metabolic function of bacterial communities were phosphotransferase system, oxygen respiration, ABC transporters and fermentation, phototrophy, nitritation (HAO), and methane oxidation. The hypoxic site commonly enriched anaerobic functional categories was fermentation, fumarate respiration, denitrification (norBC and norZ), dissimilatory sulfur metabolism, dissimilatory nitrite reduction to ammonium, hydrogen oxidoreduction, methanogenesis, nitrogen fixation, aromatic compound degradation, nitrite respiration (nirKS), cellulolysis, and chitinolysis. The vadinBC27 subgroup of Bacteroidetes bacteria has capability to digest algal detritus. The nitrogen cycling is a one of the major geochemical transformations at hypoxic zones. However, authors have reported that nitrifies (*Nitrosomonas* and *Nitrosospira*) activity is lower in hypoxic sites due presence of low oxygen. In contrast to nitrifies, the activity of denitrifies were dominated in hypoxic sites. For instance, *Pseudomonas* species have the ability to do both heterotrophic nitrification and denitrification in simultaneously. Another pathway of removal of nitrogen is ANNOMAX process during algae blooms. Further, sulfur-reducing bacteria (Desulfovibrionales or other common sulfate-reducing bacteria (SRB)) activity is more common in hypoxic sites, and they are responsible to produce H2S gas. Although many eukaryotes such as algae, protozoa, copepods, and fungi prefer to live in oxygen-rich environment, many of them have an antihypoxia capacity. The accumulation of algal scums provides suitable habitat for aquatic fungi and feeds by attachment to organic aggregates and phytoplankton-derived biopolymers. The decomposition of algal biomasses (largely comprised by *Microcystis* spp.) has released the high concentration of dissolved microcystins. Mucor is a more common fungal in hypoxic sites. They have ability to hydrolyze xylose, straw materials, and polysaccharides and directly consume acetic acid under anaerobic condition. Lei et al. (2021) stated that algal bloom containing cyanobacteria releases high amount of algal organic matter (AOM), which will enhance the growth of archaea (methanogens). These methanogens significantly contributed to increase the methylmercury (MeHg) production in eutrophic shallow lakes. Yang et al. (2020) studied the influence of eutrophication on methanogenesis pathways. Authors reveled that eutrophic lake could present a high potential of methanogenesis and more common methanogens in lake were Methanoregula, Methanolinea, Methanobacterium, and Methanosaeta. Further, long-term eutrophication causes to release high ammonia level in lake.

The phytoplankton consisting of diatoms, cyanobacteria, dinoflagellates, and green algae are responsible for eutrophication, and cyanobacteria lead a major role. Eutrophication in freshwater lake leads to hypoxia condition that is a contribution by bacterial and eukaryotic communities. Algal bloom contains cyanobacteria release high amount of AOM that will enhance the growth of methanogens, which contribute to MeHg production in eutrophic shallow lakes.

14.8 Marine Bacteria: Potential Candidates for Enhanced Bio-Remediation

Bacteria are abundant in nature owing to their capacity to adapt to extreme circumstances and perform a variety of physiological activities. Among all the ubiquitous bacteria, marine bacteria have been identified as playing a major role in a variety of biogeochemical processes. Because environmental conditions are constantly changing, the microorganisms in that environment have complicated characteristics of adaptation because they are better suited to the challenging conditions. Therefore, by forming biofilms and producing extracellular polymeric molecules, the bacteria isolated from marine habitats are expected to be more effectively used in the bioremediation of heavy metals, hydrocarbons, and many other refractory chemicals and xenobiotics. The structure, variety, and functional potential of marine microbial communities found in saltwater and sediment have recently been revealed at a global level thanks to novel developments in molecular ecology techniques. This review places particular emphasis on the use of marine bacteria in the field of bioremediation and identifies the process by which adaptive responses obtain their unique features.

Microorganisms are crucial to the maintenance and sustainability of any ecosystem because they can adapt more quickly to environmental changes and degradation. Since they are found everywhere, including in volcanic eruptions, Antarctic glaciers, and conditions on Mars, marine ecosystems are not unlike several other types of habitats. Oceans are considered a source of untold riches, which have been covered by 71% of the globe's surface by water and contain salt water (97%). Also, this water contributes 32% of global net primary production. Additionally, the seas serve as the primary global trade route and the primary climate stabilizer. The oceans are rich in a high diversity of different species, and 178,000 species of marine organisms belong to 34 phyla (UNEP report on the global biodiversity assessment). The oceans have been contaminated globally by substances like petroleum hydrocarbons (PHCs), polycyclic aromatic hydrocarbons (PAHs), antibiotics, heavy metals, and excessive nitrogen and phosphorus; also as a range of pollutants like radioactive wastes, plastics are contaminating the marine environment (Gosai et al. 2017; Dudhagara et al. 2016) as a result of urbanization and the landscape's subsequent industrialization over the past few decades. These contaminants may build up in seawater and sediments, especially in coastal locations, and eventually endanger marine ecosystems and human health. Simply put, the oceans have been indirectly impacted by overpopulation, urbanization, and increased industrialization because waste from all sources eventually makes its way to the sea or ocean. In addition, pollution is caused by offshore drilling and related activities (Gosai et al. 2017, 2018). Therefore, there is a huge requirement to find methods to remove pollutants that have become a serious issue around the globe. Previously, a variety of physicochemical techniques have been used to remove contaminants. These techniques, however, cannot be a long-term solution due to one or both of their shortcomings. To solve this issue, microorganism engagement has been given top priority, and microbial bioremediation has recently received attention (Vala and Dave 2017; Vala et al. 2018). Due to their wide range of catalytic activities and capacity to survive in challenging environments (such as hypersaline, low temperature, acidic or alkaline pH, and high pressure), marine microorganisms are the best candidates for the bioremediation of ocean toxins (Homaei 2017). In order to prove that marine bacteria can be used in enhanced bioremediation, this review summarizes the most recent findings on the unique properties of marine bacteria, their physiologic and genetic adaptation to the dynamic environmental conditions, biogeography and diversity, and the role of marine bacteria in various remediation aspects with the metagenomic approaches.

14.8.1 What Is Bioremediation?

The bioremediation process can be defined as a biological mechanism for recycling waste into another form that can be used and reused by other organisms. Currently, most parts of the world face this problem with various kinds of environmental pollution accumulation on water and land surfaces. It is primarily based on nutrient versatility, but it also relies on the ability of certain microorganisms to convert, modify, and utilize toxic pollutants in order to produce energy and biomass.

Although it can be considered as a well-organized procedural activity, which is applied to break down or transform contaminates into less toxic or nontoxic elemental or compound forms, through the use of microorganisms, bioremediation involves the removal, modification, immobilization, or detoxification of different chemicals and physical pollutants from the environment. Mainly, there are two types of contaminant that can be identified within the polluted surfaces as heavy metal inorganic compounds and organic aromatic carbon compounds. By acting as biocatalysts and accelerating the biochemical processes that breakdown the intended contaminant, microorganisms play a role in the process. Microorganisms can only effectively combat contaminants provided they have access to a variety of materials and chemicals that will enable them to produce energy and nutrients for the growth of new cells. The chemical makeup and concentration of pollutants, the environment's physicochemical properties, and the availability of the pollutants to microorganisms are only a few of the variables that affect how effective the process of bioremediation is (Abatenh et al. 2017).

14.8.2 Potential of Marine Bacteria to Adapt for Environmental Changes

They are perfect for prospective bioremediation and bio-indicator uses because they react fast to changing environmental patterns such as the sea surface temperature, the pH of the immediate surroundings, the shifting UV and light patterns, the sea level rise, tropical storms, and terrestrial inputs, which are only a few of the periodic variations that affect the marine ecosystem. Alphaproteobacteria make up the majority of the microorganisms found in the marine environment. This could be because they are better able to adapt to the changing conditions there. Numerous investigations have been made to determine the molecular basis of adaptability in this group of bacteria with the novel techniques (Gutierrez et al. 2018).

The pattern of growth rates, gene expression, physiological or enzymatic activity, and changes in close or symbiotic relationships with other species are all ways that bacteria adapt to a variety of environmental conditions. When exposed to extreme conditions like pressure, temperature, salinity, and the depletion of micronutrients, some groups of marine bacteria have also been reported to develop many novel mechanisms, such as the synthesis of bioactive compounds, while some other types of bacteria displayed mechanisms to overcome the situations of adaptation toward elevated temperature in seawater including chemotaxis and adhesion to α-galactoside receptor in the coral mucus penetration into epidermal cell differentiation into a viable-but-not-culturable state, intracellular multiplication, developmental defects, and symbiosis with other organisms, which is mostly found in pathogenic microorganisms (Beygmoradi and Homaei 2017).

14.8.3 Marine Bacteria Being a Potential Candidate in Bioremediation Process

By utilizing the ambient genome pool of microbes with incredibly precise catalytic capabilities, metagenomic approaches increase the likelihood of discovering new genes and enzymatic pathways that are supportive of bioremediation processes. On the polymer surfaces (plastic substances), dense microbial biofilms were found using fluorescence in situ hybridization and high-resolution microscopy imaging. With the help of amplicon sequencing of the 16S rRNA gene, it was revealed that the orders Flavobacteriales, Rhodobacterales, Cytophagales, Rickettsiales, Alteromonadales, Chitinophagales, and Oceanospirillales made up the majority of the bacterial communities on all types of plastic in the Mediterranean Sea (Annika et al. 2021). Proteogenomic and metabolomic methods have been used to narrow down the pathways and enzymes that the marine bacteria Mycobacterium sp. DBP42 and Halomonas sp. ATBC28 use to break down plasticizers (Wright et al. 2020). The use of bacterial species, genes, and enzymes derived from microbial communities has been found to be quite effective in the remediation of sites contaminated with diesel and petroleum pollution (Garrido-Sanz et al. 2019).

Some research has discussed the significance of biofilm-forming bacteria in the bioremediation of heavy metals, particularly mercury, in a case study. Notably, growing mercuric pollution in the ocean not only contaminates food chains but also accelerates ocean acidification (Wang et al. 2018). According to the authors, extracellular polymerase-producing bacteria like *Bacillus cereus* BW-03 are promising candidates for inorganic mercury remediation because they can produce extracellular enzymes, biosurfactants, polysaccharides, and amyloids.

Marine bacteria *Pseudomonas fluorescens* BA3SM1 was shown to be highly resistant to cadmium, copper, and zinc with the help of genomics approaches. This bacterium is considered a promising agent for the remediation of polluted waters and sediments.

In a study, it was shown that the marine actinobacterium *Brevibacterium linens* BS258 that produces urease can precipitate and dissolve calcite under various Ca^{2+} concentrations. Calcite precipitation and dissolution were connected to carbonic anhydrase (CA) activities, quorum sensing (QS), and other crucial energy metabolic activity, according to genomic sequencing, transcriptome profiling, and other assays of *B. linens* BS258. Further analysis focused on the heavy metal resistance and removal capacities of *B. linens* BS258. The final outcomes of this study provided fresh insight into the mechanisms at play the potential of bacterial carbonate bio mineralization in marine environment's metal bioremediation.

The results of an analysis of a research study of native microbial communities in seawater samples of the Northwest coast of the Iberian Peninsula showed that the oil-enriched microbial communities were dominated by hydrocarbon-degrading bacteria with no significant differences in geographical locations, such as *Alcanivorax*, *Pseudomonas*, *Acinetobacter*, *Rhodococcus*, *Flavobacterium*, *Oleibacter*, *Marinobacter*, and *Thalassospira*, which could represent prototype consortia for mitigating oil spills. Through production of H2S, sulfate-reducing microorganisms may cause oil souring in oil reservoirs, while nitrate-reducing microorganisms can inhibit sulfate reduction that leads to biosouring mitigation. It has been suggested that mitigation strategies for biosouring could be improved by monitoring volatile fatty acid concentration and microbial diversity in oil reservoirs.

The ability of marine microorganisms to adapt to rapidly changing environmental conditions is well understood, but little is known about how they can resist a toxic environment with culturable techniques. Therefore, applying genomic analysis (culture-independent) research in this area will aid in our understanding of the genetic basis of prodigies of nature. They may have a high potential to be more effective bacterial entities for enhanced bioremediation if they make some useful genetic system modifications (Beygmoradi and Homaei 2017).

14.9 System Biology Approaches in Microbial Reconstruction for Bioremediation

Environmental pollution is a serious problem today, and bioremediation can play an important role in cleaning up polluted sites. Remediation strategies such as chemical and physical approaches are not sufficient to mitigate pollution problems due to the continuous generation of new recycling pollutants due to human activities. Bioremediation using microorganisms is an environmentally friendly and socially acceptable alternative to conventional remediation approaches. Although many microorganisms with bioremediation potential have been isolated and characterized, in most cases, the target pollutants are either completely degraded or ineffective in mixed waste situations. This review envisions advances in systems biology (SB) that enable the analysis of microbial behavior at the community level under various environmental stresses.

Agricultural practices and industrial development are significant features of human civilization. Over the last few decades, excessive use of pesticides and chemical fertilizers in agricultural practices has led to environmental (water, land, and air) pollution. The industrialization has the unfortunate side effect of intentionally or accidentally releasing toxic organic and inorganic chemicals and heavy metals that adversely affect the environment. The leading pollutants from these sources are chemical solvents, paints, industrial by-products, petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), nitroaromatic compounds, industrial solvents, polychlorinated biphenyls (PCBs), trichlorethylene (TCE), phthalates, benzene, ethylbenzene, toluene and xylene (BTEX), heavy metals, and pesticides. To maintain a healthy environment and to treat existing highly polluted sites, it is necessary to remove these pollutants from waste to prevent their dispersion in the environment. Contaminant remediation was in some cases achieved using physicochemical methods such as solidification, filtration, combustion, evaporation, oxidation and reduction, reverse osmosis, chemical precipitation, electrochemical treatment, and ion exchange approaches. On the other hand, bioremediation, is a more reliable and eco-friendly approach that utilizes the natural ability of microbes and plants to remove or neutralize pollutants in the environment.

Plants and many microorganisms, including bacteria, fungi, and algae, have been reported to have high neutralizing capacity (Avlopoulos et al. 2016)

The successful use of systems biology (SB) and metabolic engineering (ME) approaches in various fields of biology makes it attractive for ecologists to use these approaches for bioremediation. These approaches to pollutant neutralization. The systems biology approach consequently provides valuable detailed information about biological processes. Then, ME can be used to exploit this information to modify microbial metabolic pathways to neutralize specific single or multiple pollutants simultaneously. This review provides insight into SB and facilitates the design and optimization of microbial cell factories for the bioremediation of pollutants at significant levels (The Financial Express 2018).

14.9.1 Systems Biology in Bioremediation

Bioremediation optimization through microbial SB approaches is novel and offers great potential. In general, SB is used to study complex biological systems to explore complex networks and their interconnections in various biological processes at the molecular, cellular, population, community, and ecosystem levels. It provides significant insight into gene expression, enzymes, biosynthetic pathways, and secondary metabolism of microbes and can reveal the alteration of pathways already present under conditions of stress caused by various pollutants. "Omics" techniques (genomics, proteomics, transcriptomics, and metabolomics) are widely used in systems biology for studying the pollutant neutralizing capacity of microorganisms. A comprehensive list of bioremediation examples with toxic chemicals using omics (Avlopoulos et al. 2016).

14.9.2 Genomics

Genomics involves gene sequencing and bioinformatics analysis using a variety of tools and algorithms. The genomes of several microorganisms involved in bioremediation have been sequenced. Genome sequence (6.2 MB) analysis of *Pseudomonas* sp. KT2440 showed the presence of genes encoding many enzymes or proteins such as dehydrogenases, oxidoreductases, oxygenases, ferredoxins, cytochromes, glutathione-S-transferases, sulfur-metabolizing proteins, and efflux pumps. These biochemicals play a significant role in the degradation of several chemicals released from industrial wastes (Zhu et al. 2017).

14.9.3 Transcriptomics

Transcriptomics is mainly used to investigate the differential expression of upregulated and downregulated genes in response to environmental pollutants. It also helps to infer the function of previously unannotated genes. Techniques such as microarrays and RNA sequencing can be applied to quantify a predetermined set of sequences. Analysis of microbial community transcriptomes requires (1) isolation and enrichment of total cellular mRNA, (2) synthesis of cDNA from total mRNA, and (3) sequencing of total cDNA or the use of microarrays for cDNA hybridization (Zhu et al. 2017).

14.9.4 Proteomics

Analysis of the set of proteins produced by bacterial cultures (proteomics) and environmental samples (metaproteomics) can be used to detect changes in the composition and production of proteins and to identify many proteins important to the physiological response of microorganisms in the presence of contaminants.

14.9.5 Metabolomics and Metabolic Flux Analysis

Metabolomics is another rapidly developing field of SB at the interface of biological sciences and chemistry. In this approach, whole set of cellular metabolites produced by microorganisms is analyzed. Microbial metabolism is tightly regulated at various genomic, transcriptional, and posttranslational levels. Metabolic flux analysis, sometimes called "fluxomics," measures the rate of metabolic reactions on a real-time basis to study cellular metabolites produced under specific environmental conditions (Klassen et al. 2017).

This article highlights the various techniques used in SB that help the bioremediation of environmental pollutants through microorganisms. Various omics-based tools in SB, such as genomics, transcriptomics, proteomics, and metabolomics, provide significant information for understanding the complex behavior of microorganisms that play an important role in bioremediation. But in natural conditions, the process is very slow and not very efficient, so SB provides a platform for the reproduction of microorganisms, and metabolic pathways are reconstructed to improve this process. Thus, the integration of SB has opened the way to realize the true potential of bioremediation (Bonifay et al. 2017).

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15

Pollution in Freshwater: Impact and Prevention

Nandan Singh and Maitreyie Narayan

Abstract

Water is the life blood of all living things and the most important natural resource on the earth planet. The water is available in liquid, solid and the gaseous forms at the surface of the earth. In the earth, only 2.5% water is present as freshwater. Of this 2.5% freshwater, 68.7% freshwater is locked in ice caps and glaciers, 30.1% is present as groundwater, and only 1.2% is present as other surface freshwater; of this 1.2% water, approximately only 0.5% freshwater is present in the rivers. It is difficult to increase the water supply to the earth, but it is possible to reduce the impact and extend of water resource pollution by managing the supply better. Water pollution is a phenomenon of contamination that means drop of water quality of land waters such as rivers, lakes, seas, oceans and groundwater. It is a major environmental problem across the world. Pollutant transport is the most important linkage between freshwater due to its severe impact on the health and integrity of ecosystems. People are not much aware of the remedies, causes and control of water pollution. The water pollution management and control measures could be through awareness, practice efforts, prevention and minimizing waste, with the help of programmes and projects, monitoring, regulations and legal framework and by avoiding the use of pesticides, fertilizers and non-toxic cleaning material.

Keywords

Pollution · Water · Glaciers · Wetland

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15.1 Introduction

The future of the earth depends on environmental components such as water, and the sustainability of these components is very important for all living organisms in the earth. All the organisms in the ecosystem are interrelated and connected to each other for sustainability. Therefore, any changes or deterioration in a part of the ecosystem can affect the whole ecosystem over time. Water is far distinct from other environmental components as it is the main source of life and cannot be substituted (Kılıc 2021). A large portion of the earth is covered with water, but the amount of freshwater available is very low. Besides all the natural causes, the damages caused by anthropogenic activities have created pressure on limited freshwater resource availability and global water problems. All domains of water quality are impacted by the natural interactions between rock and soil heterogeneities and water, including anthropogenic activities that alter natural water cycles (Trabelsi and Zouari 2019; Akhtar et al. 2021). The operation of every living thing and human health may be severely impacted by these changes in water quality. The physicochemical and biological features and the quality, quantity and availability of water resources constantly change as a result of the influence of natural and anthropogenic activities (Khatri and Tyagi 2015; Akhtar et al. 2021).

15.2 Distribution of Water

Between two-thirds and three-fourths of the earth's surface is covered with water that is present in the liquid, solid and the gaseous forms. Of the 100% water available on the earth, 96.5% is present in the oceans, 1% as other saline water, and only 2.5% is present as freshwater. Of this 2.5% freshwater, 68.7% freshwater is locked in polar ice caps and glaciers, 30.1% is available as groundwater, and only 1.2% is present as other surface freshwater; of this 1.2%, approximately only 0.5% freshwater is present in the rivers (Fig. 15.1).

15.3 Types of Water

15.3.1 Atmospheric Water

The atmospheric water mainly comes from evaporation in the atmosphere from the surface of both the ocean and the land such as transpiration and direct evaporation from the surfaces of green plants (evapotranspiration, the sum of all processes by which water moves from the land surface to the atmosphere via both evaporation and transpiration) and evaporation from ice (sublimation), which leads to an increase in the water content of the atmosphere (Table 1.3).

