Maulin P. Shah *Editor*

Modern Approaches in Waste Bioremediation

Environmental Microbiology

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Preface

Much technological advancement has already been made to check the limits but still there is a long way to go. Integrated Bioremediation Technologies in Industrial waste treatment is the most effective innovative technology that uses live naturally occurring microorganisms to degrade environmental pollutants or to prevent contamination. Microorganisms like bacteria, algae, fungi, and yeast carry the ability to utilize dye as their sole source of carbon and nitrogen thus playing a significant role in bioremediation. This is a multidisciplinary approach, but its central focus depends on microbiology. This technology includes several techniques such as biostimulation, bio-generation, bioaccumulation, biosorption, physical correction, and rhyming-emission.

The aim of this book is to describe the limitations and challenges associated with some generally accepted bioremediation strategies and evaluate the possible applications of these corrective strategies to eliminate toxic pollutants from the environment through integrated technologies in Industrial wastewater treatment. Remediation of polluted sites by the means of microbial process (bioremediation) has been established effective and dependable due to its environmentally friendly characteristics. The use of microorganisms, plants, or microbial or plant enzymes to remove contaminants in the soil and other environments is known as bioremediation. Biodegradation refers to the part of, and sometimes total, transformation or detoxification of contaminants.

Mineralization is a more qualified term for the absolute conversion of an organic contaminant to its inorganic constituents by a single species or a consortium of microorganisms. Co-metabolism is one more restrictive term for referring to the transformation of a contaminant devoid of the provision of carbon or energy for the degrading microorganisms. The process of bioremediation increases the rate of the natural microbial degradation of contaminants by providing the native microorganisms (bacteria or fungi) with nutrients, carbon sources, or electron donors (bio stimulation and bio restoration) or by adding up an enriched culture of microorganisms. This helps the microbes to develop specific characteristics that allow them to degrade the desired contaminant at a faster rate (bio augmentation). It helps to bring the level within limit as set by regulatory agencies or, ideally, to entirely mineralize organic pollutants to carbon dioxide. This process depends on invigorating the growth of certain microbes that use contaminants like oil, solvents, and pesticides as a source of food and energy. These microbes devour the contaminants, converting them into small amounts of water and harmless gases like carbon dioxide. Successful bioremediation involves combination of the right temperature, nutrients, and food. If not provided, it may take much longer for the clean-up of contaminants. If conditions are not encouraging for bioremediation, they can be improved by supplementation of "amendments" to the environment, like molasses, vegetable oil or simply air. These amendments generate the best conditions for microbes to flourish and complete the bioremediation process. The new approaches that the book highlights are Bio augmentation, Biofilters, Biosparging, Bio stimulation, Bioventing, Bioreactors, Composting and effective Land farming.

The book will picture new aspects of bioremediation that are often tailored to the requirements of the polluted site in question and therefore the specific microbes needed to interrupt down the pollutant are encouraged by selecting the limiting factor needed to promote their growth.

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Emerging Pollutants from the Industries and Their Treatment

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Abstract Environmental pollution due to various anthropogenic activities associated with industrialization and urbanization are issues of global concern in recent times. There are numerous Emerging Contaminants (EC) of concern, with the major one being: personal care products, pesticides, pharmaceuticals, surfactants and other industrial chemicals. Various methods have been employed for their removal with some of the methods having their inherent limitations. Bioremediation has been identified a reliable solution for the problems associated with emerging pollutants. There are numerous microorganisms which are vital for the remediation of the polluted environment. Bioremediation is an active process involved during the immobilization, eradication and degradation of various groups of toxic substances found within the environment. Due to the limitations of bioremediation in that it is restricted to only substances that can be acted upon by microorganisms; other advanced approaches such as membrane technologies and advanced oxidation process have been developed. This chapter discusses the various emerging pollutants from industries and their treatment. It highlights some of the limitations of some of the technologies.

Keywords Anthropogenic activities · Emerging contaminants · Bioremediation · Advanced oxidation · Membrane technology

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1 Introduction

Environmental contamination has increased rapidly in recent times as a result of increasing anthropogenic activities and industrialization. Amongst the various pollutants of concern include nuclear wastes, pesticide residues, insecticides, herbicides, green house gases and heavy metals. Discharge of contaminants into the environment occurs primarily from industrial activities. Various approaches have been adopted in time past for the removal of these toxic contaminants from the environment. Some of the processes involve complete excavation of the site which is an expensive approach. Much later, other processes such as venting of soil and vapor extraction emerged and also showed their inherent limitations such as cost and limitations with regards to high concentration of pollutants hence an incomplete solution to the identified problems. More recently, the occurrence of numerous emerging contaminants has been reported (Briffa et al. 2020; Manisalidis et al. 2020).

Emerging contaminants (EC) refer to naturally occurring or synthetic compounds or even any microbe that are not usually monitored in the environment but have the tendency of resulting to suspected or known adverse impact on the health of humans or the environment at large. Such substances include surfactants, pharmaceuticals, and personal care products which are continually rising in surface water, ground water, drinking water, food materials and municipal wastewater. Some other substances belonging to this class are analgesics, hormones, antibiotics, antidiabetis, antiepileptic and anti-inflammatory drugs (Masindi and Muedi 2017). ECs also has broad meanings and has some other terms which have subtle refinement in their definition, like pharmaceuticals, contaminants of emerging concern, as well as organic wastewater compounds. The compounds tend to be of greater concern recently because most of them have not yet been fully investigated and some of them cannot be tested for in municipal waste water. The environment is increasingly being polluted by numerous emerging contaminants which are released from industrial, urban, agricultural and other anthropogenic activities (Ofrydopoulou et al. 2022). This chapter discusses the various emerging pollutants from industries and their treatment. It highlights some of the limitations of some of the technologies.

2 Technologies for Treatment of Emerging Contaminants

Various approaches have been adopted in the treatment of various waste and contaminants released from industrial activities. Some of these methods range from physical, chemical, biological and more recently the used of various advanced techniques such as nanotechnology, advanced techniques of oxidations and membrane technologies. Bioremediation technologies have also been found highly promising in the remediation of various emerging contaminants (Patel et al. 2019) (Fig. 1).

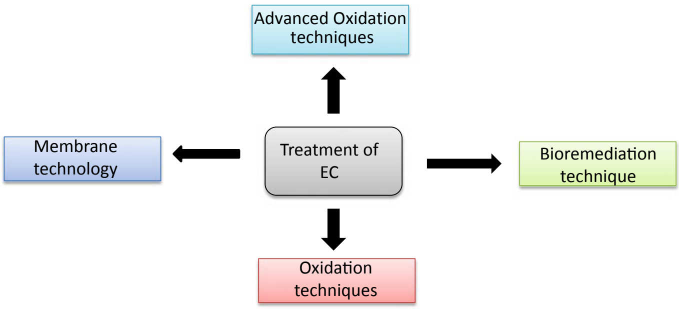

Fig. 1 Technologies for treatment of EC

3 Oxidation Techniques

Oxidation involves the transfer of electron from a donor to chemical specie. This process of electron transfer brings about a change in the oxidation number. Processes of oxidation that involve the formation of reactive intermediates usually have other oxidation steps between the oxidizing and reducing agents until the final product which is stable thermodynamically is formed. The oxidation potential is an indication of the extent to which the oxidizing agent can initiate the process of oxidation and this is a characteristic feature of each oxidizing agent (Krystynik 2021). Some of the most common oxidizing agents that have been used in the treatment of industrial contaminants found in waste water include hydroxyl radicals, fluorine, chlorine and ozone. There are various parameters that affect the processes of chemical oxidation which include the content of the contaminants in the given environmental matrix, the rate of reaction between the contaminants and the oxidizing agent employed. The oxygen concentration, pH and temperature of the media. Specific optimum conditions have been put in place for specific oxidation reactions for organic and inorganic pollutants (Ghime and Ghosh 2019; Cuerda-Correa et al. 2020).

4 Advanced Oxidation Processes

These are oxidation processes that involve the formation of various categories of radicals and other reactive intermediates such as hydroxyl radicals. Some of the processes of advanced oxidation employed for the treatment of environmental pollutants involve the application of hydrogen peroxides, ozone, and ultraviolent radiation. Most AOPs processes are dependent on the formation of the hydroxyl and other reactive intermediates in situ. These intermediates are the most active oxidants that can be adopted in the treatment of various organic contaminants most especially in aqueous media. The hydroxyl radicals that are formed go through non selective reactions which involve the degradation of the pollutants and their subsequent transformation into simple inorganic materials that are environmentally friendly (Cuerda-Correa et al. 2019). It has been documented that the processes of advanced oxidation are capable to reduce the contents of environmental pollutants to extremely low concentration that would not constitute deleterious effects on the environment. Studies have also reported the potential of AOPs in the treatment of various biodegradable pollutants such as pesticides, petroleum products, aromatic compounds and volatile organic compounds in various environmental matrices. Due to the cost of operation, there is limited commercialization of AOPs in the treatment of contaminants such as those present in waste water. The use of AOPs has however gain outstanding popularity due to the remarkable oxidation potential hence highly promising in the treatment of organic compounds, inorganic compounds and highly recalcitrant (Krishnan 2017).