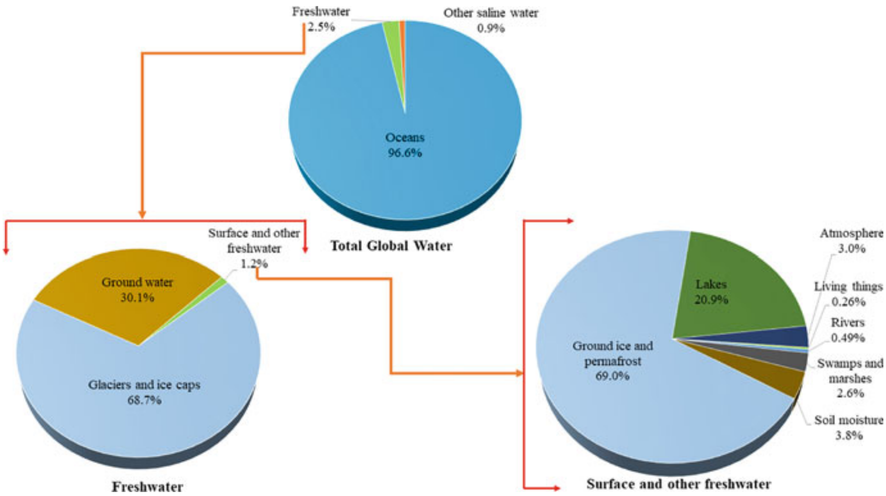

Fig. 15.1 Proportionate distribution of the earth's water (total global water, freshwater, surface water and other freshwater)

15.3.2 Oceans, Inland Seas, Costal Zones and Estuaries

An ocean is a continuous body of salt water that is contained in an enormous basin on the earth's surface. The important oceans and their marginal seas cover almost 71% of the earth's surface, with a mean intensity of 12,100 feet. Inland seas are landlocked seas that are only connected to the ocean through narrow channels. They generally contain many channels, islands, sounds and straits (Barrie et al. 2012). Due to the waves and ensuing currents, the water in the coastal area is quite erratic, the marine border of this zone is defined and is constrained by depths equivalent to ten times the wave height (Khublaryan 2009). An estuary is a partially enclosed coastal water area freely connected with the ocean, within which the seawater is considerably diluted by freshwater flowing from a river catchment area; these are transient zones between the seas and land (Table 1.3).

15.3.3 Rivers, Reservoirs, Lakes and Wetlands

A river, lake, or wetland is a body of water that flows through a self-developed bed augmented by groundwater and surface. The character and development of the water body are influenced by various factors such as the climate, its geological structure and the dimensions of its basin. The water body can be divided into mountain and plain rivers. Plain rivers flow more slowly in wide-relying valleys. A water reservoir is an artificial body of water that can be formed in a river valley to regulate its use for the benefit of the natural economy. It can be divided into three types: mixed, lacustrine and fluvial. Water in permanent or temporary reservoirs can be used for days, weeks, or even a year (Table 1.3).

A lake is a naturally occurring water storage area with a lake basin that is not immediately connected to the ocean. Basins are classified into tectonic, glacial, fluvial, coastal, sinkhole (in karst and thermokarst), volcanic and dammed (made reservoirs and ponds) categories based on their origin (Khublaryan 2009). A wetland is a region of land that experiences a constant or an excessive moistening, which encourages the growth of hydrophilic plants and particular soil processes (Table 1.3).

15.3.4 Groundwater

Water present in the earth's crust of any physical state, including massively crystalline rock cracks and sedimentary rock layers, is referred to as groundwater. There are numerous groundwater classifications based on the geological age, lithological composition, hydrodynamics, temperature, chemical composition and groundwater infiltration and dispersion (Table 1.3).

15.3.5 Soil Water

The term "soil water" typically refers to water that is both closely associated with soil skeletal particles and freely permeable through the soil profile. It may also refer to the surface area of the land as water that is localized in soil pore space, in the liquid moisture form, in the ice form as a solid component and in the soil air form, as a gaseous water form or both (Table 1.3).

15.3.6 Glaciers, Icebergs and Ground Ice

A glacier is a moving, naturally occurring build-up of ice on the land, when the solid phase of water is outweighed by the liquid phase. Only places on the earth's surface where solid precipitation exceeds evaporation are home to glaciers. When a glacier or a glacial barrier breaks, massive pieces of continental ice float in the polar and adjacent oceans as icebergs. Ground ice refers to all types of ice that form in frozen and frozen–cold ground. Ground ice, which forms in soil or rock pores, holes, cavities, or other openings, includes massive ice as one of its constituents. Buried glacier, lake, river and snow bank ice are all categorized as a single form of ground ice (Khublaryan 2009) (Table 15.1).

Table 15.1 Distribution of water over the world (Source: Chapter on "World Fresh Water Resources" by Igor Shiklomanov in Peter H. Gleick (editor), 1993's Water in Crisis: A Guide to the World's Fresh Water Resources (Oxford University Press, New York)

Water source	Water volume (miles^3)	Water volume (km^3)	Percent of freshwater	Percent of total water
Oceans, seas and bays	321,000,000	1,338,000,000	–	96.54
Ice caps, glaciers and permanent snow	5,773,000	24,064,000	68.7	1.74
Groundwater	5,614,000	23,400,000	–	1.69
Freshwater	2,526,000	10,530,000	30.1	0.76
Saline water	3,088,000	12,870,000	–	0.93
Soil moisture	3959	16,500	0.05	0.001
Ground ice and permafrost	71,970	300,000	0.86	0.022
Lakes	42,320	176,400	–	0.013
Freshwater	21,830	91,000	0.26	0.007
Saline water	20,490	85,400	–	0.006
Atmosphere	3095	12,900	0.04	0.001
Swamp water	2752	11,470	0.03	0.0008
Rivers	509	2120	0.006	0.0002
Biological water	269	1120	0.003	0.0001

15.4 Water Pollution

Water flows all over the world, regardless of borders, crossing state lines and country, which means that pollution from one part of the world can affect the other part (Kılıc 2021). This makes it more difficult to set any standard in own ways of using and conserving the earth's water. The word "pollution" can be defined as contamination, dirtying, desecration, spoiling, soiling and destruction (Karatas and Karatas 2016). Many of the sorts of pollutions caused by water pollutants are primarily widespread and affect the health of all residing organisms, mainly mankind. In the recent years, one of the biggest problems that humans are facing is related to water quantity and water quality (UNESCO 2009). Water needs to be kept safe and shielded from pollution of all kinds. Water is among the indispensable ingredients in the centre of the life. Without water, life is not possible, but because water resources are polluted by a variety of hazardous industrial pollutants, it is unhealthy for humans to drink; irrigation activities make water scarce for both people and the environment. Water sources are often unknowingly utilized and poisoned by people, endangering their offspring (Kılıc 2021). Contaminated water is unhealthy for humans to consume since it includes harmful or toxic compounds and bacteria and organisms that cause disease. The quality of water is mainly threatened by agricultural activities, cities, industries waste, mining areas and other so many causes. This pollution is then transferred to surface and groundwater (Dwivedi 2017).

15.4.1 Freshwater

Freshwater environments are a few of the most beneficial biological systems in the world (Ghermandi et al. 2008) and account for many significant services to human culture. In addition, they are extremely environmentally sensitive and flexible ecosystems (Turner et al. 2000). According to their origin, geographical location, water management strategies, water chemistry, dominant species and soil and sediment physiognomies, freshwater ecosystems exhibit a wide range of diversity (Kumaraswamy et al. 2020). In the light of their hydrological, natural and land characteristics, the freshwater ecosystems were divided into three groups: lacustrine (lakes), riverine (along waterways and streams) and palustrine (marshes and lowlands).

The world is concerned about the unfavourable economic, social and environmental consequences of declining water quality in freshwater ecosystems. In the case of small water bodies, such as tanks, lakes and lakes in the past, the problem of declining water quality is notably more alarming, affecting several social (water supply), economic (livestock, fisheries and forestry) and ecological functions (nutrient recycling and groundwater recharge).

15.4.2 Freshwater Pollution

In the recent years, there has been an obvious increase in the demand for freshwater due to the rapid growth of population and fast industrialization (Ramakrishnaiah et al. 2009). Most agricultural development activities endanger living organisms, ecosystems and human health, particularly in terms of excessive fertilizer application and unsanitary conditions (Okeke and Igboanua 2003). Human-based activities have deteriorated water quality in most part of the earth. Freshwater pollution is among the most crucial issue all over the world. Therefore, protecting freshwater sources and freshwater quality is the most urgent need, because of serious negative water pollution issues and global scarcity of freshwater resources. The freshwater pollution grade depends on the pollutant's ecological impact, its abundance and water use. Rainfall can run across the land, collecting contaminants such as pesticides and fertilizers from agricultural lands, forestry and lawns; animal waste, oil and urban road salt (Toth 2009) and toxic elements from abandoned mines are the various causes that lead to freshwater pollution.

15.4.3 Sources of Freshwater Pollution

Among the natural resources, water is the most vulnerable to pollution. Contaminants enter surface water from a specific, identifiable source or from a dense, poorly defined region. Contaminants that originate at a distinct location, such as a pipe, ditch, tank, or sewer, are examples of point source pollution. Point sources are easy to identify and hence relatively easy to stop. Direct pollution from a

Table 15.2 Properties of the point source water pollution and non-point source water pollution (Source: Kumaraswamy et al. 2020)

Point source water pollution	Non-point source water pollution
Industrial effluent and municipal pollution	Runoff from forest areas, agriculture fields and lawns
Leachate and runoff from dump sites for garbage	Runoff from the range and meadow
Runoff from stables and livestock	Runoff from locations both with and without sewers areas
Runoff from industrial locations, oil refineries and mining sites	Putrefying tank leachate
Storm water sewer overflow	Runoff from abandoned mines and building establishment
Sewage overflows caused by drainage and human waste	Deposition of atmospheric materials on a water surface
Runoff from the construction areas	Impurity-producing activities on the land including logging, wetland modification, building and the development of land or waterways

point source may enter the water. Large lagoons may have vessels that leak lubricant or deposit waste (Bhat et al. 2018). Invasive plants and animals can be transported through routine vessel operations, such as the discharge of ballast water (water needed to steady a ship), which will inevitably lead to the extinction of native species. Contaminants that come from agricultural field, livestock pens and atmosphere are examples of non-point source pollution (Kumaraswamy et al. 2020). Non-point source pollution is more difficult to control than point source pollution since it is caused by a variety of contaminants (Table 15.2).

Population growth, industrialization, urbanization, plastics materials and polythene bags, domestic sewage, pesticides and fertilizers, eutrophication, mining, agrochemical wastes, nutrient enrichment, thermal pollution, oil spills, sediment disruption, acid rain pollution, radioactive waste and climate change are the primary causes of freshwater pollution. Other causes include acid rain pollution, thermal pollution, nutrient enrichment and radioactive waste. It is recorded that 75–80% water pollution is caused by the domestic sewage. Wastes from the electroplating industries, including pesticides, sugar, textiles, paper and pulp, are causes of water pollution (Kamble 2014). Polluted water and water sources smell terrible and have fewer plant and animal species. Nearly about 80% of the world's population is facing threats to water security. Most domestic sewages are discharged into rivers, and the majority of these are untreated. Solid trash, toxins, plastic garbage and bacterial pollutants are all found in domestic sewage, and these hazardous substances pollute the water. One of the main causes to the contamination of freshwater is the discharge of various industrial wastes into rivers without any treatment (Desai 2014; Kılıc 2021).

Increasing population has a detrimental impact on freshwater pollution since it increases the production of solid waste generation (Jabeen et al. 2011). Rivers get both liquid and solid waste discharges, and human excrement contaminates the water. In contaminated water, a large number of bacteria are also found, which are detrimental for human health (Haseena et al. 2017). As more people move into towns and cities, these factors cause freshwater pollution, and there is more water consumption in these areas. Deforestation and urban growth often lead to water pollution. Wetlands are nature's means of filtering and damming water; their destruction eliminates the natural habitats and filters that may store and degrade numerous pollutants; therefore, destroying wetlands is also the cause of water pollution (Kılıc 2021).

15.5 Impact of Freshwater Pollution

Pollution destroys water's natural structure and causes a change in their properties that will harm human and living being's health (Kılıc 2021), and water pollution also has many negative effects on the entire ecosystem. An increase in water pollution has a negative impact on plant development; forests cannot grow, and the minerals required for photosynthesis cannot be obtained from groundwater sources. Freshwater pollution causes many bacterial, viral and parasitic diseases in human body. The growth of algae prevents fish and other freshwater organisms from taking oxygen and negatively affects the ecosystem. Thick, green muck generated by harmful algal blooms has an adverse effect on clear water, leisure activities, companies and property values (US Environmental Protection Agency 2022). It is a natural and an essential process for clay, silt and sand from the ground to wash off into streams and lakes. Floodplains and wetlands are built up by sediments, which also carry nutrients. However, logging, ploughing and other disturbances to the land caused by the construction of roads or buildings can cause excess sediment runoff.

Environmental risk factors that cause diseases with a high mortality in developing countries include unsafe water, lack of wastewater system and inadequate hygiene practices. Intestinal diseases are among the evidentiary results of these factors. Diseases such as diarrhoea, hepatitis and cholera stand out as the main diseases caused by freshwater pollution (World Health Organization 2022). The free circulation of toxic waters in nature poisons the soil and especially pollutes the groundwater (Kılıc 2021). Damage to the soil threatens the lives of living things in the region. With the discharge of sewage water into the seas, the lives of the creatures in the sea are adversely affected. The root causes of water pollution are improper housing, overfishing, and improper use of technology. Exposure to various chemicals causes illness and death as a result (Wear et al. 2021). The natural balance of living things that take these chemical substances into their bodies is disturbed. There is a big association between water pollution and health problems. Many waterborne illnesses and infections are transmitted from one person to another (Halder and Islam 2015).

Lakes and streams with a low pH support a lower quantity and variety of life; if water is polluted, its pH level changes. Most marine plants grow best in water with a pH of 7.0–9.2. As the pH of the water decreases, plant population also declines, which decreases nourishment for some aquatic birds (Gentili et al. 2018). If the pH continues to lower, the numbers of freshwater shrimp, crayfish, clams and some fish start to decline continuously (Food and Agriculture Organization of the United Nations 2020). The bacteria that decompose leaf litter and other waste begin to die at a pH of 5.5, cutting off the nutrient source for plankton. Waterways that already have a low pH are at the risk of serious damage from these temporary increases in acidity. Temporary acidification can completely devastate an ecosystem and result in significant fish extinction (Goolsby and Battaglin 2001). To cool a thermal plant, water is drained from the watercourse, channelled through the area that essentially to be cooled and then reverted to the river. This leads to a rise in the temperature of approximately 10 °C of the water, which is closer to the thermal plant in comparison to the other part of the water bodies. Heated water has a variety of effects on the surrounding ecosystem. Warmer temperatures upsurge the capability of plants to photosynthesize, which may outgrowth an algal bloom (Kumaraswamy et al. 2020). Warmer temperatures also put more strain on aquatic plants and animals. Cool water holds more dissolved oxygen as compared to warm water, making it more difficult for aquatic animals to breathe (Qadri et al. 2020). In water that is warmer than 35 °C, certain organisms perish; lower oxygen levels and higher temperatures could make an animal more susceptible to consequences from infections and harmful substances (Kumaraswamy et al. 2020); as a result, the biodiversity, or variety of species, of the environment may decline. Instead of producing electricity, the plant wastes two-thirds of its energy as heat. As the cooling water passes through the plant, it loses plants, eggs, larvae and young fish, including around 35% of the young striped bass in that section of the river. Some larger fish are killed or injured when they are trapped on the screens that prevent them from being sucked into the cooling system (Qadri et al. 2020).

Nutrients accumulate into lakes and streams through runoff from the land, atmospheric fallout and the recycling of plant and animal tissues within the aquatic environment. Freshwater ecosystems are directly being impacted by recent increases in fertilizer intake. Methemoglobinemia or “blue baby” syndrome, generally known as the illness in infants, is caused by high nitrate concentrations in drinking water. Due to this illness, the baby’s digestive system acclimates the nitrate to nitrite, a process that interferes with the blood’s ability to carry oxygen (Qadri et al. 2020). Smaller amounts of nutrients in a lake do not immediately kill fishes but speed up the eutrophication process.

Pesticides, flame retardants, industrial solvents and cleaning fluids contain man-made organic compounds that are commonly found in aquatic environments (Folke et al. 2002). Although these persistent organic pollutants (POPs) have many applications, some are lethal even in trace amounts. They are extremely toxic to fish and invertebrates, even in small concentrations, and are strong endocrine disruptors. They prevent both avian and mammal reproduction and development, which lowers the number and survival rates of the offspring (Qadri et al. 2020). In mammals, polychlorinated biphenyls interfere with the metabolism of thyroid hormones, which regulate a diversity of physiological processes, including brain development and metabolism. They also reduce immune system function; for example, polar bears are losing their ability to fight against common infections and are also beginning to show some endocrine effects (Okeke and Igboanua 2003) such as masculinization in some females. The exposure of polychlorinated biphenyls in humans has been linked to neurodevelopmental problems in children (Goolsby and Battaglin 2001), liver dysfunction, skin and respiratory problems, dizziness and possibly cancer. A recent study discovered that the levels of three chemicals (polychlorinated biphenyls, hexachlorobenzene and chlordane) were higher in the mothers of men with a testicular cancer than in a control group, implying that the cancer began in uterus (Carpente et al. 1998).

15.6 Prevention from Pollution in Freshwater

To prevent the negative effects of freshwater pollution, it is primarily the most important step to educate each and every individual around the world. In addition, the sensitivity and importance of the subject can be emphasized with such studies. Water is one of the most important needs of all the living organisms, and for some, it is life itself (Kılıc 2021). It keeps them alive, but polluted water is extremely dangerous. When humans drink polluted water, it has serious effects on their health. The air, the water and the soil are the main elements of our environment (Obafemi et al. 2012).

Environmental education is a key factor in an attempt to address environmental pollution and its harmful effects. The main objective of environmental and water pollution education is to equip learners with knowledge, values and skills that promote the protection and conservation of the environment (Karatas and Karatas 2016). The best way to prevent water pollution is to educate yourself and your surrounding environment. Environmental education is a process that aims to create a population that is aware of the environment as a whole and the problems that it is associated with and that has the knowledge, attitudes, motivation and skills to work both individually and collectively towards solving existing issues and preventing the emergence of new ones (Skanavis 2004). To effectively safeguard the water resources, one of the main goals of water education is to instil the knowledge of water contamination. Water pollution education is a very important and an effective tool to promote public awareness. Water pollution prevention training should be done as follows: producers should be informed by professionals about proper utilization; people should be informed about recycling process and waste materials and should be informed about the harms of excessive consumption; media should be used for promoting water pollution awareness; people should be informed about the importance of all natural resources for life; activities and presentations about the protection of water and water resources should be organized regularly. The ideal procedure to reduce diffuse pollution of waterways is to minimize or avoid the use of chemicals for agricultural, industrial and domestic purposes (Scheierling 1995).

The whole world community is currently experiencing the severe effects of water contamination, which is a global problem. It is advised to have a suitable system for disposing of trash and to treat waste before it enters rivers. To reduce pollution, seminars, workshops and educational programmes should be organized across the global level. Some measures have to be taken before such a major problem occurs.

Water pollution causes many negative effects such as diseases, death of aquatic animals, economic costs of cleaning processes, the destruction of ecosystems and the disruption of food chains.

Here, education on the dangers of freshwater pollution is extremely important, as it helps people to apply the right attitudes when dealing with the environment (Karatas and Karatas 2016). It is important to support and fund educational initiatives that inform and organize people to help safeguard water resources. Environmental education teaches individuals to weigh different sides of an environmental issue to make informed and responsible decisions (US Environmental Protection Agency 2022). Awareness-raising activities are of great importance for people before they contaminate water sources. Water contamination is the result of our impulsive behaviour and carelessness. We all know that water is so important and we should not pollute water. Without water, neither we nor any living thing can survive. Therefore, people should protect, keep saving and help prevent water pollution. Nature provides us many tangible and intangible resources in our daily lives, so let us be responsible and disciplined enough to save, protect, preserve and maintain not only water sources but also other natural resources. There are a number of things we can do in our daily lives to lessen water pollution, even though there is not a single quick action that can be made to halt it. The best way to clean polluted water is not to clean it, but to cease polluting it.

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Freshwater Pollution: Overview, Prevention, and Control

16

Pragati Srivastava and Manvika Sahgal

Abstract

All living creatures depend on water as an essential natural resource. However, increasing human population, development, and technology severely strained freshwater ecosystems, changing the freshwater's quality status by introducing massive amounts of contaminants. Increasing population tends a pressure of higher requirement for high-quality water for household use and commercial growth, which is affecting this valuable resource in becoming more and more endangered. Consequently, it is crucial to monitor the quality of this valuable resource. Freshwater pollution can be particularly harmful to the health of people and aquatic life since it is utilized as the primary source of portable water by a large portion of the global population. Chemical contaminants have long-term effects on aquatic and related biota in freshwater ecosystems. Therefore, it is vital to regularly check on the health of the freshwater ecosystem, and an attention should be paid to the treatment of effluents before they are released into the freshwater ecosystem.