Principle of AOP

Various techniques are involved in the use of AOPs in the treatment of pollutants. These include ozonation, Fenton, photo catalytic process and photo Fenton reactions. For the efficient removal of various contaminants, the continual generation of the reactive intermediates throughout the process is necessary; this is usually achieved through photochemical reactions. The formation of these radicals is achieved through the integration of various oxidizing agents such as ozone, ferrous salts, UV and hydrogen peroxides. Speeding up of the processes can also be achieved by the use of various radiation sources such as electron beams, visible rays, solar, microwaves and ultrasound (Buthiyappan et al. 2016).

5 Bio-remediation

This is a technique that involves the utilization of microorganism, fungi and plants in the enhancement of the conversion of contaminants and harmful substances into harmless substances. This is an approach that is safe, cheap and environmentally friend. The use of this technique in contaminant remediation attracted public attention around the 1960s after numerous experimentations had been done using various samples. Several achievements were documented in the 1970s in the use of bioremediation which was championed by Robinson George. The microorganisms that are used rely on environmental contaminants and it brought about the reduction in the contents of the contaminants. As the microorganism consumes the contaminants, the population of microorganism available for the bioremediation reduces continually. This is so due to the reduction in the quantity of food (contaminants) those are available for supporting the original number of the bioremediating microbes (Moenne-Loccoz et al. 2015).

This technique of waste management includes the utilization of various living organisms for the eradication or neutralization of the contaminants from the polluted site. It is a technique of treatment which utilizes organisms naturally for the breakdown of the harmful contaminants into simple inorganic materials. The process for the removal of contaminants is dependent primarily on the nature of the pollutants which include heavy metals, chlorinated compounds, agrochemicals, pesticides, xenobiotic compounds, nuclear wastes, dyes, green house gases, and hydrogen gas. Bioremediation is actively involved in breakdown, eradication, detoxification, immobilization diverse waste substances and physical toxic substances from the environment through the action of microorganisms (Das et al. 2017).

Microorganisms involved in bioremediation

The microorganisms that are employed in bioremediation of contaminants are classified into two main groups.

i. Indigenous microorganism:

These are microorganisms which are already found around the site of the contaminants. However for the purpose of stimulating the growth of this group of microorganisms, there is need for sufficient oxygen contents, efficient soil temperature, and adequate nutrients that are essential for the growth of the microorganisms.

ii. Exogenous microorganisms:

The microorganisms that fall into this group are those that were introduced into the soil to be remediated externally. The introduction is due to the absence of the necessary biological activities required for the degradation of the contaminants present in the soil (Talabi and Kayode 2019). Aside the supply of the oxygen and nutrients required for the survival of the exogenous microorganisms, the conditions of the site may require some adjustments for ensuring that the microorganism survives in the new area. It is however worth noting that the types of wastes and conditions of sites are comparable. There is need to specifically test each of the particular site that is to be remediated and thoroughly investigated for the optimization of the outcome of bioremediation (Azubuike et al. 2016).

There are various factors that determine the peculiar bioremediation technique which include: the kind of microorganisms present, the conditions of the site and the amount and toxic effect of the contaminants. Different types of microorganisms act on specific types of contaminants and are capable of surviving under varying conditions (Fig. 2).

Types of bio-remediation

i. Microbial bioremediation

This type of bioremediation relies on the activity of microbes, for the transformation of toxic contaminants into non harmful forms. This is achieved through the interactions between the contaminants and the microorganisms bringing about the compartmentalization, immobilization and concentration of the contaminants.

ii. Phytoremediation

This is a cheap cleanup technology that is driven by solar energy. This involves the utilization of plants for in situ degradation, elimination or the containment of

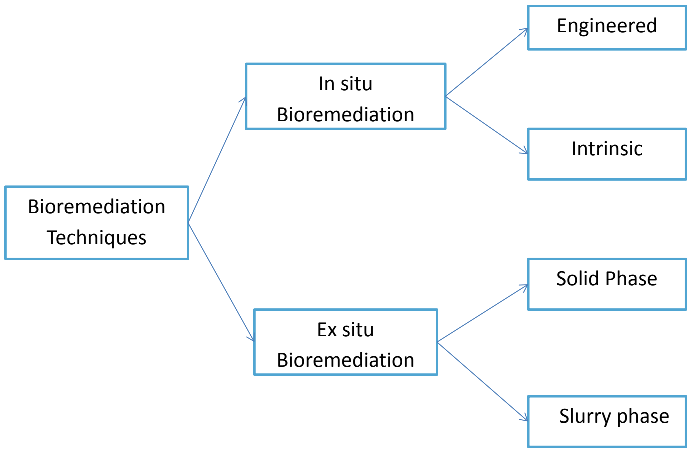

Fig. 2 Techniques in bioremediation of EC

the contaminant in soil, sludge, sediments and ground water. There are popular plants that are specially employed for such purpose. However, if the contents of the contaminants are high, the plants that is employed for the purpose of the remediation might die. For efficient bioremediation, there is need for a larger surface area of plant due to the need for a large surface area.

iii. Mycoremediation

This involves the use of fungi for the purpose of bioremediation. The group most commonly employed is the mushrooms hence the approach is dependent on the efficiency of the enzymes that are produced by the mushroom during the degradation of different substrates. Aside the enzymatic form of bioremediation in biosorption, fungi could also be used. Biosorption is an approach during which contaminants are taken in by the mushrooms into their mycelium thereby making the mushrooms not edible. The following are fungi that have been found useful in this regard: Aspergillus, Pleurotus, and Trichoderma used for the removal of lead, nickel, cadmium, arsenic, iron, mercury in marine environment (Abatenh et al. 2017).

Techniques of bioremediation

i. In-situ technique

This approach does not need the excavation of the polluted soils; hence it is a cheaper technique in bioremediation as opposed to ex-situ approach. The polluted soil is treated within through the use of specifically selected bioremediation. Even though this approach is cheaper and generates very few quantity of dust in comparison to the ex situ approach, it may be difficult and slower to manage. It is the most efficient on site with soils that are permeable (Sharma 2019).

In aerobic in situ technique, oxygen and nutrient are supplied to the organism that is involved during the bioremediation. The specific techniques that are commonly employed in achievement of adequate supply of oxygen and nutrient are Injection of hydrogen peroxide and bioventing.

ii. Ex-situ technique

This involves the removal of polluted soil or the pumping of the underground contaminated water before the treatment process. This technique is easier, cheaper and has been successfully adopted for various categories of contaminants (Talabi and Kayode 2019). The ex-situ technique consists:

Slurry phase bioremediation: It is very important when quick remediation is highly needed. The solid phase bioremediation is comparably easier to operate and needs more space whereas the cleanup process require more time when compared to the slurry phase technology.

Application of bioremediation technologies

i. Treatment of oil contaminants: Oil spillage is one of the most common pollution around countries that have a high reserve of oil. Such pollution brings about the death of aquatic life. It involves the introduction of bacteria that consume the oil thereby inducing the depletion of the oil spilled in the water bodies. The process is usually aided using dispersants (Cordes et al. 2016).
ii. Treatment of estuaries, streams and rivers: This technique is used in the removal of pollutants such pesticides, fertilizers etc. from water bodies.
iii. Treatment of sewage: Sewage is a mixture of chemicals, and wastes which can be treated in the process of recycling. This process proffers the most cheapest and efficient technique.
iv. Compost bioremediation: The removal of pollutants in contaminated soils sites could also be achieved through the mixing of the composts. The pollutants are removed by the microbes that are present within the compost. This is a very efficient method of bioremediation.
v. Bioaugmentation: Bioremediation is paramount for the development of highly effective decomposers. The development of such decomposers aids the fast removal of environmental contaminants. This is readily achieved through the application of genetic engineering manipulations on the natural decomposers for the production of super-decomposers.