Keywords

Pollution · Bioremediation · Freshwater

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16.1 Introduction

The oceans, having an average depth of 3.8 km with water which is extremely saline, encompass the majority of the earth's surface (71%) and are extremely large. The remaining 2.5% of the world's water, which is freshwater, is contained in the oceans and seas, and 68.9% of this freshwater is trapped in glaciers and ice sheets, with the majority of the remaining freshwater (29.9%) being groundwater. Rivers and lakes hold the majority of the 0.3% of freshwater in the world (Okafor 2011). However, freshwater, which makes up a very small percentage by the volume of the earth's water, is required by human population for their domestic and industrial needs. Although it is possible to evaporate the plentiful ocean and seawater to produce freshwater, the current techniques are not cost-effective. As a result, freshwater is a rather rare resource. Because of this, it is cleansed and reused in several nations. Water bodies are a crucial component in the production of electricity and are used for the disposal of sewage and industrial effluents (Eunice et al. 2017), but the regular disposal of these effluents has raised environmental concerns due to its pollution and causes enormous threats to aquatic life and decrepit for general human use.

Biotic, chemical, and physical elements from various sources are a major cause of water pollution (Richardson et al. 2007). There are innumerable distinct reasons for water contamination, including the discharge of field and industrial waste into bodies of water, oil tanker leaks, the overuse of fertilizers and pesticides for crop protection, sewage sludge, and many more (Aboyeji 2013; Bhat et al. 2017). According to Bhat et al. (2014, 2018), the excessive use of chemicals, filth, dust, and debris and overcrowding in metropolitan areas are the main causes of water body pollution (Master et al. 1998; Carpenter et al. 1998; Kalff 2002; Moss 2008). Assimilative capacity (the ability of water bodies to assimilate contaminants) to a degree may be harmful to the life of biota in some form, variety, or structure (Adekunle 2009; Adekunle and Eniola 2008). There is a direct corelation between contaminants and organism health (Otokunefor and Obiukwu 2005). Various authorities employ a wide range of factors as biomarkers of water pollution. Biochemical oxygen demand (BOD), dissolved oxygen (DO), pH, specific conductance, water temperature, and chemical components such as nitrates and phosphates are some of the influencing factors. Across the globe, an estimated value of municipal and industrial discharge in freshwater bodies is calculated to be 80% without any antecedent ministration (Lin et al. 2022). The shortfall of adequate sanitization of freshwater bodies led to increased commencement of threatful waterborne diseases such as cholera, trachoma, helminthiasis, and gastrointestinal illness enabling the absorption of nutrients in children causing malnutrition (Wright et al. 2004).

16.2 Origin of Pollution

16.2.1 Agricultural Wastes

To feed the increasing population, an immense pressure is provoked on enhancing the productivity of agricultural crops via the usage of chemical pesticides and fertilizers, which has dramatically led to deterioration of water quality, making it unfit for drinking and farm sluicing (Mali et al. 2015). Although the use of pesticides has a number of advantages, such as improving food quality and quantity and reducing damage attributed by insects, but it has also aroused concerns about possible negative impacts on the environment, notably those on water supplies. The corresponding environmental effects are primarily caused by the various herbicides' pervasive endurance in the environment, leading to the disturbance of biodiversity. The pesticide chemical composition and climate components decide its dissolution in the soil, and if the process of dissolution is not readily done it will move from one environment entity to other, leading to its pollution "eutrophication" with unknown health risks in the food chain (Sharma et al. 2019). Notably, long-term absorption of pesticides through water can imitate human hormones, impair hormone balance, causes problems with reproduction, have cancerous effects, and impair intelligence, especially in youngsters still in the development stage of body (Yadav and Samadder 2018; Syafrudin et al. 2021).

Coagulation–flocculation, adsorption, filtering, and sedimentation are common pesticide treatment procedures that depend on the phase transfer of contaminants. These techniques frequently have a considerable production expenses and may result in secondary contamination, such as the production of sludge (López-Loveira et al. 2017). For the treatment of water containing tenacious and biorefractory contaminants such as pesticides, advanced oxidation processes (AOPs) are acknowledged as clean technology. Because of its wide range of applications and thermodynamic viability, it has become a new popular water purification method (Galeano et al. 2019). The fundamental hypothesis that governs the water purification via AOPs is based on the production of highly reactive hydroxyl radicals that randomly oxidize a variety of resistant organic pollutants in order to completely break down the organic contaminants into carbon dioxide, water, and mineral salts and efficient conversion of pesticides into more biodegradable species (Zapata et al. 2010).

Establishing regulatory bodies to regularly check on pesticide release in freshwater bodies, this development could be a significant step toward addressing health concerns related to pesticide pollution of freshwater bodies. The management of pesticides and insecticides in rural areas can benefit from an effective integrated pest management approach that ensures proper application for a long-term reduction of pesticides naturally. Water bodies where pesticide compounds have been detected should be regularly monitored for national water security, and drinking water should, as needed, undergo advanced water treatment methods.

16.2.2 Sewage

Sewage discharge in freshwater bodies is the major setback acquired in changes of species composition and deterioration of biodiversity and a drift in aquatic habitats, which ultimately affect the country's economy, environment sustainability, and downfall in the quality of life. Various components in sewage potentially disturb the microbiota through various means (Bhatt et al. 2021). Many physical changes are observed in the freshwater bodies due to sewage discharge. There is a temperature shift above the optimum temperature, which is undesirable for the sustenance of aquatic life. An increase in temperature also affects the solubility of gases such as oxygen (O_2), which is a major requirement for the breakdown of waste by bacteria (Lecerf and Chauvet 2008).

Sewage consists of domestic waste and effluent discharge from commercializing industrial setup. It attains 99.9% water and 0.1% suspended solids and dissolved solids. Microbial population is diverse in sewage water. It consists of many pathogenic population such as *Shigella*, *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, cysts, fungi, viruses, and parasitic eggs (Grandclément et al. 2017; Yu et al. 2019). Freshwater bodies encountering more of untreated and halfway treated sewage have a higher incidence of biodiversity loss and species physiological alteration and fish mortality. Nutrient-rich sewage has a higher ratio of nitrogen (N) and phosphorous (P), which are major governing factors responsible for eutrophication in freshwater bodies. Untreated sewage has immensely affected the trophic status of freshwater habitats (Kirchmann et al. 2017).

16.3 Urbanization and Hiking Population

Most of the growing population is accommodating themselves in urban cities for the scope of development and exposure to new technologies (Strokal et al. 2021). A 2:3 urbanization ratio contributes to global domestic products while altering the composition of freshwater bodies and increasing their use in industries and agriculture (Best 2019; Keeler et al. 2019). A multipollutant approach should be taken into consideration rather than a single-pollutant approach. Advanced research is required to gain a thorough understanding of the interactions that occur between pollutants, their driving sources, and pressures (e.g., nutrients N and P, pathogens, and plastics) (Strokal et al. 2021).

16.4 Metal Mining Impact on Global Freshwater Resources

Although freshwater usage in the mining sector is very low both worldwide and at the national level, when the usage exceeds the carrying capacity set by the standard quantity, it has an impact on the scarce supply of freshwater resources (Meißner 2021). The process of mining extraction and purification requires a large amount of water for the processing (extraction and purification) of the mining plant, which is an energy-intensive process (Gunson et al. 2012).

16.5 Nutrient Imbalance (N and P)

The science behind the nutrient pollution is rather simple. Phosphorus and nitrogen are both found in soil and water, but nitrogen is also found in the air we breathe. Through intensive farming practices and chemical fertilizers, these nutrients are deposited in the ecosystem (Jwaideh et al. 2022). Despite the fact that its use increases the productivity of various agricultural crops, its ineffective soil absorption causes it to run off through rain into freshwater bodies, where it promotes algal bloom and fish mortality, depletes oxygen concentration, and reduces water quality, disrupting aquatic metabolism (Liu et al. 2022; Mishra et al. 2022).

16.6 Heavy Metal Contamination

The environment contains trace amounts of heavy metals in major forms. Lead and mercury, for instance, are fundamental elements of all rocks created in the crust of the earth, whether intrusive, igneous, or volcanic in origin. Plants only need heavy metals such as vanadium, copper, cobalt, zinc, and iron (Fe) to perform biological reactions (Mehmood et al. 2019). For instance, Fe in hemoglobin is substantially necessary to transmit O_2 in the blood. However, after or over a certain threshold value, it is biologically harmful to humans, animals, and plants (Singh et al. 2018).

Mercury, the most dangerous heavy metal, is released into the environment when coal is burned. Hazardous forms of mercury are released into the atmosphere when household, municipal, and medical trash is burned. These toxic forms of mercury bioaccumulate in the form of aerosol droplets. These aerosol droplets have a propensity to travel thousands of miles before depositing in bodies of water and land. Sediment deposition of this mercuric aerosol is acquired by the bacteria that convert mercury into its more toxic form, which is eventually consumed via fish, leading to its mortality. It is most menacing for humans' brain, kidney, and liver (Rashid et al. 2019; Kumaraswamy et al. 2020).

16.7 Conclusion

The physiological and biological characteristics of the freshwater bodies may be hampered by untreated sewage discharge. Untreated sewage, oil spills, industrial and mining effluent, and domestic waste (fecal matter and urine) discharges all contain persistent contaminants that degrade the quality of quiescent freshwater bodies and cause eutrophication. As a result, lakes and wetlands have less capacity to hold water, and dissolved oxygen levels are brought down to a level where its bioavailability to aquatic life is decreased. Due to all of these limitations, freshwater resources are not utilized to serve mankind and other forms of life. Given the value of the scarce, nonrenewable resource to the biodiversity of the ecosystem and to humanity, multidisciplinary methodologies should be used to target many pollutants simultaneously before they are dumped into freshwater bodies, both spatially and temporally.

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Iron-Degrading Bacteria in the Aquatic Environment: Current Trends and Future Directions

17

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Abstract

Iron (Fe) is an abundant element found in the earth's crust and is required by organisms to perform their metabolic activity. However, its toxicity in excess amounts in the aquatic environment can negatively impact the organism's habitat. Therefore, the extra amount of iron in the aquatic environment needs to be checked or converted into a nontoxic form to sustain the well-being of the organisms. Biologically, this can be achieved by targeting iron-degrading microbes that can degrade iron in the environment. In this chapter, we present the role of various iron-degrading bacteria and their importance in the environment. Moreover, we highlight some essential mechanisms involved in the degradation of iron through microbes. *Gallionellaceae* sp. in the KS culture, for example, could oxidize iron in such a way that an electron is gained through iron oxidation and transmitted through the electron transport chain (ETC) where nitrate (NO_2) is reduced step by step to nitric oxide (NO). Thus, this chapter explores the possibility of key microbial groups in remediating iron-toxic environments and the underlying mechanisms involved in the process to benefit the degrading environment.

Keywords

Anaerobic photosynthetic · Acidophilic · Aquatic environment · Iron-degrading microbes · Neutrophilic · *Gallionellaceae*

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17.1 Introduction

Iron is one of the fourth most abundant key elements and also the redox-active metal in the earth's crust. The iron requirement in the organisms is also remarkable; without it, the microorganisms cannot conduct their metabolic activity significantly. For instance, it is involved in cellular respiration and oxygen transport (Bury et al. 2001). Although it is imperative for organisms, excess of it affects the organisms. Most countries face the problem of excess iron in their environment, such as water bodies and sediment, which subsequently lead to iron toxicity to the organism. The ability of microbes to oxidize iron may convert the negative impact of iron to the positive impact in the environment that ultimately utilized by the microbes. Therefore, in this chapter, we discuss the key role of iron-degrading bacteria in nature and their importance in the aquatic environment. In addition, we also highlight some important mechanisms concerned in the degradation of iron by using microbes.

17.2 Toxicity of Iron

The toxicity of iron to organisms, particularly fish, can be broadly classified as acute or chronic depending on the time and strength of the toxic substance that the organisms are exposed to. While the first addresses a brief duration with a greater concentration of the toxic substance that causes adverse effects and leads to mortality in organisms, and the second addresses a prolonged exposure with a lower concentration of the agent that causes gradual adverse effects in the organism. Many researchers have studied iron toxicity to organisms, including fishes. For instance, according to Bury et al. (2001), the ferrous form (Fe^{2+}) is more harmful to European plaice (*Platichthys flesus*) than the ferric form (Fe^{3+}), and iron intake occurs largely in the latter section of the gut. The iron toxicity in the fish mainly affects the fish's gills, subsequently causing respiratory problems (Zahedi et al. 2014). In addition, animals on iron-rich diets may experience stunted development, low feed conversion, food refusal, death, and histopathological injury in the liver cells, which retain excessive iron (Bury and Grosell 2003). Fish can be employed as ecological indicators, displaying physiological changes including opercular beating, abnormal swimming, gill modifications, and feeding changes or problems responding to changes in the aquatic ecosystem (Hundley et al. 2018). According to Gemaque et al. (2019), both the forms of iron ions (Fe^{2+} and Fe^{3+}) are detrimental to the "piau" (*Leporinus friderici*), with Fe^{2+} being the most toxic.

17.3 Classification

Iron-degrading bacteria may be classified broadly into two groups: iron-reducing bacteria (IRB) and iron-oxidizing bacteria (IOB), based on their potential to reduce and oxidize iron, respectively, in the nature. While the first one applies to the ability of the bacteria to convert ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions under both aerobic and anaerobic environments, the latter one is the ability of the bacteria to transform ferrous ions to ferric (Fe^{3+}) ions under anaerobic and/or microaerobic conditions (Ebrahiminezhad et al. 2017). The majority of IOB are found in a variety of phyla within the bacteria domain, along with Firmicutes and Nitrospirae, the lion's share being Proteobacteria (Hedrich et al. 2011). Moreover, IOB may be classified into four classes based on their physiological diversity: (a) acidophilic, aerobic iron oxidizers; (b) neutrophilic, aerobic iron oxidizers; (c) aerobic iron oxidizers; and (d) anaerobic photosynthetic iron oxidizers. With the exception of nitrate-dependent iron oxidizers, the majority of the species in this phylum belong to one of the Proteobacteria classes. Furthermore, the Zetaproteobacteria are iron-oxidizing neutrophilic chemolithoautotrophs found in estuarine and marine settings all over the world.

17.3.1 Acidophilic, Aerobic Iron-Oxidizing Proteobacteria

These groups play an essential role in creating acidic and metal-fortified mine drainage waters. Acidophilic prokaryotes utilize the pH gradient across their cell membranes to drive ATP synthesis through F_1F_0 ATP synthase, but the accompanying proton influx is balanced by gaining electrons from the oxidation of ferrous iron (Hedrich et al. 2011). For example, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus ferrivorans*, *Acidiferrobacter thiooxydans*, *Thiobacillus prosperus*, *Mariprofundus ferrooxydans*, *Ferrovum myxofaciens*, and *Thiomonas* spp are included in this group of proteobacteria (Colmer et al. 1950; Hallberg et al. 2010; Hedrich et al. 2011).

17.3.2 Neutrophilic, Aerobic Iron-Oxidizing Proteobacteria

These are the stalk-forming organisms where they divide at a limited partial pressure of oxygen in anti-gradients of oxygen and Fe^{2+}, viz. *Crenothrix*, Ferritrophicales, *Ferritrophicum radicicola*, *Gallionella*, *Leptothrix cholodnii*, *Leptothrix discophora*, *Leptothrix mobilis*, *Metallogenium*, *Siderocapsa*, *Sideroxydans* spp., *Sideroxydans*, *Sideroxydans* sp. ES-1, *Sideroxydans paludicola*, and *Sphaerotilus natans* (Emerson and Moyer 1997). Transmission electron microscopy of *Gallionella ferruginea* or *Mariprofundus ferrooxydans* revealed that stalks are made up of certain fibrils, which accommodate few millimicrometer-sized iron oxyhydroxide crystals (Chan et al. 2011). In 1838, *Gallionella ferruginea* was first proclaimed by Ehrenberg as a leading neutrophilic iron oxidizer, where it can grow mixotrophically or autotrophically utilizing an electron donor (ferrous iron) (Hallbeck and Pedersen 1991). *Mariprofundus ferrooxydans* is an autotrophic, mesophilic, marine iron oxidizer, which has an immediate morphological resemblance to *G. ferruginea*, although phylogenetically it is amazingly isolated from the freshwater bacterium and is related to Zetaproteobacteria (Emerson et al. 2010). In addition, it was announced that such heterotrophic *Pseudomonas* or

Pseudoalteromonas-like gammaproteobacteria separated from a seamount of volcanic sands under microaerobic conditions could catalyze ferrous iron oxidation and consequently lead to the development of iron mats in the deep seas (Sudek et al. 2009).

17.3.3 Neutrophilic, Anaerobic Iron Oxidizers

These groups have been created in freshwater, brackish, marine, and anaerobic sediments. Microbes that have a characteristic to oxidize iron and reduce nitrate under anaerobic conditions are classified as autotrophic and heterotrophic. These unique bacteria may thrive in organic acids when nitrate or oxygen served as an electron acceptor (Straub et al. 1996) such as *Acidovorax* sp., *Aquabacterium*, *Dechlorosoma suillum* strain PS, *Dechloromonas* spp., and *Geobacter* (Chaudhuri et al. 2001). In the presence of nitrate, *Thiobacillus denitrificans* also oxidized iron sulfide (FeS) (Straub et al. 1996). Comprehensive research is still being done to convert nitrate to nitrogen gas under anoxic conditions, in a process known as "nitrate-dependent anaerobic ferrous oxidization (NAFO)" with the help of ferrous iron as the electron donor (Su et al. 2017; Zhang et al. 2015). This is because iron is the most redox-active metal in organisms, sediment, and soil (Li et al. 2014).

Pseudogulbenkiania strain 2002, an isolate from a sediment of a freshwater lake, could reduce nitrate and oxidize ferrous iron during the time of growing as an autotroph (Weber et al. 2006), but it can also grow under heterotrophic conditions on a variety of organic substances. 16S rRNA gene sequence analysis disclosed that this isolate was nearly linked to the beta proteobacterium, *Pseudogulbenkiania subflava* (99.3% sequence similarity) (Weber et al. 2009). Surprisingly, the discovery of iron-oxidizing proteobacteria explained nitrate-dependent iron (Fe^{2+}, ferrous) oxidation by the stringent anaerobe *Geobacter metallireducens* (Finneran et al. 2002). Moreover, Raiswell and Canfield (2012) proposed that, anaerobically, ferrous form Fe^{2+} is oxidized with nitrate in the nitrate-containing oxygen minimum zones (OMZs) of present sea.

Anaerobic iron oxidation with nitrate (NO_3) produces nitrite (NO_2^-), nitrogenous gases (nitrogen [N_2] or nitrous oxide [N_2O]), or ammonium (NH_4^+) (Carlson et al. 2013), and a range of authigenic Fe minerals, based on surrounding water chemistry and pH (Carlson et al. 2013), for example,

$$2Fe^{2+} + NO^{-3} + 5H_2O \rightarrow 2Fe(OH)_3 + NO^{-2} + 4H^+ \quad (17.1)$$

$$6Fe^{2+} + 2NO^{-2} + 14H_2O \rightarrow 6Fe(OH)_3 + N_2 + 10H^+ \quad (17.2)$$

In the presence of nitrite, such as via heterotrophic nitrate reduction, iron oxidation accompanied by nitrite in Eq. (17.2) has been demonstrated to occur abiotically at around neutral pH, peculiarly when reactive Fe oxyhydroxide mineral surfaces are accessible to catalyze the process (Picardal 2012). The phrase "nitrate-dependent iron oxidation" refers to the microbial and partially abiotic mechanisms with the aid of ferrous iron oxidized using nitrate as the terminal electron acceptor (Picardal 2012; Klueglein et al. 2014).

Nitrate-dependent iron oxidation has been occurred in a diverse freshwater and marine sediments and also shown in laboratory cultures (Straub et al. 1996; Scholz et al. 2016). This might be a crucial mechanism in biogeochemical cycling, especially in OMZs. Despite the fact that sediments under the Peru OMZ discharge large quantities of iron into the anoxic column of water (Noffke et al. 2012), primary production in the eastern equatorial Pacific high-nitrate-low-chlorophyll (HNLC) zone off Peru has been demonstrated to be iron-restricted (Hutchins et al. 2002). Furthermore, ample of iron liberated from the sediments of Peru margin is restored and buried adjacent to its origin rather than being moved offshore within the OMZ (Scholz et al. 2014). Both of these surprising data might be explained by nitrate-dependent iron oxidation inside the anoxic column of water (Scholz et al. 2016).

17.3.4 Anaerobic Photosynthetic Iron (Fe) Oxidizers

The first proof that microbes might oxidize ferrous iron (Fe^{2+}) in anaerobic settings came from phototrophic purple proteobacteria. The class Alphaproteobacteria is placed in the majority of iron-oxidizing phototrophs that have been discovered. *Thiodictyon* strain L7, a gammaproteobacterium, is the striking exception. This type of bacterium uses ferrous iron as a cause of carbon dioxide reductant, as revealed in the following equation, where CH_2O represents fixed biomass carbon:

$$4Fe^{2+} + CO_2 + 11H_2O + hv \rightarrow CH_2O + 4Fe(OH)_3 + 8H^+ \qquad (17.3)$$

At the same time, the majority of photosynthetic microbes that oxidize ferrous iron consume such a reaction for the assimilation of carbon; it can further be utilized as a detoxification mode of action. Moreover, phototrophic iron oxidizers can employ soluble ferrous iron and other minerals, namely FeS or ferrous carbonate ($FeCO_3$), as origins of reductants although they are not allowed to access ferrous iron in more crystalline minerals like magnetite (Fe_3O_4) or pyrite (FeS_2) (Kappler and Newman 2004). So far, most of the identified and validated phototrophic iron oxidizers have been placed under the thoroughly varied family of Rhodobacteraceae within the class of Alphaproteobacteria including aerobic and anaerobic facultative heterotrophs, facultative methylotrophs, fermentative bacteria, and photoheterotrophs (Imhoff 2005).

Rhodobacter sp. strain SW2, the foremost iron-oxidizing phototroph identified, oxidizes ferrous iron exclusively when supplied with an organic carbon origin and also uses hydrogen (H_2) and organic molecules (Ehrenreich and Widdel 1994). Additional iron-oxidizing photosynthetic purple bacteria in the Rhodobacteraceae family comprise *Rhodovulum iodosum* and *Rhodovulum robiginosum*; both of them are found in marine environments and oxidize sulfide and ferrous iron when fed an organic cosubstrate like acetic acid (Hedrich et al. 2011). Heising and Schink (1998) revealed that a phototrophic isolate of *Rhodomicrobium vannielii*, a heterotrophic nonsulfur purple bacterium from the Hyphomicrobiaceae family, can oxidize ferrous iron, a trait that was later validated in the species' type strain. Tentatively, Widdel et al. (1993) had already identified *Rm. vannielii* as the sole of the iron-oxidizing phototrophic isolates from freshwaters they had collected. Acetate or succinate was introduced to strain BS-1 as cosubstrates to promote growth when ferrous iron was present. According to Heising and Schink (1998), ferrous Fe oxidation is just a small act for *Rm. Vannielii* strain BS-1. Alternatively, the Hyphomicrobiaceae species, *Rhodopseudomonas palustris* strain TIE-1, segregated from an iron-rich mat was utilized as a model species for a genetic research by Jiao et al. (2005). The two photosynthetic iron-oxidizing gammaproteobacteria of *Thiodictyon* strains have been reported. One of the two, strain L7, was desegregated from the identical origin as *Rhodobacter* sp. SW2 (Ehrenreich and Widdel 1994), while the other *Thiodictyon* sp. strain f4 was from a marsh (Croal et al. 2004). In addition, among all the isolated phototrophic iron-oxidizing bacteria, *Thiodictyon* sp. strain f4 shows iron oxidation at elevated rates (Hegler et al. 2008). However, Widdel et al. (1993) observed no one at all of the confirmed *Thiodictyon* spp. they experimented with which could oxidize ferrous iron, implying that such a feature is unusual in this genus.

17.4 Iron-Reducing Bacteria's Role

In the sediment column, the presence of anaerobic dissimilatory Fe(III) iron-reducing bacteria makes distinguishing the type of reactive iron challenging (Lovley and Phillips 1986). These organisms seem to be present in both bacteria and archaea and have a broad phylogenetic range (Kashefi and Lovley 2003). The Geobacteraceae, a wide phylogenetically coherent group that oxidizes acetate to carbon dioxide (CO_2) using Fe(III) as the only electron acceptor (Lonergan et al. 1996), and also H_2-oxidizing Fe(III) reducers including *Shewanella putrefaciens* and *S. alga*, are among them (Lonergan et al. 1996). It was proved that Fe(III) reducers may decrease Fe(III) sheet silicates and magnetite (Kostka et al. 1999). Because Fe sheet silicates and magnetite are resistant to inorganic acid leaching, they are normally allocated as unreactive or weakly reactive iron phases in sequential leaching systems. Numerous sulfate-reducing bacteria (SRB) are linked to Fe(III)-reducing bacteria (FeRB) on a phylogenetic level, and many Geobacteraceae species reduce S(0). The sulfate and iron reducers may be an element of a compact ecosystem as the FeRB uses acetate, which is a typical by-product of SRB metabolism. As a result, these organisms are expected to have a big influence on the iron in the sediment's oxic and transition zones. The proportion of Fe(III) that is reduced by iron-reducing bacteria and reacting with the Fe(II) produced will be determined by the relative rates of the bacterial reduction reactions and sulfidation and the time spent by Fe(III) before being exposed to S(-II) in the bacterial iron-reducing subenvironment. Sequentially, this will change depending on sedimentation characteristics such as the degree of advection in the column of water, sediment grain size, the concentration of organic matter, and the velocity of sedimentation (Rickard 2012).

Moreover, the Fe-reducing bacteria may play a vital role in oil degradation. For instance, ferric iron is used by the hyperthermophile *Ferroglobus placidus* to consume benzene at 85 °C (Holmes et al. 2011; Waikhom et al. 2020). In oil field brine enrichment cultures, iron-reducing bacteria have been detected (Magot 2005); nevertheless, the availability of iron oxide surfaces may restrict the degree of oil biodegradation in the deep below. In sandstones, Fe(III) is found in the range of 0.4–4.0 oxide weight percent on pore filling or grain coating material (Pettijohn 1963). Although the energy gained by Fe(III) reduction is equivalent to aerobic respiration or NO_3 reduction, the oxidant amount required is significantly more. According to the volume, around 10 L of hematite would be required to biodegrade 1 L of hydrocarbon approximately. Due to the inadequate biological availability of ferric iron in hematite, such a procedure would be extremely slow, made even more difficult by the fact that hematite is frequently destroyed in course of diagenesis, and the majority of the iron in sedimentary reservoir rocks is constrained in surprisingly less reactive silicates and sulfides (Prince and Walters 2016).

17.5 Mechanism of Iron Oxidation

17.5.1 Mechanisms

Mechanisms utilized by anaerobically nitrate-reducing iron oxidation (NRFeOx) bacteria to oxidize Fe(II) are yet to be more explored, even though it appears to have different mechanisms, even if the bacteria are autotrophs, chemodenitrifiers, or mixotrophs.

For autotrophic NRFeOx bacteria, three mechanisms have been put forward for Fe(II) oxidation (Fig. 17.1) such as (a) a devoted Fe(II) oxidoreductase, (b) a nonspecific performance of the nitrate reductase, or (c) the *bc1* complex that receives electrons from Fe(II) and passages down the quinone pool (Bryce et al. 2018). The first scenario has dominated research in the recent years, intending to discover a particular outermost membrane Fe(II) oxidoreductase seen in these bacteria (Beller et al. 2013). Metagenomics investigation of the NRFeOx in the KS culture revealed homologs of the cytochrome c putative Fe(II) oxidase *Cyc2*, which have been detected in other identified and validated Fe(II) oxidizers in the draft genomes of *Gallionellaceae sp.* and *Rhodanobacter* sp. observed in KS culture (He et al. 2016).

Furthermore, the *Gallionellaceae* sp. and *Dryobalanops aromatica* RCB in the KS culture, which was suspected to be autotrophic but was not confirmed, both include homologs of the porin cytochrome c porin complex MtoAB (He et al. 2017). Figure 17.1a depicts a possible process for the autotrophic Fe(II) oxidizer to oxidize Fe(II) in the KS culture, in which an electron is gathered through Fe(II) oxidation and transmitted through the ETC, where nitrate is reduced step by step to nitric oxide (NO). NO has the ability to be absorbed by the surrounding population or to react with aqueous Fe(II) outside the cell.

Some NRFeOx bacteria may undergo an enzymatic process to oxidize Fe(II), but whether or not these organisms are real mixotrophs is still up for debate. Many

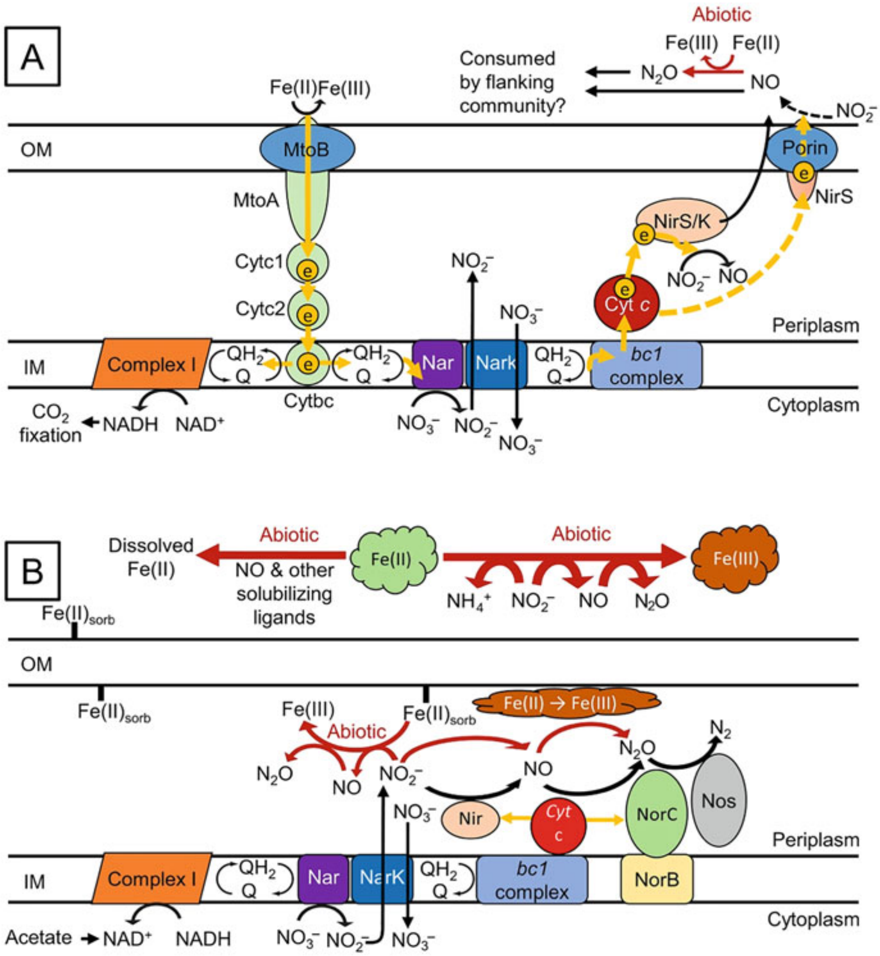

Fig 17.1 The existing hypotheses on the mechanism of iron oxidation in (**a**) *Gallionellaceae* species, considered autotrophic nitrate reducers and Fe(II) oxidizers in the KS culture and (**b**) the reduction of nitrate might be mediated by *Nap* in place of *Nar*, and the reduction of nitric oxide could be aided by *NorZ* instead of *NorC*. Notably, *NorZ* utilizes electrons from quinols as opposed to cytochrome c (Reprinted from Bryce et al. (2018), copyright 2022 Society for Applied Microbiology, with permission from John Wiley and Sons). Abbreviations: OM, outer membrane; IM, inner membrane; Cyt c, cytochrome c; NO, nitric oxide; N_2O, nitrous oxide; NAD, Nicotinamide adenine dinucleotide

people assume that Fe(II) oxidation is powered by denitrification (Brons et al. 1991). It has been hypothesized by Carlson et al. (2013) that all heterotrophic denitrifiers can increase nitrate-dependent Fe(II) oxidation. Several nitrate reducers, including *Escherichia coli*, can oxidize an organic molecule when it is combined with Fe (II) (Brons et al. 1991).

Although the rate of dissolved Fe(II)-mediated abiotic nitrate reduction by nitrite to N_2O is a slow process, the rate of Fe(II)-mediated nitrite reduction to N_2O is kinetically advantageous under environmental conditions if reactive chemical substrates act as catalysts (Colman et al. 2008). Heterogeneous surface catalysis, for instance, can decrease nitrogen species by Fe(II) on viable surfaces such as cell surfaces, green rust, crystalline Fe(III) oxyhydroxides, and pyrite (Bryce et al. 2018). Many researchers have shown that microbially driven NRFeOx, heterotrophic nitrate reduction, and Fe(III)-coupled ammonium oxidation, that is, iron-ammox can result in the development of reactive nitrogen species (nitrite, NO_2^-, or nitric oxide [NO]) as a metabolic intermediate (Picardal 2012), providing a sufficient stock of compounds that could react rapidly with Fe(II) (Bryce et al. 2018).

17.5.2 Mechanisms of Ferrous Iron Oxidation by Photoferrotrophs

The insight mechanisms engaged in Fe(II) oxidation by phototrophic anoxygenic Fe (II)-oxidizing bacteria are yet a mystery. However, *Rp. palustris* TIE-1 has been the subject of the most scientific research for the process of phototrophic bacterial Fe (II) oxidation. Besides, the *pioABC* operon, which stands for “photosynthetic Fe (II) oxidation,” is assumed to be essential for electron transport by Fe(II) oxidation in this species. This three-gene operon encodes three proteins: PioA, PioB, and PioC (Jiao and Newman 2007), where PioA is a periplasmic decaheme c-type cytochrome, PioB is an outer membrane beta-barrel protein, and PioC is a periplasmic, strong potential iron–sulfur cluster protein. MtrA and MtrB, which are expressed by the Fe (III) reducer *Shewanella oneidensis* MR-1, are the same as PioA and PioB. (Jiao and Newman 2007). Iro, the putative Fe(II) oxidoreductase of *A. ferrooxidans*, is linked to PioC. *Rp. palustris* TIE-1 carries electrons from *PioA* to *PioC*, which gives them to the *bc1* complex. The electrons may be shifted to the phototrophic reaction center in the inner membrane, according to some studies (Bird et al. 2014). The pioABC operon in *Rhodomicrobium vannielii* acts similarly to the operon in *Rp. palustris* TIE-1 (Bryce et al. 2018).

In *Rp. palustris* TIE-1, the deletion of *pioA* consequences in nearly full loss of Fe (II)-oxidizing capacity, although the deletion of *PioB* and *PioC* consequences in only partial loss when correlated to the wild type (Bose and Newman 2011). When Fe(II) is utilized as an electron donor, the pio genes are expressed at their highest levels, but they are transcribed and translated under all anoxic growth conditions. The global regulator *FixK* controls the expression of the pio operon (Bose and Newman 2011). Supplementary clue in what way Fe(II) influences cellular activities in *Rp. palustris* TIE-1 has come from transcriptome and proteome analyses. These findings reveal that high levels of Fe(II) cause stress reactions unexpectedly in anoxic settings, despite the fact that Fe(II) toxicity caused by oxidative stress created by the Fenton’s reaction is not be a concern. The induction of many metal efflux pathways characterizes the cellular response, which was observed throughout both short- and long-term periods of time (Bird et al. 2013). The evidence of PioA’s location in the cell is currently contradictory. Based on the sequencing results, Jiao and Newman (2007) suggested that PioA appears to be a periplasmic protein.

Nevertheless, Bose et al. (2014) revealed that *Rp. palustris* TIE-1 can oxidize the crossed-valent Fe(II)–Fe(III) mineral magnetite (Fe_3O_4) in the solid phase and utilize electrons straight from positioned electrodes. PioA can oxidize Fe(II) with the association of its electron transport mechanism, which must thus be existing on the cell's outer membrane, according to this discovery. Furthermore, *Rp. palustris* TIE-1 can only approach surface-bound Fe(II) in magnetite, emphasizing the necessity for a straight surface–mineral interaction mechanism (Byrne et al. 2015, 2016). *Rhodobacter ferrooxidans* SW2 is another well-studied "anoxygenic phototrophic Fe(II) oxidation" pathway. This organism's foxEYZ operon permits it to oxidize Fe (II) (Croal et al. 2007).

The genomic sequencing of *Chlorobium phaeoferrooxidans* reveals that these green sulfur bacteria have yet another mechanism. In *Mariprofundus ferrooxidans* PV-1, which is a microaerophilic Fe(II) oxidizer, the outer membrane cytochrome (*Cyc2PV-1*) encoded by this genome is thought to be engaged in Fe(II) oxidation (Crowe et al. 2008). *Cyc2PV-1* is a far relative of *Cyc2*, a lithotrophic Fe(II)-oxidizing bacteria found in a broad range of obligatory lithotrophic bacteria (He et al. 2017). Besides, *Cyc2* is encoded in the genome of *C. ferrooxidans* DSM13031 (He et al. 2017). The electron transport pathways and proteins involved in Fe(II) oxidation are likely varied, and no one mechanism exists across every single one of the physiological groups of Fe(II) oxidizers, neither within a single group of Fe(II) oxidizers, like phototrophs (Bryce et al. 2018).

17.6 Environmental Significance

The NRFeOx mechanism aids biochemically and microbially driven redox shifts of iron and nitrate species (Liu et al. 2019). The potency and remodeling of pollutants like chlorinated organic compounds and heavy metals (arsenic [As], antimony [Sb], and uranium [U]) are created by the "Fe III–Fe II redox wheel."

Nitrate reduction is intimately linked to N pollution in numerous natural and anthropogenic systems of waters, and the generation of N_2O (greenhouse gas) has the ability to affect global climate change.

As a result, an in-depth investigation of the redox reactions that may occur in the middle of reactive Fe and N species will assist in an improved insight into the Fe–N cycle in nature. It has long been understood that immobilizing these pollutants requires the absorption of heavy metals and radioactive nuclides onto Fe and manganese (Mn) oxides (Means et al. 1978a, b). A practical bioremediating technique for heavy metals including iron and radioactive nuclide fixation in reducing settings might be devised depending on the reported anaerobic NRFeOx employing *Dechlorosoma* species. The findings show that the oxidation of Fe(II) by *D. suillum* is a unique strategy for heavy metal and radionuclide stabilization and immobilization in the ecosystem. Senn and Hemond (2002) revealed that nitrate altered the arsenic (As) cycling in anoxic circumstances in an urban Upper Mystic Lake located in Massachusetts, the United States, by oxidizing Fe(II) to generate arsenic-adsorbing particulate hydrous iron oxides and creating the oxidation of As(III) to As(V).

After large-scale Fe(II) oxidation started, arsenate was discarded from the water adjacent to the injection at the U.S. Geological Survey (USGS) test site on Cape Cod, Massachusetts, the United States, by integration into hydrous Fe oxide, which was precipitated in the iron-reduction zone in a sandy aquifer under an anoxic condition (Senn and Hemond 2002; Höhn et al. 2006), revealing the significance of NRFeOx to arsenic immobilization. The inclusion of nitrate in paddy field soils decreased the Fe(II) concentration in the soil solution while increasing NRFeOx bacterial density, resulting in considerably reduced dissolved Fe(II) concentrations in the rhizosphere soil solution (Liu et al. 2019). It was proposed that NRFeOx bacteria activation might result in arsenic coprecipitation with or adsorption to Fe (III) ions in the soil, followed by reduced arsenic absorption utilizing rice plants (Chen et al. 2008). The findings of continuous-flow sand-filled columns revealed that microbial oxidation of arsenate and Fe(II) associated with denitrification boosted aqueous As immobilization in anaerobic conditions by creating hydrous iron oxides–coated sands with adsorbed pentavalent arsenic (Sun et al. 2009). Despite the fact that it does not affect the anaerobic NRFeOx bacteria metabolism, arsenate efficiently immobilized arsenic during Fe(II) oxidation (efficient for more than 96%), lowering the residual dissolved arsenic concentrations to levels approaching or even below the acceptable limit of drinking water of 10 μg/L (Hohmann et al. 2010). The ability of a single anaerobic NRFeOx *Citrobacter freundii* strain PXL1 to reduce and remove As(III) and nitrate from water synchronously was investigated, and it was disclosed that these bacteria are potential microorganisms for in situ remediation of arsenite- and nitrate-contaminated groundwater in China (Li et al. 2015). The concurrent usage of Fe(II) and nitrate successfully reduced the inflation of arsenic (As) in rice paddy plants by intensifying arsenic oxidation or immobilization intervened by biotic or abiotic iron redox transformation and mineralization (Wang et al. 2018), and the conclusions delivered an understanding about the As/Fe/N biogeochemical cycles, which are essential from the perspective of agricultural crop management activity of arsenic toxicity and its mitigation in arsenic-affected area.