Factors affecting bioremediation

Enzymatic metabolic routes of microorganisms aid the progress of chemical reactions that aid in the degradation of the contaminants. The microorganisms act on the contaminants only when they come in contact with the compounds which aid the generation of nutrients and energy for the nutrient multiplication. The efficiency of the bioremediation is dependent on several factors such as concentration of contaminants, chemical nature of the contaminants, and their ease of accessibility to the original microorganisms. The major factors include the population of the microorganism, nature of the environment, soil pH, temperature, nutrients and oxygen (Talabi and Kayode 2019).

i. Biotic factors

These are useful in the breakdown of various organic contaminants by microorganisms having less sufficient sources of carbon, antagonistic association among the protozoa or microorganisms and bacteriophages. The rate of degradation of this is mostly dependent on the contents of the pollutants and the quantity of catalyst in the biochemical process. The primary biological factors include activity of the enzymes, mutation, and biomass production, horizontal transfer of genes, compositions and size of the population (Manisalidis et al. 2020).

ii. Abiotic factors

The successful interaction existing between the pollutants and microbes is dependent on moisture, pH, soil structure, nutrients, oxygen content, water solubility, redox potential, solubility, toxicity, types and concentration.

The association of the environmental pollutants with metabolic activities, physicochemical properties of the microbes targeted in the process are all vital during the remediation process. The successful association between the microbes and contaminants is affected by temperature, moisture, deficiency, oxygen content, physicochemical, concentration, chemical structure, solubility, toxicity and degradation kinetics (Prasad and Yadav 2022).

Biodegradation of pollutants can take place under a pH range of 6.5–8.5 which is basically optimal for biodegradation in most terrestrial environment. Moisture affects the breakdown of pollutants because it is dependent on the nature and quantity of the soluble components that are accessible together with the osmotic pressure and pH (Azubuike et al. 2016).

Types of in situ bioremediation

There are two major types of in situ bioremediation technique. These are the engineered and intrinsic bioremediation.

i. Intrinsic bioremediation

This is also known as the natural reduction process which is an in situ bioremediation technique; it involves a passive remediation of contaminated area with any external influence or intervention by humans. The process involves the stimulation of the local or natural population of microbes. The process is based on anaerobic and aerobic processes in the biodegradation of the contaminating constituents that contain the portions that are recalcitrant. The non availability of the external forces therefore implies that the approach is not expensive when compared to the other in situ techniques (Abetenh et al. 2017).

ii. Engineered in situ bioremediation

In this approach, certain microorganisms are introduced into the site of pollution. The genetically engineered microbes that are employed aid in the acceleration of the remediation process through the enhancement of the physicochemical factors to induce the growth of microbes.

iii. Bioventing

In this technique there is controlled stimulation of the flow of air through delivering of oxygen to the unsaturated area so as to increase the activity of the localized microorganisms for the bioremediation. The amendments used in this process are produced through the addition of moisture primarily to enhance the bioremediation process. Among the various in situ techniques this technique has gain remarkable attention (Prasad and Yadav 2022).

iv. Bioslurping

In this technique soil vapor extraction, vacuum aided pumping and bioventing are integrated in achieving ground water and soil remediation through indirect provision of oxygen and inducing of the pollutant breakdown. This approach is designed for products recovery. It is suitable for the remediation of soils that are polluted by semi volatile and volatile organic compounds. Though this approach is not reliable for less permeable soil remediation, it is however efficient in cost advantage as a result of lower amount of ground water, treatment, minimization of storage and cost of disposal (Prasad and Yadav 2022).

v. Biosparging

It is a technique that is close in principle to bioventing. It involves the injection of air into the contaminated soil sub layer for the improvement of the microbial degradation thereby inducing the removal of the contaminants within the area. The efficiency of biosparging is dependent on two primary factors which are contaminant biodegradability and permeability of the soil. This technique has been employed generally in the treatment of aquifers polluted by kerosene and diesel.

vi. Reactive barrier

It is commonly seen as a physical approach for the remediation of polluted ground water. The biological phenomena involved are sorption of contaminants after precipitation degradation. It is an in situ approach utilized for the remediation of chlorinated and heavy metal polluted sites.

Bioremediation has numerous merits. These include its functionalities with respect to cost when compared to the traditional techniques that involve the general cleanup of the harmful substance during the process. Bioremediation also makes it possible to achieve a high removal of the contaminants. This technique does not require much effort and can easily be done at the site without altering the usual activities of the microorganisms. This therefore saves the cost of conveying the waste off area and the potential threats to the environment and human health. This approach is also fast, requiring less time. It is a nonintrusive approach hence permits the incessant use of the site (Azubuike et al. 2016).

Bioremediation in spites of its numerous advantages also has certain disadvantages. It can only be employed for contaminants that can be biodegraded. Not all pollutants are amenable to microbial action. In some other cases, the contaminants are converted to intermediate compounds that could constitute more harm than the original compounds. Also, bioremediation is also considered to be time consuming in comparison to other methods such as total excavation of the polluted soil. Scaling process for bioremediation is also difficult from the various batches and small scales to larger scales application (Abatenh et al. 2017).