Even though nitrate respiration has long been established as a general process in microbes (Ducluzeau et al. 2009), little has been written about the effects of iron oxidation on nitrate reduction and releasing of a greenhouse gas (N_2O) due to a lack of understanding of nitrogen cycle through abiotic and biotic procedures. However, these abiotic N_2O generation mechanisms have been recognized since the nineteenth century; they are typically disregarded in contemporary environmental studies, making it critical to analyze their importance (Zhu-Barker et al. 2015). Following an early idea (the ferrous wheel theory) for abiotic nitrate immobilization in forest soils, reactive Fe(II) species might convert nitrate to nitrite. Nitrite was then combined alongside dissolved organic materials to form dissolved organic nitrogen (Davidson et al. 2003). Despite the fact that the abiotic and biotic interactions were unclear, the data highlighted the realness activity and significance of NRFeOx in the renowned N cycle scenario. The potential of iron-dependent nitrogen cycling in riparian forest sediments also discussed the Fe–N interaction (Clement et al. 2005). Under a variety of environmental circumstances, dual (N and O) isotope structures were used to investigate the reduction of abiotic nitrite by Fe(II). The findings showed that Fe(II)-mediated nitrite reduction could be a significant origin of abiotic environmental N_2O, particularly in an iron-rich environmental condition with potent redox fluctuations (Buchwald et al. 2016).

17.7 Bioremediation

Various biological approaches, like bioremediation including phytoremediation, have been used to remediate environmental toxins, and they are not only cost-effective but also environmentally beneficial. Bioremediation is a broad word that refers to the transformation of harmful or complicated pollutants into simpler, less toxic forms, or even full degradation, using biological mechanisms including bacteria or microbial products (Datta et al. 2020; Singh et al. 2021). The process of eliminating pollutants from the environment in the absence of the use of chemicals is known as intrinsic bioremediation (Lovley 2003). By altering the environment in which the microorganisms are grown at the polluted location, microbial activity can be increased to achieve higher clean-up rates. The bioremediation technique is regarded as designed in these circumstances (Mishra et al. 2021).

Iron oxidation occurs in most aquatic habitats that include elemental or reduced forms of iron, that is, ferrous iron (Eggerichs et al. 2020). Microorganisms that accelerate iron oxidation gain the energy they need to multiply when they use either nitrate or oxygen as an electron acceptor (Emerson et al. 2010). Iron oxidation of microorganisms can be divided into four categories that have been already mentioned above somewhere in the content. Zagury et al. (1994) demonstrated heavy metal removal by native IOB from polluted soil. Bacteria such as *Acidithiobacillus ferrooxidans* can be used to reduce the soil solution pH lower than 2.5 and to increase the oxidation–reduction potential at room temperature when adjusted to a pH of 4. Iron oxidation through such bacteria was accelerated by the addition of ammonium sulfate [$(NH_4)_2SO_4$] and potassium phosphate (K_2HPO_4). Zinc and manganese had higher percent removal rates than copper. Metal speciation, together with the soil types studied, affected metal removal by IOB. A potential technique for the efficient removal of arsenic from groundwater was discovered to be the biotic oxidation of iron by the bacteria *Gallionella ferruginea* and *Leptothrix ochracea*, which offered an economical and environmentally beneficial alternative. The microorganisms and iron oxides that were formed in the upflow filtration columns, during this process, provide an ideal environment for the absorption and arsenic removal from the aqueous streams (Katsoyiannis and Zouboulis 2004). Blais et al. (1993) described their research on the function of IOB in the sequestration of toxic metals from sewage sludge. Iron-oxidizing microorganisms lower the pH of sewage sludge and increase its oxidation–reduction potential. These were found to be the metals removed from sludge by *A. ferrooxidans* (previously known as *Thiobacillus ferrooxidans*) in the following ascending order: manganese, zinc, nickel, cadmium, copper, lead, and chromium, with the sludge form used during the experiment having a little effect. Xiang et al. (2000) conducted another batch of investigation aimed at the heavy metal removal from sewage sludge utilizing indigenous IOB. When iron was supplemented to inoculated sets as the ferrous sulfate form, the sludge pH fell from 2.0 to 2.5. Following 16 days of treatment with iron oxidizers, there was a significant decline in the metal contents (chromium, copper, lead, nickel, and zinc) available in sewage sludge, attaining agriculturally acceptable levels, indicating that biological agents can be used to remediate metal contaminants. Recently, the contribution of microaerophilic IOB in arsenic (As) removal was reported by Tong et al. (2019) through the adsorbed/immobilized of arsenite and arsenate that resulted in ferric oxyhydroxide formation following the prominent activity of iron oxidizers with a significant arsenic adsorption affinity. The As content was reduced from 600 to 4.8 g/L following iron oxidizer treatment. The presence of an arsenite oxidase gene, which is accountable for the formation of arsenite from arsenate in microbes, was also discovered in the study. The generation of biogenic iron oxides as a result of the iron oxidation by the bacteria *Gallionella* and *Leptothrix* has a tremendous potential for phosphate elimination from the solution phase (Buliauskaite et al. 2020). Moreover, *Gallionella* sp. has been shown to have an overall phosphate removal capacity than *Leptothrix* sp. When compared to chemically manufactured iron oxides, biologically derived iron oxides had a greater phosphate sequestration performance, implying that they could be used in phosphate recovery treatment systems. Adsorption and precipitation play a substantial role in phosphate elimination by biogenic iron oxides, according to their findings. Another recent study showed that IOB can fortunately bioremediate groundwater contaminated with prominent levels of iron and manganese (Aziz et al. 2020). Not long ago, the ability of bacteria *A. ferrooxidans* to extract heavy metals (chromium, copper, nickel, and zinc) from electroplating sludge under low-voltage stimulation paves the door for industrial-scale metal contamination treatment (Wu et al. 2020). Under laboratory conditions with a pH of 2.0–3.0 and Fe concentrations near 11 g/L, *A. ferrooxidans* catalyzes the synthesis of crystalline biogenic tooeleite, which is an iron-arsenic-based mineral and is thought to remove roughly 95% of As(III) (Li et al. 2020a, b). The research revealed key principles driving biogenic mineral formation, which could lead to the advancement of a treatment system that uses rapidly proliferating IOB to decontaminate As-damaged environments (Li et al. 2020a, b; Singh et al. 2021).

17.8 Future Direction and Conclusion

In the future, nitrate-reducing bacteria may be a valuable microbe for the Biofloc Technology of fish culture to reduce nitrate, which is a severe concern in the system. However, more research into this topic is required. Besides, the pathogenic nature of these bacteria needs to be thoroughly studied to avoid any disease outbreak in the future. Moreover, bioremediation with iron-degrading bacteria can minimize iron toxicity, which is both economical and ecologically sound. Furthermore, this technology will be a boon. However, it is critical to investigate all of the mechanisms involved in the process. Furthermore, the microorganisms must be completely defined before they can be deployed in the bioremediation of iron in an environment where in situ bioremediation has limited. In addition, the remediation of environments contaminated by metalloids, metals, and hazardous organics may benefit from the use of IOB that are resilient to greater metal concentrations and can swiftly adapt to changing natural environmental circumstances.

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Bioactive Compounds from Aquatic Ecosystem

18

Surendra Puri, Rohit Mahar, and Gunjan Goswami

Abstract

Aquatic ecosystems, especially marine, which cover nearly 67% of the earth and 50% of the whole globe's biodiversity, offer scientists and researchers a promising sustainable source of many bioactive substances and their potential applications in pharmaceuticals, cosmeceuticals, and nutraceuticals industries. They contain different specific habitats characterized by a wide range of temperatures, hydrostatic pressures, and salinity. To survive in such harsh and challenging conditions, marine living beings have the potential to produce unique and valuable bioactive compounds. In the recent decades, the course of investigations of bioactive compounds from the aquatic ecosystems has greatly expanded and gained much importance because of their therapeutic uses for numerous diseases. Compounds isolated from aquatic or marine biotopes possess a broad spectrum of biological activities such as antitumor, anticancer, anti-human immunodeficiency virus (HIV), antidiabetic, antioxidant, antimicrotubule, antiproliferative, antibacterial, anti-inflammatory, cytotoxic, photoprotective, antibiotic, and antifouling. The huge chemical complexity, unique skeleton diversity, broad spectrum of biological activities of the compounds isolated from aquatic beings, and their therapeutic applications have engrossed the attention of scientists and researchers in the recent years.

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Keywords

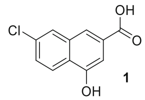
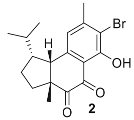
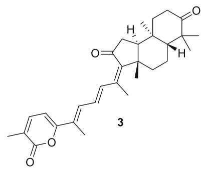
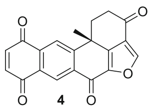
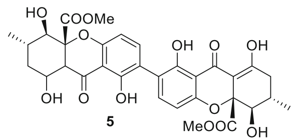
Aquatic · Bioactive compounds · Ecosystem · Anti-inflammatory · Antioxidant

18.1 Introduction

Natural products have been used for the treatment of human ailments since the ancient time, and the remedial properties of these natural products are well described in Rigveda (2500–1800 BC), Charak Samhita, and Sushruta Samhita. Oceans cover nearly 67% of the earth and 50% of the whole globe's biodiversity and remain as one such storehouse for natural products. The importance of natural product discovery from microorganisms started only after the large-scale production of penicillin during World War II, although penicillin was discovered in 1928 by the Scottish bacteriologist Alexander Fleming from the *Penicillium* mold (Landau et al. 1999), and at that time, it was believed that soil microorganisms are the major source of novel drugs. The aquatic ecosystems are less explored than their terrestrial counterparts (Lordan et al. 2011; Rasmussen and Morrissey 2007) due to the time, cost, and geographical constraints, yet they possess infinite hidden sources for many useful chemical substances (Schröder 2010). The aquatic biotopes undergo harsh climate changes; live in stressful habitats; experience a cold, lightless, and high-pressure conditions; and biosynthesize a wide variety of secondary metabolites (SMs) for their defense against other microorganisms. These fascinating and structurally complex SMs serve as a source of bioactive compounds for use in human therapies (Bhatnagar and Kim 2010). Secondary metabolites (SMs) are generally defined as organic compounds formed as bioproducts in organisms and do not directly involve in the growth, development, and reproduction of thereof (i.e., hormones, antibiotics, and vaccines). The metabolites derived from marine bacteria, proteobacteria, cyanobacteria, microalgae, seaweed, fungi, marine invertebrates, fishes, and actinomycetes showed antibacterial (Lu et al. 2009), antiviral, antifungal (Rajanbabu and Chen 2011), antihypertensive (angiotensin-converting enzyme [ACE] inhibitor) (Ono et al. 2003), antioxidant (Mendis et al. 2005; Nalinanon et al. 2010), antimicrobial (Zhang et al. 2004), antitumor (Guha et al. 2013; Li et al. 2005), antithrombin (Salte et al. 1995), antidiabetic (Zhu et al. 2010), antilarvicidal (Kirst 2010), antineoplastic (Smith et al. 2000), antituberculosis (Barry et al. 2000; Jensen et al. 2007), and antimalarial (Jensen et al. 2007) activities.

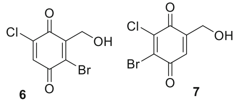
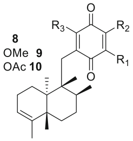
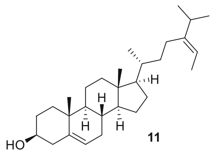
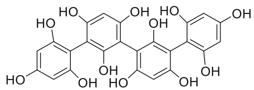
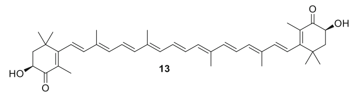
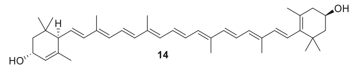
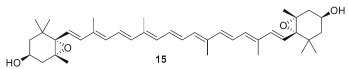
Having a broad range of bioactivities, aquatic organisms and microorganisms have not been fully explored yet, and limited research and discoveries are available in literature to date. To fill this gap, there is still a scope for a collaborative research to explore the therapeutic potential of novel molecules of an aquatic biotope. In 2004, Blunt et al. reported that among the marine organisms and microorganisms, sponges (37%), coelenterates (21%), microorganisms (18%), algae (9%), echinoderms (6%), tunicates (6%), molluscs (2%), bryozoans (1%), etc. are the major contributors to the production of bioactive compounds (Leal et al. 2012). Table 18.1 summarizes the medicinal applications and activities of the molecules isolated from the marine ecosystems.

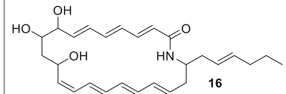
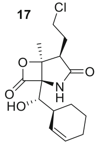
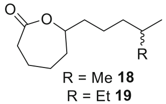
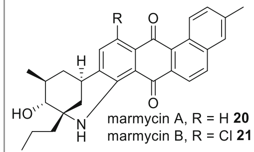
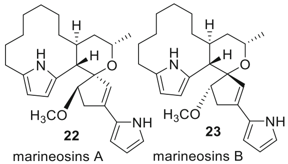
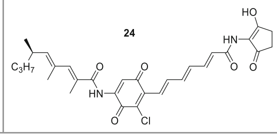
Table 18.1 Structures and biological activities of secondary metabolites isolated from marine sources

Sources	Order/species	Bioactive (secondary metabolites)	Biological activity	Structures
Marine sponge	*Streptomyces* sp. SBT345	Ageloline A	Antioxidant and antichlamydial activities	 1
	Hamigera tarangaensis	Hamigeran B	Antiviral activity against polio virus	 2
	Jaspis stellifera	Stellettin B (Wu et al. 2016)	Significant cytotoxic effects against the human glioblastoma cell line SF295	 3
	Xestospongia carbonaria	Halenaquinone (Budke et al. 2013)	Inhibits the secondary DNA binding of RAD51	 4
Marine fungi	*Penicillium oxalicum*	Secalonic acid D	Antitumor activity and antimicrobial activity (against the gram-positive bacteria and gram-negative bacteria); antifungal and antialgal activities	 5

(continued)

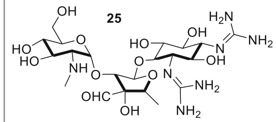
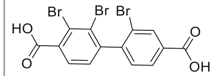
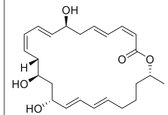
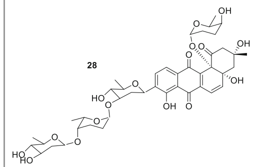
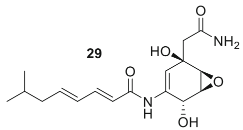
Table 18.1 (continued)

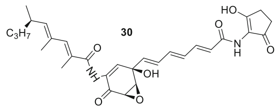
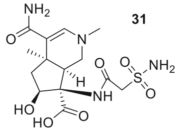
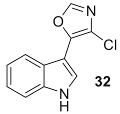
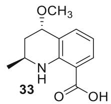
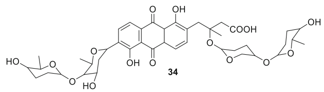
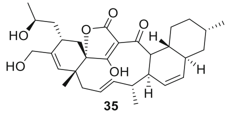
Sources	Order/species	Bioactive (secondary metabolites)	Biological activity	Structures
	Phoma herbarum	Bromo-chloro-gentisyl-quinones A and B	Antioxidant activity	 6 7 A B bromochlorogentisylquinones
	Dysidea avara	Avarol and avarone (Loya and Hizi 1990)	Antiviral activity against HIV and anti-inflammatory activity	R1 = H, OH **8** R2 = H, OH, OMe **9** R3 = H, OH, OAc **10** 
Marine macroalgae	*Pelvetia siliquosa*	Fucosterol	Prevent the degradation of blood glucose	 **11**
	Ecklonia kurome	Phlorotannins	Inhibition of amylase precipitate proteins	 **12**
Marine microalgae	*Haematococcus pluvialis*	Astaxanthin	Antioxidant, anticancer, and anti-inflammatory activities	 **13**
	Chlorella vulgaris	Lutein	Antioxidant and anticancer activities	 **14**
	Chlorella ellipsoidea	Violaxanthin	Anti-inflammatory and anticancer activities	 **15**

Marine bacteria	*Streptomyces aureoverticillatus*	Aureoverticillactam	Cytotoxicity against various types of cell tumors	
	Salinispora tropica	Salinosporamide A (NPI-0052) (Beer and Moore 2007)	Potential anti-cancer agents	
	Streptomyces sp.	Caprolactones	Anticancer activity	
	Streptomyces sp.	Marmycin A and marmycin B	Marmycin A displays potent cytotoxicity against numerous cancer cell lines	
	Streptomyces sp.	Marineosins	Significant inhibition of human colon carcinoma and selective activities in diverse cancer cell types	
	Streptomyces sp.	Chinikomycins	Antitumor activity	

(continued)

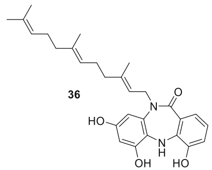
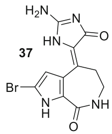
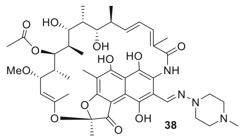
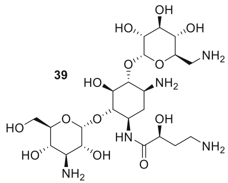
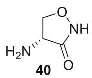
Table 18.1 (continued)

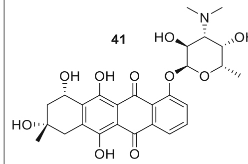
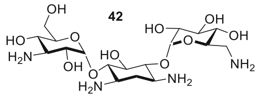
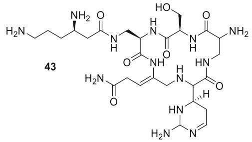
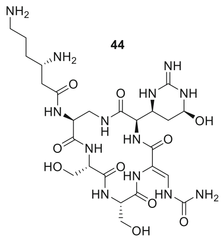
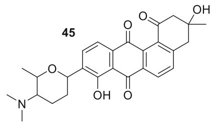
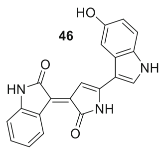
Sources	Order/species	Bioactive (secondary metabolites)	Biological activity	Structures
	Streptomyces griseus	Streptomycin	Potent antibiotic used to treat numerous bacterial infections	 25
	Pseudoalteromonas	MC21-B	Antibacterial activity	 26
	Bacillus sp.	Macrolactin S	Antibacterial activity against *E. coli* and *S. aureus*	 27
	Streptomyces fradiae	Urdamycin A	Antibacterial and anticancer activities	 28
	Streptomyces strain CNQ-085	Daryamide D	Cytotoxic activity against human colon carcinoma and antifungal activity against *Candida albicans*	 29

	Streptomyces sp. M045	Manumycin	Antitumor activity	 30
	Streptomyces sioyaensis	Altemicidin	Antitumor and acaricidal activities	 31
	Streptomyces sp.	Streptochlorin	Potent chemotherapeutic agent	 32
	Janibacter limosus	Helquinoline	Antibacterial, antifungal, and antimicroalgal activities	 33
	Streptomyces sp.	Himalomycins	Antimicrobial activity against gram-positive bacteria	 34
	Micromonospora sp. GMKU326	Maklamicin	Antimicrobial activity against gram-positive bacteria	 35

(continued)

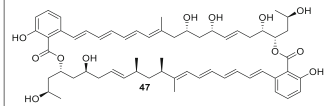
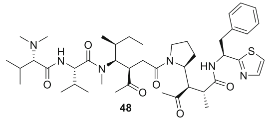
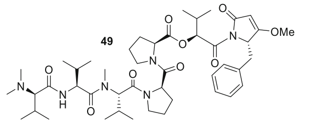
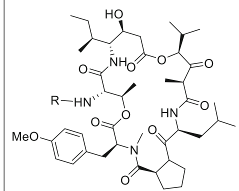
Table 18.1 (continued)

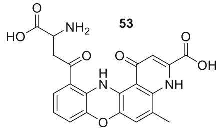
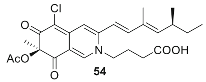
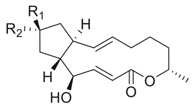
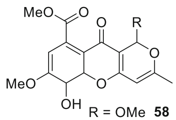
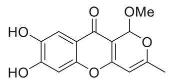
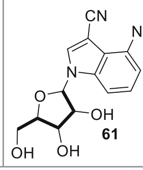
Sources	Order/species	Bioactive (secondary metabolites)	Biological activity	Structures
	Micromonospora sp.	Diazepinomicin (ECO-4601)	Antitumor, antioxidant, and antiprotease activities	 36
	Agelasidae, Axinellidae, and Halichondriidae	Hymenialdisine (Meijer et al. 2000)	Anti-inflammatory activity	 37
	Amycolatopsis rifamycinica	Rifampicin	Potent antibiotic used to treat various bacterial infections	 38
	Sporosarcina newyorkensis	Amikacin	Powerful activity against antibiotic-resistant clinical isolates	 39
	Streptomyces sp.	Cycloserine	Antitubercular activity	 40

	Streptomyces sp.	Komodoquinone A (Itoh et al. 2003)	Induces neuronal cell differentiation in the neuroblastoma cell line	**41** 
	Streptomyces kanamyceticus	Kanamycin	Antibiotic used to treat severe bacterial infections and tuberculosis	**42** 
	Streptomyces sp.	Capreomycin	It is a second-line anti-TB drug used to treat drug-resistant tuberculosis.	**43** 
	Streptomyces puniceus	Viomycin	Viomycin is a peptide antibiotic exhibiting anti-TB activity	**44** 
	Streptomyces griseus	Frigocyclinone	Antibacterial activities against gram-positive bacteria	**45** 
	Chromobacterium violaceum and *Janthinobacterium lividum*	Violacein	Antiprotozoal activity	**46** 

(continued)

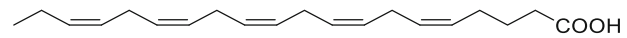
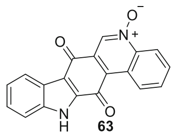
Table 18.1 (continued)

Sources	Order/species	Bioactive (secondary metabolites)	Biological activity	Structures
Marine actinomycetes	*Marinispora* sp. strain CNQ-140	Marinomycin	Inhibits cancer cell proliferation	 **47**
Marine invertebrates	Mollusca (*Dolabella auricularia*)	Dolastatin 10	Anticancer activity, promising antiproliferative activity	 **48**
	Mollusca (*Dolabella auricularia*)	Dolastatin 15	Anticancer activity	 **49**
	Ascidians and tunicates (*Tridemnum* sp.)	Didemnin	Exhibits antitumor activity, strong antiviral agent against both DNA and RNA viruses, exhibits strong activity against murine leukemia cells	 R = *N*-Me-L-Leu : Didemnin A **50** R = Lac-Pro-*N*-Me-L-Leu : Didemnin B **51** R = Lac-*N*-Me-L-Leu : Didemnin C **52**

Marine fish	Giant squid (*Dosidicus gigas*)	Xanthommatin	Antioxidant activity	 53
Marine fungus	*Penicillium* sp.	Isochromophilone	Anti-inflammatory activity	 54
	Penicillium sp.	(+)-Brefeldin A, (+)-brefeldin C, and 7-oxobrefeldin A	Antifungal activity against *Microsporum gypseum* SH-MU-4	 **33** R1 = OH, R2 = H **55** **34** R1 = R2 H **56** **35** R1 + R2 = O **57**
	Chaetomium sp.	Chaetocyclinones A–C	Antifungal activity	 R = OMe **58** R = H **59**  **60**
	Streptomyces sp.	Toyocamycin	Antifungal activity	 **61**

(continued)

Table 18.1 (continued)

Sources	Order/species	Bioactive (secondary metabolites)	Biological activity	Structures
	Shewanella sp.	Eicosapentaenoic acid (EPA) (Braune et al. 2021)	Used to treat heart disease, anti-inflammatory activity	 62
	Chaetoceros sp.	Calothrixin	Antimalarial and anticancer activities	 63

18.2 Bioactivity of Secondary Metabolites Isolated from Marine Ecosystems

18.2.1 Anticancer Activity

In general, human cells grow and proliferate through the cell division process and form new cells. When cells become old, get damaged, or die, new cells take their place. Sometimes cell division process breaks down, and the abnormal or damaged cells grow up and multiply themselves uncontrollably. These abnormal cells have the ability to invade and destroy normal body tissue or spread to other parts of the body and cause cancer. The probable signs and symptoms of cancer are abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. There are more than hundred types of cancer, and the most common types are lung cancer, prostate cancer, breast cancer, stomach cancer, cervical cancer, and colorectal cancer (Cakir et al. 2012). Worldwide the number of cancer patients and the cost of treatments are increasing simultaneously, which remain a huge socioeconomic burden. Therefore, there is an urgent need to discover new anticancer drugs. A few natural compounds such as taxol, camptothecin, vincristine, and vinblastine were isolated from plants and used to treat cancer (Sung et al. 2017). In the recent decades, secondary metabolites isolated from marine biotopes have shown a promising activity when administered at different cancer stages and opened another dimension for the development of new anticancer drugs. Secalonic acid D (SAD, **5**) obtained from *Penicillium oxalicum* and marine-derived fungus shows promising anticancer properties and acts as a deoxyribonucleic acid (DNA) topoisomerase I inhibitor (Cherigo et al. 2015; Steyn 1970). It demonstrates a potent cytotoxic activity and induces apoptosis in K562 and HL60 myeloid leukemia cell lines by blocking the G1 phase of the cell cycle in the GSK-3β/β-catenin/c-Myc pathway. It also shows a potent antimicrobial activity against gram-positive bacteria such as *Bacillus megaterium* and the gram-negative bacteria *Escherichia coli*, an antifungal activity against *Microbotryum violaceum*, and an antialgal activity against *Chlorella fusca* (Zhang et al. 2008). Salinosporamide A **(17)** is a potent anticancer agent obtained from marine bacteria *Salinispora tropica* and *Salinispora arenicola.* In preliminary screening, a high percentage of the organic extracts of Salinispora strains displays antibiotic and anticancer activities. Compound **17** shows potent in vitro cytotoxicity against HCT-116 human colon carcinoma with an half-maximal inhibitory concentration (IC_{50}) value of 11 ng mL^{-1} and inhibits proteasome activity by covalently modifying the active site of threonine residues of the 20S proteasome (Groll et al. 2006). Caprolactones (R-10-methyl-6-undecanolide **(18)** and 6R, 10S-10-methyl-6-dodecanolide **(19)**) are isolated from the extract of a marine *Streptomyces* sp. B6007. These two compounds are found active against human cancer cell lines and show concentration-dependent inhibition of the cell growth of HM02 (gastric adenocarcinoma), MCF-7 (breast adenocarcinoma), and HepG2 (hepatocellular carcinoma). Cell cycle analysis was carried out in HepG2 cells, which showed an increased number of cells in the G1 phase than the S phase on the treatment of 5 μg mL^{-1} for 2–24 h (Stritzke et al. 2004). Marmycins A and B

(20, 21) isolated from the genus *Streptomyces* display significant cytotoxicity against numerous cancer cell lines at nanomolar concentrations. It is noteworthy that Compound **21** was found less potent than Compound **20**. Compound **20** induces apoptosis and arrests the G1 phase of the cell cycle (Martin et al. 2007).

The secondary metabolites marineosins A **(22)** and marineosins B **(23)** are obtained from actinomycete of the genus *Streptomyces.* Marineosins A **(22)** shows significant cytotoxicity against HCT-116 human colon tumor cell line with an IC_{50} value of 0.5 μM. Marineosins B **(23)** displays significantly weaker cytotoxicity ($IC_{50} = 46$ μM). Compounds **22** and **23** show very weak antifungal activity against *Candida albicans* with a minimum inhibitory concentration (MIC) value of 100 μg/mL (Boonlarppradab et al. 2008). Similarly, Aureoverticillactam **(16)** (Mitchell et al. 2004), Chinikomycins (**24**) (Li et al. 2005), Urdamycin A **(28)** (Henkel et al. 1989), Daryamide D **(29)** (Asolkar et al. 2006), Manumycin **(30)** (Li et al. 2005), Altemicidin **(31)** (Takahashi et al. 1989), Streptochlorin **(32)** (Shin et al. 2007), Diazepinomicin (ECO-4601) **(36)** (Sung et al. 2017), Cycloserine **(40)** (Barry et al. 2000), Marinomycin **(47)** (Liu and Jiang 2017), Dolastatin 10 **(48)** (Pettit et al. 2017), Dolastatin 15 **(49)** (Pettit et al. 2017), and Didemnin **(50, 51** and **52)** (Rinehart et al. 1981), etc. are isolated from marine ecosystems and show a promising activity against a panel of cancer cell lines. Table 18.1 summarizes the potential applications of these compounds.

18.2.2 Antimicrobial Activity

Natural products are being widely and frequently utilized for antimicrobial therapy and other related diseases since the ancient times (Suleria et al. 2016). SMs secluded from marine ecosystem show a broad range of antimicrobial activities against various human pathogens. Fucosterol **(11)**, isolated from *Fucus vesiculosus* (macroalgae), inhibits the germination of macroconidia in *Fusarium culmorum* (Meinita et al. 2021; Tyśkiewicz et al. 2019). In the recent years, the outbreak of drug-resistant tuberculosis (TB) increases the mortality rate across the globe and drew the attention of researchers to search for new potent anti-TB agents. A few marine-derived compounds such as Rifampicin **(38)** (Barry et al. 2000), Streptomycin **(25)** (Barry et al. 2000), Capreomycin, Viomycin, and Kanamycin (Barry et al. 2000) show promising anti-TB activity and are used as second-line anti-TB drugs. Compound **25** is also found active against several aerobic gram-negative bacteria. Macrolactin S **(27)** isolated from a culture broth of marine *Bacillus* sp. inhibits the bacterial growth against *E. coli* with an MIC of 0.2 mg mL^{-1}. Macrolactin S also shows an antifungal activity and inhibits the hyphal growth of *Pyricularia oryzae* (Lu et al. 2009). MC21-B is isolated from marine bacterium Pseudoalteromonas phenolica O-BC30^{T} and shows an antibacterial activity against various isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) (Isnansetyo and Kamei 2009). Helquinoline **(33)**, another secondary metabolite obtained from the cultures of *Janibacter limosus*, shows significant antibacterial and antifungal activities against *Streptomyces viridochromogenes*, *Staphylococcus aureus*, *Mucor miehei*, and

Chlorella vulgaris (Maskey et al. 2004). Similarly, Himalomycins **(34)** ("Himalomycin A and cycloheximide-producing marine actinomycete from Lagos Lagoon soil sediment" 2015), Frigocyclinone **(45)** (Bruntner et al. 2005), Amikacin **(39)** (Barry et al. 2000), and Maklamicin **(35)** (Igarashi et al. 2017) show a potent antimicrobial activity against gram-positive bacteria.

18.2.3 Anti-inflammatory Activity

Anti-inflammatory drugs reduce inflammation or swelling by binding to cortisol receptors in case of steroidal anti-inflammatory drugs (SAIDs) or inhibit cyclooxygenase (COX) enzymes in case of nonsteroidal anti-inflammatory drugs (NAISDs). The frequent use of anti-inflammatory drugs causes gastrointestinal ulcer, bleeding, heart attack, stroke, and hepatotoxicity. Because of severe side effects, natural products–based drugs are preferred over synthetic drugs for the treatment of inflammation. Azaphilone derivative, isochromophilone IX **(54)**, and sclerketide C isolated from coral-derived fungus *Penicillium sclerotiorin* exhibit potent anti-inflammatory activity (Liu et al. 2019). Fucoidans are polysaccharides containing a significant fraction of L-fucose and sulfate ester groups obtained from brown algae *Fucus vesiculosus*, which possess potent anti-inflammatory activity via inhibiting pro-inflammatory cytokine production (Vo et al. 2015). Marine lectins (protein) also show an anti-inflammatory activity due to their carbohydrate-binding site (Cheung et al. 2015).

18.2.4 Antioxidant Activity

Janibacter melonis and *Pseudomonas Stutzeri* are marine bacteria that produce antioxidant compounds (Shahidi and Santhiravel 2022). The extract of actinobacterium *Streptomyces* sp. *S2A* shows antioxidant and cytotoxic activities against different cell lines (Siddharth and Vittal 2018). Bromo-chloro-gentisylquinones A **(6)** and B **(7)**, obtained from a marine fungus *Phoma herbarum* strain, show a significant radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Trisuwan et al. 2009). Xanthommatin **(53)** is an ommochrome obtained from jumbo squid skin (JSS) and shows a potent antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Swain et al. 2017; Williams et al. 2019).

18.2.5 Antifungal Activity

An antifungal medication, also known as an antimycotic medication, is a fungicide used to treat and prevent mycosis such as ringworm, athlete's foot, and candidiasis (thrush). Three compounds named (+)-brefeldin A **(55)**, (+)-brefeldin C **(56)**, and 7-oxobrefeldin A **(57)** are isolated from the marine fungi of the genus *Penicillium* sp. Compound **55** shows an excellent antifungal activity against *Microsporum gypseum* with an MIC value of 228.57 μM, while the rest of the compounds are found inactive (Nenkep et al. 2010). Chaetocyclinones A–C **(58, 59,** and **60)** are the marine SMs obtained from the cultures of *Chaetomium* sp. Compounds **59** and **60** are found inactive, while Compound **58** shows a potent antifungal activity against *Phytophthora infestans* at a concentration of 89.1 μM. Toyocamycin **(61)** is an adenosine analog produced by *Streptomyces diastatochromogenes*, which shows an antifungal activity (Burja et al. 2001; Chan-Higuera et al. 2019). Isochromophilone **(54)** derivatives isolated from the culture broth of *Penicillium* sp. show a broad range of activities, such as inhibition of acyl-CoA cholesterol acyltransferase (ACAT) and diacylglycerol acyltransferase (DGAT) (Arai et al. 1995).

18.2.6 Miscellaneous Activity of Marine-Derived Secondary Metabolites

Violacein **(46)** is a bisindole, water-insoluble purple pigment, produced by *Chromobacterium violaceum* and *Janthinobacterium lividum.* This compound displays antiprotozoal and antiparasitic activities and also shows a cytotoxic effect against several tumor cell lines (Almeida et al. 2021; Matz et al. 2008). Toyocamycin **(61)** isolated from culture broth of an *Actinomycete* strain, which belongs to a class nucleosides, shows significant antifungal, antibiotic, and antitumor activities (Katritzky and Rees 1984). Calothrix obtained from cyanobacteria shows a potent antimalarial activity and inhibits ribonucleic acid (RNA) polymerase and DNA topoisomerase-I poisoning activity. Compound **61** also shows a significant antiproliferative activity against human HeLa cancer cells (Ramkumar and Nagarajan 2013; Rickards et al. 1999). Eicosapentaenoic acid (EPA, **62**), also known as omega 3-fatty acid, obtained from microalgae is used to treat heart and inflammatory diseases. EPA is also effective against rheumatoid arthritis and immunodeficiency disease (Braune et al. 2021) (Table 18.1).

18.3 Conclusion

The various structurally diverse compounds and extracts obtained from marine biotopes possess a broad range of biological activities including anticancer, antibacterial, antiviral, fungicidal, cytotoxic, antimalarial, and antioxidant and have been utilized for the treatment of numerous human ailments. The well-documented medicinal applications of these marine compounds as evidenced from the past and current research make oceans so special and remain a large producer of many bioactive compounds. The hidden unexplored marine diversity would be a great treasure for scientists and researchers to discover the novel drugs. Under this paradigm, a growing and sustainable research and development programs must be fostered by the scientists, researchers, academicians, and industries under collaborative partnerships. It is also encouraging that few molecules obtained from marine sources are under clinical and preclinical trials, and many of them are on the market for the treatment of many diseases.

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Freshwater Blue–Green Algae: A Potential Candidate for Sustainable Agriculture and Environment for the Welfare of Future Planet Earth

19

Arun Kumar Rai, Binu Gogoi, and Rabina Gurung

Abstract

Different types of microorganisms are known to exist in freshwater habitats. These microbes function similarly to the microorganisms found in soil and air. Freshwater, brackish, marine and terrestrial cyanobacteria (blue–green algae [BGA]) are a diverse group of prokaryotes and are also the most successful and oldest life forms on the planet. They play an important role in maintaining and improving soil fertility, increasing plant growth and yield as a natural biofertilisers, nutrient cycling, nitrogen (N_2) fixation and environmental protection. Cyanobacteria demonstrate the potential for effectively converting light energy into chemical energy. The aim of this chapter is to provide valuable information about the potential role of freshwater cyanobacteria in solving the agricultural and environmental problems on the planet earth.

Keywords

BGA · Nitrogen fixation · Plant growth · Bioremediation · Biofuels

19.1 Introduction

Crop production has mostly relied on conventional agriculture for thousands of years. However, the world is evolving (Mutale-Joan et al. 2022). By 2050, there will be roughly nine billion people on the planet, according to the current growth rates. Abiotic stresses, global climate change and instances of land degradation will all rise in tandem with this (Gr et al. 2021). Producing adequate food for an

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expanding population is one of the issues faced by agriculture today (Iniesta-Pallares et al. 2021). The development of chemicals like synthetic fertilisers and pesticides has significantly increased crop yield over time. However, the widespread use of such chemicals has an adverse effect on the ecology and ecosystem (Mutale-Joan et al. 2022). The continuous increase of human population and growing concerns about energy crisis, food security, disease, global warming and other environmental problems require a sustainable solution from nature, and one of the promising resources is cyanobacteria which need simple materials to grow and have relatively simple genomes (Zahra et al. 2020). Blue–green bacteria, sometimes known as cyanobacteria, are Gram-negative prokaryotes that may grow in photoautotrophic environments. They occupy a unique place in the earth's evolutionary process. In addition to living in other harsh environments, cyanobacteria may survive in deserts and soil that has been impacted by salt. Based on their physical characteristics and molecular approaches, around 150 genera and 2000 species have been discovered (Li et al. 2019). Cyanobacteria are classified as members of the kingdom Monera and the division Cyanophyta (Singh et al. 2005). They are also known as blue–green algae, found in a variety of environments including freshwater, oceans, soil and bare rock (Mehdizadeh Allaf and Peerhossaini 2022). They frequently form vast colonies, are tiny and are typically unicellular. Cyanobacteria are made up of a wide variety of bacteria in various sizes and forms. They can be found in 150 of the currently recognised genera. They resemble the earliest fossils, which date back to more than 3.5 billion years ago (Chittora et al. 2020) and also have an evolutionary significance, as they are necessary for today's oxygenic climate (Awasthi and Singh 2021). Being prokaryotic oxygenic phototrophs, cyanobacteria are resilient to adverse environmental conditions, such as temperature extremes, ultraviolet radiation, drought, salt and quick hydration/dehydration cycles. As a result, they may colonise a wide range of environments (Chamizo et al. 2018). Their cellular structure is simple prokaryotic, and they perform photosynthesis like plants, but without plant cell walls like primitive bacteria. These are also similar to animals in that they have complex sugars on their cell membranes, such as glycogen (Singh et al. 2005). Cyanobacteria are one of the most important organisms to have ever evolved on our planet. Their ancestors developed the ability to oxidize photosynthesis, making it possible to oxidise the earth's atmosphere and oceans (Sánchez-Baracaldo et al. 2022).

19.2 Diversity in Freshwater Ecosystem

Algae predominantly are a group of photosynthetic organisms, having a nucleus like plants but lacking true roots, stems and leaves. Algae are divided into two classes based on cellularity: microalgae and macroalgae. Microalgae are unicellular species and comprises red algae, green algae, brown algae and blue–green algae. Macroalgae are multicellular and commonly known as seaweeds. Based on their pigments, macroalgae are divided as red algae (Rhodophyta), green algae (Chlorophyta) and brown algae (Phaeophyta).

Blue–green algae are also known as cyanobacteria. They are Gram-negative prokaryotes. Because of the presence of the blue pigment phycocyanin, they are able to fix atmospheric nitrogen and help in carbon assimilation. Their structural organisation comprises simple unicellular to complex multicellular forms. They have highly differentiated cell types. The cells of cyanobacteria are differentiated into special cell types that are known as heterocysts. With the aid of this structure, cyanobacteria are able to carry out nitrogen fixation (Waterbury 2006). Photosynthesis is carried out by vegetative cells present in them. Cyanobacteria are present in paddy fields, soil, saline habitats and brackish, fresh and marine waters (Thajuddin and Subramanian 2005). Numerous studies have been conducted on the diversity of blue–green algae from various sources of their existence. Blue–green algae consist of 2000 species in 150 genera.

19.2.1 Classification

Blue–green algae (cyanobacteria) are divided into five orders (Rippka et al. 1979).

1. Chroococcales.
2. Oscillatoriales.
3. Pleurocapsales.
4. Nostocales.
5. Stigonematales.

This classification was based on the observation of morphological characteristics. These orders are further divided into families, subfamilies, genera and species. Blue–green algae are made up of the genera *Anabaena, Aphanizomenon, Calothrix, Cylindrospermopsis, Gloeotrichia, Microcystis, Nostoc, Nodularia, Oscillatoria planktothrix, Phormidium, Scytonema*, etc.