6 Conclusion and Future Trends

The concept of emerging contaminants has now become trendy and fashionable research area. The increasing number of these compounds has constituted a serious challenge for regulatory agencies. Various approaches have been employed for the treatment and remediation of the compounds. The approach of bioremediation has proven to be efficient in the restoration of areas that have been contaminated by various classes of pollutants. Microorganisms have been identified as the major driving force for this process. The achievement of remarkable bioremediation is affected by the degradation potential of the microbes, availability of nutrients and population of the microorganisms. There is need for more researches for the development and advancement of site specific bioremediation techniques, for complex groups of contaminants. Bioremediation is limited in that it is only applicable to compounds that can be acted upon by microorganisms. Other reliable technologies include oxidation processes, advanced oxidation processes and membrane technologies.

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Bioremediation: The Remedy to Expanding Pollution

Shreya Anand and Padmini Padmanabhan

Abstract The worldwide population is growing at an astounding rate, with evaluations suggesting the increase to approximately 9 billion by 2050. The exhaustive agro-system and the industrial systems required to upkeep this huge number of societies will unavoidably become the basis of pollution (air, water, soil) buildup. Hydro systems have slight improved fare, having an approximation of 70% industrial waste that are discarded into nearby water bodies. The global generation of garbage is 1.3 billion tons per year, the mainstream trash is deposited in the sites of landfill or discarded in the oceans. The microorganisms are commonly acknowledged for its capability to disrupt the enormous variety of organic compounds and engross the inorganic substances. Presently, microorganism are used in treatment of pollution treatment through a process known as 'bioremediation'. Bioremediation is the effective green process of removing stubborn contaminants from the environment through microorganism to decrease the level of pollution using the approach of biological degradation of pollutants into non-toxic substances.

Keywords Agricultural waste · Waste water · Contaminants · Microorganism · Bioremediation

1 Introduction

> Bioremediation is a waste management technique that includes the use of living organisms to eradicate or neutralize pollutants from a contaminated site.

The pollution dilemma has become a big issue all across the world. Every year, it has a negative impact on millions of people, resulting in numerous health problems and deaths. Although urban regions are typically more polluted than rural ones, pollution can spread to far-flung locations; pesticides and other chemicals have been discovered under the Antarctic ice sheet, for example. The Great Pacific Garbage Patch is a

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vast concentration of microscopic plastic particles discovered in the centre of the northern Pacific Ocean. Pollutants can be transported from one location to another by land, water, and the atmosphere, progressively aggravating the situation. Pollution is carried by air and water currents, ocean currents and migrating fish, and the wind can pick up and spread radioactive material mistakenly discharged from a nuclear reactor or smoke from a factory from one country to another (Doney et al. 2012). As a result, pollution is unconcerned about geographical boundaries. Oil spills, fertilizers, waste, sewage disposals, and poisonous chemicals are only a few of the major pollution-causing substances found around the world. They all contribute to global pollution by polluting the soil, air, water, and marine environments. Among 87,000 commercial compounds, the US Environmental Protection Agency has designated 53 substances as persistent, bio-accumulative, and hazardous (USEPA 2007).

Contamination is defined as the presence of high quantities of substances in the environment that are either detrimental to society or not. Pollution, on the other hand, is the deliberate introduction of harmful elements into the environment by humans, resulting in a hazardous effect. These pollutants can be produced by a variety of natural and anthropogenic activities, such as large-scale chemical synthesis, processing, and handling. Pollution levels are rapidly rising over the world, posing a huge threat to both industrialized and developing countries. Despite the fact that statistical data differs widely between countries and years, most contaminants have a long residence duration and can travel considerable distances. As a result, they change into very poisonous compounds, exacerbating the problem. Pollutants can easily travel vast distances since they don't respect geographical boundaries, as evidenced by the presence of xenobiotics in Arctic regions where no local sources of pollution-causing agents can be detected. As a result, pollution has become a worldwide issue that must be addressed regardless of political or geographical boundaries.