19.3 Role in Agriculture

19.3.1 Biofertilisers (Nitrogen Fixation)

The ongoing increase in human population and the depletion of energy resources pose a threat to the environment's requirements and to the sustainable production of food and energy. Anthropogenic activities can lead to soil barrenness, degradation and salinity, which can reduce the area of arable land and threaten food security. The demand for food like rice increases as the world's population increases, necessitating a greater use of nitrogen fertilisers to boost crop yields (Song et al. 2021). The most environmentally friendly method, or 'green technology', has been used to prepare biofertilisers. The prokaryotic organisms that have evolved and survived the longest are cyanobacteria (Chittora et al. 2020). Biofertilisers are organisms that improve soil nutrient availability for crops (Awasthi and Singh 2021). Cyanobacteria, since they have the ability to fix atmospheric nitrogen, might be utilised as a biofertiliser for the growth of commercially significant crops like rice and beans (Chittora et al. 2020). Cyanobacteria have unique properties of nitrogen fixation in paddy fields, resulting in enhanced rice growth and productivity. The biological nitrogen fixation process is aided by the use of cyanobacteria as biofertilisers, which also increases the organic matter level of the soil. They improve the grain weight, yield, fresh and dry weight, root and shoot length and uptake of micronutrients by the crop (Kalyanasundaram et al. 2020). The agricultural importance of cyanobacteria in rice cultivation is based on their ability to fix nitrogen and other beneficial effects on soil and plants (Zahra et al. 2020). In rice fields, green manure, particularly nitrogen-fixing cyanobacteria (NFC), has been shown to be an environmentally benign N_2 source. NFC inoculation has been proved in studies to greatly boost the nitrogen-fixing capacity in rice fields, hence improving the rice yield (Song et al. 2021). Cyanobacteria called diazotrophs are helpful in producing affordable, conveniently accessible biofertilisers that are friendly to the environment. They can reduce plant nitrogen shortage, aerate the soil, increase soil water retention and supply vitamin B12 (Awasthi and Singh 2021; Chittora et al. 2020). Rice crop cultivation area contains the most effective nitrogen-fixing cyanobacteria, including *Nostoc linckia, Anabaena variabilis, Aulosira fertilisima, Calothrix* sp., *Tolypothrix* sp. and *Scytonema* sp. These species act as biofertilisers, sequestering atmospheric nitrous oxide and allowing it to be converted to ammonium, helping to maintain soil fertility and sustainability over the long term (Zahra et al. 2020). In addition to being more affordable and less noxious to the environment than chemical fertilisers, biofertilisers are also a renewable energy source. Therefore, if we want to boost the productive capability of land on the earth, we must utilise biofertilisers (Tiwari et al. 2022). The use of consortia or biofilms of green algae and cyanobacteria as biofertilisers has recently been shown to be effective (Jemilakshmi 2021).

19.3.2 Soil Fertility Improvement

The soil microbiota are essential for preserving the soil's health, carbon sink and nutritional balance (Abinandan et al. 2019). Cyanobacteria have a wide range of contaminant-digesting abilities and serve a number of roles in the soil ecosystem to maintain soil fertility (Koul and Kumar 2022). Cyanobacteria are not dominant microorganisms in the soil, but they have a significant impact on soil fertility, microbial community and soil productivity (Li et al. 2019). It is undeniably true that cyanobacteria and microalgae play a significant part in preserving the fertility and health of the soil required for long-term agriculture. The balance in the dynamics of nutrients like carbon, nitrogen and phosphate for the yield of agricultural crops is the main benefit of utilising microalgae and cyanobacteria (Abinandan et al. 2019). So, by utilising microbes, researchers have used 'green technology' to create eco-friendly environments. The use of cyanobacteria to boost soil fertility and agricultural yield is covered in a number of green technology articles (Chittora et al. 2020). By storing significant volumes of water, cyanobacteria promote soil stability, decrease water and wind erosion, increase surface moisture and boost soil fertility by fixing carbon (C) and elemental nitrogen (N) (Chamizo et al. 2018). They are advantageous over rivals in numerous ecological niches because they can withstand a variety of stressors, including salt, pH, high or low temperatures and drought. They bind soil particles together, causing soil aggregation, an increase in organic matter and an improvement in the soil's top layer's ability to store water (Mutale-Joan et al. 2022). Through atmospheric nitrogen fixation, phosphate solubilisation and nutrient release, cyanobacteria increase soil fertility and crop yield (Gr et al. 2021). The improvement and reclamation of soil infertility can be facilitated by plant growth promoting rhizobacteria (PGPRs) and cyanobacteria that produce exopolysaccharides (EPS) (Awasthi and Singh 2021). The ability of *Nostoc* sp. to absorb nitrogen in the environment improves soil fertility and plant growth (Maltseva and Maltsev 2021).

19.3.3 Wasteland Reclamation

A sizeable amount of land on the earth is classified as wastelands. They are deteriorated and underutilised lands as a result of many factors. They include places impacted by waterlogging, riverine lands, shifting cultivation, degraded forest land, sandy area and salt-affected land. One of the most pressing issues today is the recovery of natural ecosystems in areas disturbed by human activities (Maltseva and Maltsev 2021). Global warming is contributing to an increase in the desertification of soil, and water shortage makes traditional restoration techniques ineffective (Roncero-Ramos et al. 2022). Due to the full loss of topsoil and the ecological instability of these wastelands, they are unsuited for farming. Salinity is a significant issue among the causes of wastelands and may be brought on by improper agricultural practises, waterlogging and mining (Devi et al. 2021). Soil deterioration and salinisation are caused by both natural and human-made processes. The security of our food supply is threatened by the loss of cropland. Around one billion acres of salt-affected soils can be restored using chemical, physical and biological methods. Exceptional abilities of *Nostoc, Anabaena* and other cyanobacterial species include the capacity to fix nitrogen from the atmosphere, build an extracellular matrix and synthesise suitable solutes (Li et al. 2019). Therefore, a substitute technique based on cyanobacteria that create biocrusts on deteriorated soils has arisen. Communities of mosses, lichens, cyanobacteria or fungi called biocrusts invade the soil's surface and create a permanent and fertile layer (Roncero-Ramos et al. 2022). Since native cyanobacterial strains have developed an innate resilience to salt and osmotic stress from repeated exposure to such stress over time, using them as biofertilisers for the restoration of salt-affected soil is anticipated to be more effective. Utilising native cyanobacterial strains that are osmo- and halotolerant and have high levels of stress tolerance and adaptations as biofertilisers will help add organic matter, promote the development of other microbial communities, maintain nutrient cycles, reduce soil erosion by improving soil aggregation and structural stability and facilitate increased crop yield (wheat and pearl millet) under water scarcity (Nisha et al. 2018). Species from the genus *Nostoc* are frequently listed as dominants among cyanobacteria. It is proof that the closest steppe phytocenoses and agrocenoses have a substantial impact on group formation in revegetation habitats, where cyanobacteria typically play a key role (Maltseva and Maltsev 2021). There are large expanses of unproductive usar lands in many Indian states, such as Uttar Pradesh (12.95 lakh hectares) and Rajasthan (12.14 lakh hectares). Salinity-affected or alkaline lands are considered to be usar soils. Thus, the need to reclaim such barren terrain has been recognised in order to meet the growing need for food from a growing world population. Usar soil can be reclaimed using a variety of techniques that lower its salt content, including irrigation with freshwater, gypsum treatment, the growing of crops that can withstand salt and the use of cyanobacteria (Rai et al. 2019). By promoting plant growth and development and boosting soil fertility in unfavourable environmental conditions, algae are crucial to the rehabilitation of soil. Use of algae in soil reclamation is not only economical, but it will also improve the soil fertility status and plant growth conditions under adverse environmental stresses (Kalyanasundaram et al. 2020).

19.3.4 Biocontrol

The majority of chemical pesticides are extremely hazardous to plants and should not be used. These chemical pesticides are dangerous for soil and plants to use because they cause crops to accumulate a variety of harmful toxins. By producing hydrolytic enzymes such as carboxymethyl cellulase (CMCase), chitosanase and 1,3-endoglucanase, cyanobacteria inhibit the growth of phytopathogens (Kalyanasundaram et al. 2020). The development of hydrolytic chemicals and biocidal mixtures, such as benzoic acid and majusculonic acid (Renuka et al. 2018), has resulted in microalgae, particularly cyanobacteria, being considered as potential biocontrol agents because of their antagonistic effects against a variety of plant pathogens, such as microscopic organisms, parasites and nematodes. Microalgae and cyanobacteria contain a wide range of bioactive chemicals; therefore, using them (or their extracts) can help crops be adequately protected from both biotic and abiotic influences (Gonçalves 2021). The existence of organisms like insects, nematodes, bacteria and fungi, among others, can have a detrimental effect on the productivity of crops (Pan et al. 2019). Polysaccharides are frequently used to combat these pathogenic organisms because they recognise the signalling molecules found in the pathogenic organism cell wall and trigger a number of defensive reactions (Chanda et al. 2019). Regulation of signalling pathways, gene expression and induction of particular biosynthetic pathways are examples of common defence reactions. These actions typically produce secondary metabolites with antioxidant, antimicrobial and fungicidal properties (such as phenolic compounds, terpenoids and other compounds) (Renuka et al. 2018; Farid et al. 2019). Microalgae and cyanobacteria, which are both used in agricultural crops and are a major source of polysaccharides, may help to strengthen these defence mechanisms (Chanda et al. 2019). Indeed, the induction of plant defence mechanisms in the presence of microalgae and cyanobacteria has been described previously. For example, inoculating spice crop seeds with the cyanobacteria *Anabaena laxa* and *Calothrix elenkinii* significantly increased the activity of the enzyme 1,3-endoglucanase in shoots and roots, which is responsible for the breakdown of pathogen cell wall components. The authors also reported an increase in fungicidal activity, plant dry weight and shoot and root length (Kumar et al. 2013). Increased activity of plant defence enzymes is linked to the activation of metabolic pathways that result in the production of thousands of metabolites, such as phenolic compounds, which are toxic to pathogenic organisms. For example, phenylalanine ammonia-lyase causes an increase in the phenylpropanoid pathway, which produces phytoalexins, phenolic substances with antimicrobial and antioxidant properties (Chanda et al. 2019). The seaweeds *Spatoglossum variabile, Stokeyia indica* and *Melanothamnus afaqhusainii* were found to have a significant suppressive effect against eggplant and watermelon pathogens (e.g. the root-rotting fungi *Fusarium solani, Fusarium oxysporum* and *Macrophomina phaseolina* and the root-knot nematode *Meloidogyne* spp.). Apart from the inhibitory effect on these organisms, the authors reported improved plant growth (increased fresh weight of shoots, vine length in watermelon and shoot lengths in eggplant) (Baloch et al. 2013). In addition, the cyanobacterium *Anabaena minutissima* provides bioactive substances such polysaccharides and phycobiliproteins (PBPs). Pre-harvest treatments with polysaccharides on strawberry fruits decreased sporulation and symptoms of *Botrytis cinerea* by 50% and 67%, respectively (Righini et al. 2022).

19.3.5 Crop Productivity Enhancement

The physiologically active chemicals produced by microalgae and cyanobacteria are a key source of molecules that can greatly increase agricultural productivity. It is possible to use microalgae and cyanobacteria as pure extracts or as a crude algal compost due to the large variety of substances they contain and the several roles they play in crop production (Gonçalves 2021). It has already been established that cyanobacteria have advantageous impacts in agricultural soils for the growth of various crops. For instance, applying a cyanobacterial inoculum in a rice paddle boosted the yields of grain and straw while also increasing the soil's ability to hold onto nitrogen (Jha and Prasad 2006). The strains of *Anabaena variabilis* encouraged an overall improvement in the rice crops by using them in a rice crop field (e.g. increase in plant height and leaf length, improvement of seeds, grain and straw productivity) (Singh and Datta 2007). Despite the fact that the majority of studies focus on the utilisation of cyanobacteria in rice fields, more recent research has also highlighted the benefits of cyanobacteria in other cultures. After introducing the cyanobacterial species *Nostoc entophytum* and *Oscillatoria angustissima* to the soil used to cultivate pea plants, there was a considerable improvement in the germination rate, photosynthetic pigments and growth characteristics of this plant. The authors also noted that both cyanobacteria had greater N_2-fixing activity in correlation with this increase (Osman et al. 2010). Using a biofilm made by the cyanobacterium *Anabaena torulosa* in a wheat crop considerably enhanced the amount of nitrogen that was available in the soil (Swarnalakshmi et al. 2013). Consequently, the addition of microalgae and cyanobacteria to soils can increase the availability of macro- and micronutrients for plant growth (Gonçalves 2021).

19.4 Role in Environment

19.4.1 Bioremediation

The health of the ecosystem has been severely harmed by the release of untreated sewage effluents into the environment, which has led to an alarming rise in pollution. A novel and efficient method for removing both organic and inorganic toxins is to use cyanobacteria for bioremediation. Cyanobacteria are effective at combating with various pollutants due to their capacity to fix atmospheric nitrogen and their special ability to adapt to adverse environmental circumstances (Cui et al. 2022). Because cyanobacteria exopolysaccharides are anionic, they have the ability to remediate heavy metals (Potnis et al. 2021). A study showed how *Spirulina* sp. was highly tolerant and helpful in biosorption of heavy metals like cobalt and chromium (Murali et al. 2014). Bioremediation of phenol, which is another harmful organic pollutant discharged by industries and is known to cause severe health issues, was carried out using *Leptolyngbya* sp. (Guha Thakurta et al. 2018). Bacteria and cyanobacteria are capable of cleaning up hydrocarbon-contaminated environment (Kumar et al. 2016). A study on heavy metal removal by *Cyanothece* and *Cyanospira capsulata* strains showed high metal adsorption (De Philippis et al. 2001). Some cases have demonstrated how a consortium of green algae such as *Ankistrodesmus* sp. and cyanobacteria such as *Nostoc* and *Anabaena* were used to remove heavy metals such as iron (Fe), copper (Cu), lead (Pb), chromium (Cr) and zinc (Zn) through biosorption and bioaccumulation (Iqbal et al. 2022).

Cyanobacteria also have the capability for soil bioremediation. They are known to increase the soil organic content. When cyanobacteria are added to the soil, they bind to the soil particles, resulting in soil impermeability and improved aeration. Cyanobacteria and microalgae, when compared to other species, are more environment-friendly, have large cell sizes and generate extra biomass, making them the greatest and most sustainable solution to the problems with soil fertility and accessible water resources. Remediation by microalgae and cyanobacteria may be a harmless and long-term solution for removing heavy metals found in municipal sewage water and for assisting in the breakdown of contaminants in industrial waste water (Koul et al. 2022).

19.4.2 Carbon Dioxide (CO_2) Sequestration

The rise in the earth's temperature, because of the increase of CO_2 gas in the atmosphere, is a major cause of global warming. The disruption in the amount of

Table 19.1 Blue–green algae species contributing to CO_2 sequestration and fixation

Microalgae/cyanobacteria	Application	References
Limnothrix redekei, *Planktolyngbya crassa*	CO_2 sequestration	Manjre and Deodhar (2013)
Leptolyngbya tenuis, *Chlorella ellipsoidea*	CO_2 fixation	Satpati and Pal (2021)
Thermosynechococcus sp. *Nannochloropsis* sp.	CO_2 fixation	Hsueh et al. (2009)
Desmodesmus sp.	CO_2 tolerance and assimilation	Xie et al. (2014)
Oscillatoria sp.	CO_2 capture	Anguselvi et al. (2019)

CO_2 gas majorly affects all the biogeochemical cycles. Higher amount of CO_2 emitted in the atmosphere due to the burning of fossil fuels must be removed. This can be achieved by carbon sequestration that is the method of holding and storing carbon dioxide present in air (Gayathri et al. 2021). This is carried out through biological, chemical and physical processes. Carbon sequestration helps in maintaining the ecological balance by placing carbon from its origin. The amount of fossil fuel emissions is related to carbon sequestration. If the amount of fossil fuels is limited, there will not be any need to go for carbon sequestration. An efficient way by which carbon sequestration is accomplished is through photosynthetic organisms. Such microorganisms that can carry out carbon sequestration are able to withstand a high temperature. Microalgae have the ability to convert CO_2 present in the atmosphere and the emitted CO_2 from flue gas, thus reducing greenhouse gases. The application of microalgae has elevated the CO_2 sequestration in a sustainable way (Cheah et al. 2015) (Table 19.1).

Literature studies have shown that microalga-based carbon sequestration is quite beneficial over other methods as it involves quick biomass production, a high rate of photosynthetic conversion for bioremediation and the ability to produce various value-added products. Other conventional methods of carbon sequestration are high power driven and are of high cost. As microalgae grow in aquatic habitats, they are able to capture CO_2 gas passing through them. Microalgae under light are able to take in solar energy and assimilate CO_2 based on the air and nutrients present in the aquatic habitat (Anguselvi et al. 2019). Microalgae utilise the carbon dioxide present in the atmosphere for their growth by converting it into glucose (Ho et al. 2014). Photosynthesis involves two reactions: light and dark reactions. Microalgae in the first stage of photosynthesis, which is a light-dependent reaction, captures the energy so obtained from the sunlight, thereby transforming it into adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) to energy carriers ATP and NADPH. Besides their role in ATP and NADPH formation, microalgae also capture CO_2-producing organic compounds in the light-independent stage. This ability of microalgae in capturing and storing CO_2 for bioconversion makes them an excellent choice for carbon sequestration (Zhao and Su 2014).

By using microalgae for carbon sequestration, greenhouse gases can be moderated. In addition to this, microalgae are also known to produce a variety of products like biofuels. There is an urgent need to reduce carbon emissions so that carbon sequestration does not have to be considered.

19.4.3 Biofuels

Algae are an alluring source for various bioproducts in agriculture, pharmaceuticals and other industries, particularly as biofuels. Blue–green algae (cyanobacteria) can convert solar energy and carbon dioxide into biofuels. They are also able to produce biofuels because of the high fatty acid and lipid content (Quintana et al. 2011). Among the members of cyanobacteria, the highest lipid content is present in *Oscillatoriales.* Cyanobacteria produce biofuels in the presence of alcohol as an acyl acceptor and a catalyst. According to Mata et al. (2010), the two different ways in which cyanobacteria produce biofuels are excess production and transesterification of fatty acids to produce fatty acid methyl esters.

The ability of microalgae in growing fast either on brackish or on saline water as compared to other plants makes them a good option to be considered as a renewable biofuel (Dismukes et al. 2008). Due to their numerous beneficial characteristics, cyanobacteria have a high potential for producing cyanodiesel. Some of the advantages are listed below: (Sarsekeyeva et al. 2015).

1. Cyanobacteria do not claim farmlands.
2. Cyanobacteria growth gathers biomass quickly.
3. They have the ability to fix atmospheric CO_2.
4. They have less requirement of elements like sunlight, water and a few inorganic trace elements.
5. They serve as a medium for genetic modification of metabolic pathways.

By utilising cyanobacteria for the production of biofuels, we can mitigate issues concerning the dependence, depletion and overuse of oil and gas reserves.

19.4.4 Methane (CH_4) Oxidation Augmentation

Methane (CH_4) is one of the chief constituents of natural gas, and it also accounts for the most potent greenhouse gas. It is the simplest among the members of the paraffin series of hydrocarbons. The major sources of methane are from geological deposits and coal bed methane extraction. Rivers, lakes and gas hydrates are some other sources of atmospheric methane gas. Another contributor of CH_4 is the methanogenesis in anaerobic flooded paddy soils. The amount of CH_4 increases also from the burning of fossil fuels and excessive waste generation. The application of cyanobacteria is a sustainable approach for limiting the amount of CH_4 generated (Cuellar-Bermudez et al. 2017; Singh et al. 2016).

Cyanobacteria are known to increase the amount of oxygen in the rhizosphere region of paddy. This increases the methanotrophs to take in the methane. These microbes carry out N_2 fixation, which aids in combating global warming. By releasing oxygen into the flooded soils that are unsuitable for methanogenesis, cyanobacteria generate an aerobic environment during this process. By boosting methanotroph activity and population, the oxygen supplied also promotes methane oxidation (Singh et al. 2011; Singh and Pandey 2013).

19.4.5 Food Supplements

The demand for food supplements made from microbes has increased due to the increase in global population. Many blue–green algae species like *Arthrospira platensis*, *Aphanizomenon flosaquae*, *Oscillatoria funiformis*, *Limnospira maxima* and *Nostoc sphaeroides* have been consumed by humans since a long time (Mutoti et al. 2022). Due to their great nutritional value, such blue–green algae species are available as food supplements in the form of pills, capsules and liquids. Blue–green algae are considered to be both food and feed supplements. Some species of blue–green algae like *Spirulina* are excellent food sources. *Spirulina* is a microscopic, filamentous cyanobacterium that grows in alkaline water bodies. It was described as the best food for the future by the United Nations World Food Conference in 1974. The food and feed supplement of *Spirulina* are made mainly from two species of cyanobacteria: *Arthrospira platensis* and *Arthrospira maxima*. Their dried biomass is produced on a large scale due to the abundant nutrient content (Shinde et al. 2022).

The biological components of blue–green algae include minerals, like sodium, potassium, calcium, iron, manganese, selenium, magnesium and zinc, and roughly 70–80% protein content, polysaccharides, polyunsaturated fatty acids, carotenoid colour and the presence of vitamins. The other constituents of spirulina include lipid, protein and chlorophyll, which have high antioxidant properties. Another important constituent found in *Spirulina* sp. is gamma-linoleic acid, which is known for its various health benefits like boosting hair growth, strengthening bone and maintaining the reproductive system (Raja et al. 2016).

Blue–green algae's anti-inflammatory and antioxidant properties have been found to stimulate neurological development, prevent nutrient deficiencies and protect against an overactive immune system. In addition to this, blue–green algae have also been known to maintain cholesterol level and are known to possess numerous dietary benefits boosting the immune system (Al-Thawadi 2018).

In a study by Appel et al. (2018), two commercial extract products of spirulina, Immulina® and immunLoges®, showed anti-inflammatory properties by inhibiting the immunoglobulin E (IgE)–antigen complex-induced production of TNFα, histamine, interleukin-4 (IL-4) and leukotrienes by also releasing histamine from mast cells.