Microorganisms, in this perspective, play a critical role in the maintenance and sustainability of any ecosystem since they are more capable of quickly adapting to environmental changes and deterioration. Microorganisms are thought to be the first life forms to evolve; they are adaptable to a variety of harsh environmental circumstances. Microorganisms are everywhere, and they have a huge impact on the environment. They are important regulators of biogeochemical cycles in a variety of habitats, including cold environments, acidic lakes, hydrothermal vents, deep ocean bottoms, and animal small intestines (Seigle-Murandi et al. 1996). Microorganisms govern global biogeochemical cycling by performing carbon fixation, nitrogen fixation, methane metabolism, and sulphur metabolism (Das et al. 2006). They produce a variety of metabolic enzymes that can be used to safely remove contaminants, which can be done either by destroying the chemical directly or by transforming the toxins into a less harmful intermediate (Dash and Das 2012).

Microbes are particularly helpful in remediating the contaminated environment; bioremediation and natural reduction are also seen as solutions for emerging contamination problems. The bioremediation process involves a variety of microbes, including aerobic and anaerobic bacteria as well as fungi. Through the all-inclusive and action of microorganisms, bioremediation is heavily involved in the degradation, eradication, immobilization, or detoxification of various chemical wastes and physical dangerous chemicals from the surrounding. The fundamental premise is to degrade contaminants and transform them to less harmful forms. The pace of deterioration is determined by two types of factors: biotic and abiotic environments. Bioremediation is now carried out using a variety of technologies and tactics.

Increased human activities such as population explosion, unsafe agricultural practices, unplanned urbanization, deforestation, rapid industrialization, and non-judicious use of energy reservoirs, among other anthropogenic activities, have resulted in increased environmental pollution in recent decades. Chemical fertilizers, heavy metals, nuclear wastes, pesticides, herbicides, insecticides, greenhouse gases, and hydrocarbons are among the pollutants that cause environmental and public health concerns due to their toxicity. Thousands of hazardous waste sites have been identified, with more expected to be discovered in the future decades. Illegal dumping by chemical businesses and industry releases contaminants into the environment. Many of the previous site cleanup approaches, such as digging up the contaminated soil and shipping it away to be landfilled or burnt, were prohibitively expensive and did not provide a long-term solution. Recent solutions like vapor extraction and soil venting are less expensive, yet they're still insufficient.

2 Bioremediation

> Bioremediation is a 'treatment techniques' that uses naturally occurring organisms to break down harmful materials into less toxic or non-toxic materials.

Bioremediation is a metabolic process that uses biological organisms to remove or neutralise an environmental pollution. Microscopic organisms such as fungi, algae, and bacteria are included in the "biological" organisms, as is the "remediation"—the treatment of the issue. Microorganisms thrive in a diverse range of environments across the biosphere. They thrive in a variety of environments, including soil, water, plants, animals, the deep sea, and the frozen ice. Microorganisms are the ideal candidates to function as our environmental stewards because of their sheer numbers and desire for a wide spectrum of pollutants.

Bioremediation technologies became widely used and are still increasing at an exponential rate today. Because of its environmentally benign characteristics, bioremediation of polluted places has proven to be effective and trustworthy. Recent advancements in bioremediation techniques have occurred in the last two decades, with the ultimate goal of successfully restoring damaged areas in an economical and environmentally beneficial manner. Different bioremediation approaches have been developed by researchers to recover polluted ecosystems. Bioremediation can involve either indigenous or non-indigenous microorganisms supplied to the contaminated site.

Most of the issues connected with pollution biodegradation and bioremediation can be solved by indigenous microorganisms found in disturbed areas (Khan et al. 2015). Bioremediation has a number of advantages over chemical and

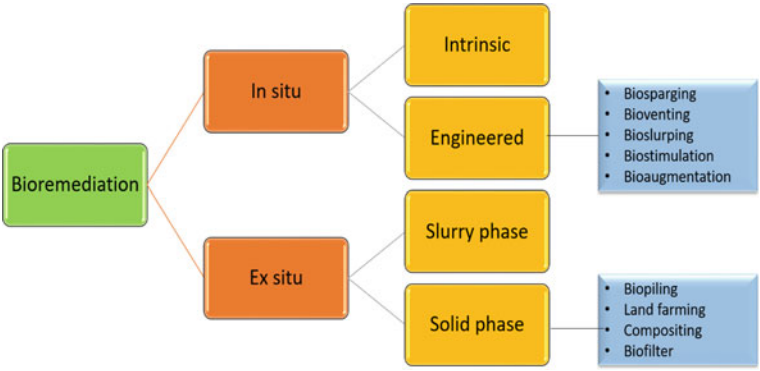

Fig. 1 Various approaches of bioremediation involved in control of pollution

physical remediation approaches, including being environmentally benign and cost-effective. Bioremediation works by reducing, detoxifying, degrading, mineralizing, or transforming more hazardous contaminants into less toxic ones. Pesticides, agrochemicals, chlorinated compounds, heavy metals, xenobiotic compounds, organic halogens, greenhouse gases, hydrocarbons, nuclear waste, dyes, plastics, and sludge are among the pollutants that can be removed. Toxic waste is removed from a polluted environment using cleaning techniques. Bioremediation is highly involved in degradation, eradication, immobilization, or detoxification diverse chemical wastes and physical hazardous materials from the surrounding through the all-inclusive and action of microorganisms (Fig. 1).