19.5 Climate Change and Its Impacts on Freshwater Cyanobacteria Species

Globally, the effects of climate change have profoundly changed ecosystems, with freshwater lakes being especially sensitive because of their propensity to absorb atmospheric and terrestrial reactions. Northern latitudes have seen the fastest rates of change as several locations have warmed to previously unheard of levels. In reaction to increasing temperatures, northern latitude lakes are now experiencing 'longer summers' (warmer and longer ice-free situations) and hydrological intensification (wetter climates) (Erratt et al. 2022). Among the physical responses of lakes to climate change include changes in mixing regimes, loss of ice cover, water budget changes and increasing surface water temperatures. Fish deaths brought on by hypoxia in shallow lakes, the growth of cyanobacteria and modifications to the energy and carbon cycling are only a few of the effects of climate change (Cremona et al. 2022). A greater proportion of cyanobacteria was found in the mat-forming algae specifically as a result of warming and even more so as a result of warmth plus acidity. Only around 25% of the benthic cover was made up of cyanobacteria under control conditions; however, heat and acidity increased this proportion to over 75% while decreasing the amount of edible algae (Hofer 2018). The two most common climatic variables are temperature and precipitation, which have been investigated as potential drivers of cyanobacteria bloom production (Erratt et al. 2022). Toxic cyanobacteria (cyanobacterial harmful algal blooms [cyanoHABs]), which favour warmer temperatures and thrive under increasingly intense oscillations in the wet/dry cycle, have been particularly affected by global warming and have seen an increase in their species that are associated with harmful algal blooms (Zeppernick et al. 2022).

19.6 Future Prospects and Constraints

Cyanobacteria are a resource with potential for use in many different fields. They are an appealing renewable source of bioactive substances and other fine chemicals because their production has a generally minimal negative influence on the environment. However, due to the high degree of variety seen in cyanobacteria in natural ecosystems, this field of microorganisms is currently understudied. In particular, there are no genetic improvement studies on cyanobacteria with a view to eco-sustainable agriculture. Large-scale production and commercialisation of blue–green algae are required as they have high applicability and they can be used in the development of various products. Therefore, more attempts are required to study and screen blue–green algae species that show major potential traits of bioremediation as it has a high market potential in the coming few years.

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Factors Affecting Fish Migration

20

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Abstract

Multiple environmental and endogenous elements work together to cause fish migrations. Abiotic and biotic environmental conditions, such as seasonal variations in temperature and photoperiod, changes in the number of food sources, and the behavior of conspecifics, are all known to impact migration timing. The size, age, and sex of fish and energy reserves are endogenous variables affecting the time of migration. For instance, temperature and photoperiod influence juvenile salmonid movements, which are further altered by endogenous variables such as size and metabolic rate. In order to survive, thrive, and depend on the river flow regime, particularly the time and predictability of flows for spawning and raising their young, fish have evolved features and life history characteristics. It is unknown how much environmental factors affect migratory behavior, such as pulsed flows from hydropower plants. Research on some fish species' exceptional migrations is still ongoing. The adaptive benefit of adopting higher productive habitats for growth is probably how diadromy arose. This chapter explains all the possible aspects and the factors affecting fish migration.

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Ecological barrier · Factors · Fish · Migration

20.1 Introduction

Many organisms exhibit migration behaviors (Dingle 2014). Animals migrate for the same reason humans do: to maximize an individual's chances of growth and survival (Dingle and Drake 2007). Individuals must constantly adjust their metabolic demands for somatic growth, maturation, and reproduction with the energy available throughout their lives (Zera and Harshman 2001). Differences in food availability between marine and freshwater habitats are hypothesized to have led to the evolution of diadromy, migration between these two types of environments (Gross et al. 1988). All species in the Salmonidae family spawn in freshwater, but many are anadromous, meaning that individuals move to the sea at some point to take advantage of the more abundant food supplies (Jonsson and Jonsson 1993). It is common for salmonid population to include both permanent freshwater residents and seasonal marine migrants (Chapman et al. 2012). Salmonids' body mass positively correlates with their reproductive success (Elliott 1995). If the fitness gains from a larger body outweigh the costs of migrating, such as an increase in mortality, disease, and the inability to reach spawning grounds, then marine migration is beneficial (Klemetsen et al. 2003; Thorstad et al. 2016; Eldøy et al. 2021).

The unidirectional flow of river and narrow channels give migrating fishes definite reference points. In a dendritic river system, physical and chemical flow properties may vary spatially and temporally, which may confuse migrants but also allow branch detection. River migration requires upstream and downstream components. Early life history stages usually have the former and vice versa. However, life history stage migration may entail downstream and upstream components. Upstream migration must be active. Juveniles and adults migrate downstream nocturnally but diurnally in murky rivers. Some riverine and anadromous species spawn downstream, while growing adults usually spawn upstream. The timing of an upstream migration can vary from night to day (Northcote 1984; Jungwirth et al. 1998; Smith 2012; Tsukamoto et al. 2009; Morais and Daverat 2016).

20.2 Fish Migration

Sometimes, a single habitat cannot meet the needs of a population of fish (e.g., foraging and reproduction) (Wang et al. 2020). There are several reasons for this, including variations in habitat conditions (e.g., temperature) or changing needs of the population (e.g., foraging habitat vs. spawning habitat) (Zorn and Kramer 2022). An alternate habitat can benefit an individual's fitness in such a situation. This has led many fishes to develop a life history that involves coordinated movement across habitats (Cooke et al. 2022). The movement between discrete habitats of part or all of a population is what is known as "migration" (Dingle and Drake 2007). Migratory fish make up about 2.5% of all fish species. Some species of coastal- and stream-dwelling fish migrate hundreds of meters, while others migrate thousands of kilometers, such as eels (e.g., *Anguilla* species) and tunas (e.g., *Thunnus* species) (Kumari 2020; Cooke et al. 2011). Most species migrate on a seasonal basis, although some show coordinated daily movements (e.g., vertical migrations or tidal migrations) (Secor 2015). Many authors consider this to be migratory activity, while others view it as a specialized form of foraging.

20.3 Energetics of Migration

The long-distance migrations are energy-demanding, and feeding occurs rarely during long-distance migrations. Due to energy reallocation for foraging and digestion, feeding reduces the metabolic scope available for migratory activity (Naisbett-Jones et al. 2017; Eliason et al. 2011). Most species accumulate energy reserves prior to migration, so they rely heavily on those reserves for survival (Churova et al. 2021; Olsen et al. 2021; Paton et al. 2020). The most energy-dense form of energy storage is lipids, which have an energy density of 9.45 kcal per gram that is roughly twice as high as that of proteins and carbohydrates, which have 4.8 and 4.1 kcal per gram, respectively (Cooke et al. 2011). Fishes store their lipid reserves throughout a range of organs, including adipose-like tissue in the viscera, the muscle tissue, and the liver, in contrast to higher vertebrates, which mostly store lipids in adipose tissue (Azeez et al. 2014; Sheridan 1988). Except during spawning migrations, where the energy is used for gonadal growth, the majority of energy generated during migration is utilized by the muscles (da Silva Souza et al. 2020; Yin et al. 2020). Fishes use their energy reserves to varying degrees depending on the species and their associated population. Energy depletion can reach up to 85% of total energy stored during various anadromous spawning migrations (Birnie-Gauvin et al. 2021; Baktoft et al. 2020). The frequency of semelparity in fish population is correlated with the amount of energy depleted during migration; the more the energy depleted, the less likely they are to be a repeat spawner. In semelparous communities, mortality is most likely influenced by the depletion of energy supplies, although it is not the only reason (Tentelier et al. 2021; Kern and Gems 2022). It is clear that there are additional genetic and/or environmental factors at work because certain semelparous population travel just a moderate distance, which is significantly less than what would deplete their energy stores (Birnie-Gauvin et al. 2021).

20.4 Marine Migration Behavior

Among sea trout individuals, marine migration behavior varies widely. Prior to migration in the spring, the majority of sea trout had poor nutritional conditions (Jönsson et al. 2010). Low plasma triglyceride levels and body condition parameters in sea trout were associated with earlier sea entrance and increased likelihood of migration to sea. When compared to fish in better health, fishes with poor physical condition are more likely to stay in the sea longer and migrate farther offshore. Males were less likely than females to move to the sea. More often than the smaller fish, the larger fish scattered farther from the river and were also more likely to migrate to the sea rather than staying in freshwater and estuaries (Eldøy et al. 2021).

Fish nutritional status can be assessed using a blood plasma sample-derived nutritional correlation in addition to the body condition component. Low levels of plasma triglycerides, total protein, and calcium may be signs of inadequate nutrition, which has been linked to an increase in brown trout and Atlantic salmon migration in the ocean (Halttunen et al. 2018; Congleton and Wagner 2006). Cortisol levels that are elevated, perhaps as a result of scarce food sources, have been linked to earlier seaward migration (Birnie-Gauvin et al. 2019). Sex and body size have been observed to affect sea trout migration behavior. Consequently, genetically determined sex and physical size were also included. Body size, sex, and nutritional status all have an impact (Ferguson et al. 2019). Sea trout population from two different fjord systems in Northern Norway were studied for their maritime migration patterns (Eldøy et al. 2021; Berg and Berg 1989). Fish who were in poorer physical condition before migrating spend more time at sea and move further from the fjord than fish that were in better condition. When migrating, sea trout with low body condition parameters tended to spend more time at sea and migrate further from shore, presumably indicating a larger need for reconditioning compared to those with higher body condition scores (Davidsen et al. 2014; Eldøy et al. 2015; Bordeleau et al. 2018; Pemberton 1976; Jensen et al. 2019). Due to the close association between a female's body size and the number of eggs they may lay, females likely gain more from additional feeding chances than do males (Elliott 1995). A well-known phenomenon called sexual bias in migratory behavior has been documented in the range of salmonid species (Dodson et al. 2013).

20.5 Prediction of Fish Migration

The significant rise in global ocean temperatures has caused several issues, including ecological repercussions, the relocation of some traditional fisheries, increased competition among fisheries, and effects on biological reproduction. Investing in the consequences of ocean temperatures on fisheries is vital, which accounts for a significant amount of some nations' economies and affects people's ability to support themselves. Therefore, models for predicting fish migration to address the issue was explored and presented. The impacts of seawater temperature and salinity was studies and an ArcGIS-based model for predicting fish school migration was developed. Using this ArcGIS software, kriging interpolation, and nuclear density analysis, a thermodynamic map of fish school dispersion was generated from these data. In addition, linear interpolation and fitting are utilized to determine the link between the yearly fish movement distance and the year to anticipate fish migration paths over the following 50 years (Yipeng et al. 2020; Yalçin 2019).

The explosion of weather data has made weather forecasting more difficult (Belavadi et al. 2020). Artificial intelligence algorithms based on machine learning have steadily given meteorological research new ideas and ways to handle such massive data. Research now uses support vector machine (SVM), convolutional neural network (CNN), back-propagation (BP), etc. methods. These approaches have good temperature prediction results but cannot analyze the time correlation of time series data, such as forgetting premature data and reflecting periodic changes. However, ocean temperature data restrict prediction accuracy in a typical time series and influence migratory fish prediction. Researchers developed a recurrent neural network (RNN) (Ma et al. 2021) to include meteorological data time series information into the prediction model and improve forecast outcomes (Olivetti et al. 2021). The classic RNN model can process time series data, but its deep memory is short-term. Because numerous Jacobian matrix multiplication is required to calculate connections between distant nodes, it will struggle with long-term dependencies. The gradient disappears and explodes. RNN cannot handle long-term timing concerns due to these drawbacks. The distorted long short-term memory (LSTM) network may use previous data to help make current decisions and overcome the problem that existing approaches are hard to learn vast data and do not account for temperature time series data's time correlation (Jiao 2021; Zhang et al. 2021).

20.6 Ecological Barrier

Dams are obvious vertical barriers in fixed locations along river courses. Fish cannot swim upstream due to a dam, but they can swim downstream using the dam's structures, including the spillways or turbines (in hydroelectric dams). It is not uncommon for fish to congregate below the dam wall, particularly during spawning season (Loures and Pompeu 2012). Because of this problem, which can be seen at any dam, fishways have been proposed by scientists, managers, and regular people for a long time. Ladders, elevators, bypass channels, locks, and trap-truck systems are examples of technologies that help migratory fish get through dams and fulfil their life cycle needs by reuniting once-separate river sections (Agostinho et al. 2007; Clay 2017; Larinier 2002). However, most fish passes are insufficient (Brown et al. 2013; Noonan et al. 2012), and fishways may only assist many upstream passages depending on the area and the species. For a selected number of species, fishways in South America allow for the passage of just a tiny percentage of migratory individuals (Fernandez et al. 2004). From the time the first ladders were set up in Brazil until now, there has been much room for improvement in terms of both planning and engineering and hydraulics. Increased understanding of the swimming skills of local species and the development of more efficient fish passages have contributed to improved conservation efforts (Agostinho et al. 2007; Pelicice et al. 2015).

First and foremost, when a river is dammed, the hydrophysical and morphological structure of the water flow is altered, producing a distinct biotopic pattern that influences the life activities of fish, including their migratory behavior. Ecological barriers, such as the water reservoir and the dam, are established in a dammed river, fundamentally altering the conditions that determine the features of the downstream migration from the higher reaches to the lower reaches of a natural river. Both the physical complexity of a body of water and the intensity of water exchange play crucial roles in the establishment of these barriers and in regulating downstream migration. These influences operate not just at the scale of the entire water reservoir but also at small biotopes, where there is a downstream migration (Pavlov et al. 2019; Larinier, 2000).

20.7 Abiotic Factors

20.7.1 Climatic Factors

Migration is an important, expanding, and an extremely complex phenomenon (Black et al. 2011). Changes in temperature were primarily linked to variations in migration and reproduction timing, age at maturity, age at juvenile migration, growth, survival, and fecundity (Crozier and Hutchings 2014). Migration is one option among the many that might be used to address the effects of climate change, and it has drawn more and more interest from policymakers and researchers. The underlying hypothesis of most of these reports is that climate-related migration essentially reflects a failure to slow down climate change and/or a failure to adapt. Together, they have generated some concerning projections for the overall magnitude of migration that such failures might cause (Douglas et al. 2008; Warner et al. 2009; Renaud et al. 2007; Conisbee and Simms 2003). However, migration can also be considered to be a legitimate coping strategy for the increased stresses and shocks that may be brought on by climate change and a number of other causes (Tacoli 2009). The claim that climate change is already affecting marine ecosystems is supported by scientific research. The structure of marine communities, changes in the intensity and timing of coastal upwelling, and increases in ocean temperature all have an impact on the primary and secondary productivity (Leonard et al. 2015; Sissener and Bjørndal 2005).

20.7.2 Anthropogenic Factors

A significant anthropogenic contributor to the reduction in biodiversity, particularly in aquatic ecosystems, is habitat fragmentation. The effects of habitat fragmentation in rivers are influenced by the interactions of biota migration characteristics, temporal hydrological change that mediates connection, and the location of anthropogenic obstacles (Rolls et al. 2014). Anthropogenic factors are typically believed to have significant impacts on migration rates. The anadromous fish Hilsa exhibits the same migratory patterns and breeding habits as the Atlantic salmon (*Salmo* sp.) (Shohidullah Miah 2015).

20.7.3 Global Warming

Ocean temperatures have been gradually rising in the recent years as a result of global warming. Fish population (such as those of herring and mackerel) will migrate in order to survive and reproduce (Xu et al. 2021). The research by Nicolas et al. found that the distribution of several typical estuarine species has shifted northward, which is consistent with the idea that these species will migrate north in response to climate change (Nicolas et al. 2011). Global warming may make fish in streams in the southern Great Plains and southwest North America particularly susceptible to extirpation or extinction. The streams in this area already have some of the warmest free-flowing water on the planet, and fish continue to survive occasionally extremely close to their lethal thermal limits. In the case of global warming, fish in these prairie stream systems cannot migrate northward to colder temperatures, unlike many terrestrial and marine animals or fish of some rivers (Matthews and Zimmerman 1990).

20.7.4 Effects of the Photoperiod

Numerous physiological and biochemical systems in fish experience daily variations (circadian rhythms), and the endocrine system regulates these processes (Björnsson 1997). Different photoperiod modes cause the parr–smolt transformation and smoltification, which are linked to increased endocrine and Na^+ and K^+-ATPase activity, an increase in lipid and nitrogen metabolism, modifications in the bioconversion of physiologically important polyunsaturated fatty acids (PUFAs) like eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid from essential fatty acids and linoleic acid (Nemova et al. 2020). The physiological state of the organism, including age, gut fullness, fitness, reproductive status, maturity, and water temperature, determines how the fish react to different light types (Sautin 1989). Light conditions (not less than 16,000 and not more than 80,500 lx) limit the salmon parr's ability to move around and hunt (Nesterov 1985).

20.8 Bioaccumulation of Organic Compounds

Due to habitat loss and shrinking biodiversity, persistent organic pollutants (POPs) and metals can have a negative impact on an entire aquatic ecosystem. They can also be toxic to aquatic organisms, either chronically or acutely (Teunen et al. 2021a). Pollutants that result from a variety of anthropogenic activities (such as industry, agriculture, and combustion by-products) can enter the environment through discharge, leaching, erosion, and atmospheric deposition (Schweitzer and Noblet 2018). The Stockholm Convention has significantly reduced the use and production of numerous pollutants over the past few decades, but previous contamination is still pervasive in the aquatic environment (Maes et al. 2008). As hydrophobic organic compounds (HOCs) do not dissolve easily in water, they are not easily measured in the water phase (Belpaire and Goemans 2007; Jürgens et al. 2013). Pollutant bioavailability can be influenced by sediment properties such as clay concentration and total organic carbon (TOC) and water qualities such as oxygen content, pH, conductivity, and dissolved organic carbon (DOC). The bioavailability of lipophilic substances may be decreased by greater organic complexation caused by high DOC or TOC levels (Dittman and Driscoll 2009; Li et al. 2015; Moeckel et al. 2014). Consequently, it is advised to measure polycyclic aromatic hydrocarbons (PAHs) in bivalves or crustaceans rather. Active biomonitoring has frequently been used to monitor bioaccumulative pollutants using translocated individuals (Babut et al. 2020; Catteau et al. 2021). This standardized sampling method is based on exposing a specific species to various sampling locations that reflect the level of local pollution under controlled low background concentrations, sizes, or circumstances. For this, the bivalve genus Dreissena has been employed frequently (Bashnin et al. 2019; Potet et al. 2018; Teunen et al. 2021b).

20.9 Environmental Cues

The study of migratory biology frequently centers on how environmental factors affect migration. The fact that environmental variables are frequently highly associated with one another makes it challenging to determine which variables are the most relevant for affecting a migration, which presents the biggest obstacle in investigating how environmental factors influence migration (Gahagan et al. 2010; Davidsen 2010). The local features of the habitat where the migration is taking place may affect the relative impact of each environmental element. Nevertheless, despite these difficulties, it has consistently been demonstrated that a variety of environmental conditions affect fish migration (Ahn et al. 2020). Discharge-related occurrences can significantly affect migratory behavior in fluvial habitats. Changing discharge often correlates with changes in other environmental factors (Sykes et al. 2009; Bhat and Qayoom 2021; Schwevers and Adam 2020). A significant environmental factor that stimulates migratory movement is changing light levels. Changes in photoperiod on a seasonal scale offer calendar information that is used to start and synchronize migratory behavior among individuals within a group. The photoperiod appears to be particularly important for coordinating migratory activity during long-distance migrations, as the spawning migrations of Pacific salmon and Pacific lampreys (*Lampetra tridentata*) (Cooke et al. 2011). Fish migratory activity can be synchronized and triggered by temperature. Migration can be seen as a type of behavioral thermoregulation when temperature serves as a trigger (Haesemeyer 2020; Archer et al. 2020). The temperature may fluctuate in thermally diverse situations beyond what a specific population can tolerate. The fish are forced to look for a new thermal environment as a result. The population's thermal needs could alter. The ideal temperature, for instance, may not be the same for development and reproduction (Haesemeyer 2020; Wheeler et al. 2020; Volkoff and Rønnestad 2020).

20.10 Conclusion

The term "migration" refers to the periodic changes in habitat that are an integral component of the life cycle of many species and are used for survival, development, and reproduction. An expansion or relocation of a migration loop may cause diadromy or speciation in diadromous fishes, respectively; hence, migration loop models might be helpful for conceptualizing the process of speciation in these organisms. According to their phylogenetic relationships, salmonids originated in freshwater. They became increasingly dependent on the sea through anadromous migrations, while anguillid eels originated in the ocean and appeared to be facultatively catadromous in temperate regions, with some individuals not entering freshwater for growth (Tsukamoto et al. 2009). Changes in productivity between marine and freshwater ecosystems at high and low latitudes appear to cause these variations. Using the upstream migration of amphidromous Ayu as a behavioral model to try to figure out what makes fish move, a behavioral approach suggests that both internal and external factors, like an increase in water temperature, fish density, or hunger level, can raise the drive level and release behavior associated with upstream migration.

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