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3 Factors Involved in Cleaning Pollution Through Bioremediation

The bioremediation process involves bacteria, fungus, algae, and plants degrading, eliminating, altering, immobilizing, or detoxifying various chemicals and physical contaminants from the environment. Microorganisms' enzymatic metabolic pathways aid in the progression of biochemical events that aid in pollution breakdown.

Table 1 Factors affecting the bioremediation and the role of the associated factors

Factors	Role
Microbial factors	• Growth of microorganisms take place till the biomass has reached its critical production • Mutation and horizontal gene transfer • Enzyme induction • Enrichment of the capable microbial populations • Production of toxic metabolites
Environment factors	• Depletion of preferential substrates • Lack of nutrients • Inhibitory environmental conditions
Substrate factor	• Too low concentration of contaminants • Chemical structure of contaminants • Toxicity of contaminants • Solubility of contaminants • Biological aerobic versus anaerobic process • Oxidation/reduction potential • Availability of electron acceptors • Microbial population present in the site
Growth of microbes, and co-metabolism	• Type of contaminants • Concentration • Alternate carbon source present • Microbial interaction
Bioavailability of pollutants	• Equilibrium sorption • Irreversible sorption • Incorporation into humic matters
Mass transfer limitations	• Oxygen diffusion and solubility • Diffusion of nutrients • Solubility/miscibility in/with water

Only when microorganisms come into touch with substances that assist them generate energy and nourishment to multiply cells do they act on pollution. The chemical composition and quantity of contaminants, as well as the physicochemical properties of the environment and their accessibility to existing microorganisms, all influence the success of bioremediation (Fantroussi and Agathos 2005). The key contributors include the microbial population's ability to degrade pollutants, contaminants' accessibility to the microbial population, and environmental factors like type of soils, pH, temperature, oxygen and nutrients (Table 1).

3.1 Biological Factors

Biotic factors aid in the breakdown of organic compounds by microorganisms with limited carbon sources, antagonistic interactions between microorganisms, and protozoa-bacteriophage interactions. The pace of contaminant degradation is frequently influenced by the amount of catalyst present in the biochemical reaction as well as the concentration of the pollutant. Enzyme activity, interaction (competition, succession, and predation), mutation, horizontal gene transfer, biomass production growth, population size, and composition are among the major biological parameters (Naik and Duraphe 2012; Boopathy 2000).

3.2 Environmental Factors

Environmental pollutants interact with metabolic activity and the physicochemical properties of the microorganisms targeted throughout the procedure. The success of the microbe-pollutant interaction is determined by the environmental conditions. Temperature, pH, moisture, soil structure, water solubility, nutrients, site conditions, oxygen content and redox potential, resource deficiency and physico-chemical bioavailability of pollutants, concentration, chemical structure, type, solubility, and toxicity are all factors that influence microbial growth and activity. The dynamics of degradation are controlled by the components listed above (Adams et al. 2015).

In most aquatic and terrestrial environments, contaminant biodegradation can occur in a pH range of 6.5–8.5, which is generally ideal for biodegradation. Moisture affects the metabolism of contaminant because it depends on the kind and amount of soluble constituents that are accessible as well as the pH and osmotic pressure of terrestrial and aquatic systems (Cases and Lorenzo 2005).

4 Techniques Used in Bioremediation

There are various types of bioremediation or technologies or strategies used in bioremediation, but the following are the most common ways in which it is used are bio-pile, windrows, land-farming, bioreactor, bioventing, bio-slurping, bio-sparging, phytoremediation, and permeable reactive barrier.

Bio-pile: To increase bioremediation by microbial metabolic activities, above-ground stacking of dug toxic soil is followed by aeration and nutrient replenishment. Aeration, irrigation, fertilizers, leachate collection, and treatment bed systems are all part of this technique. This unique ex-situ technology is increasingly being evaluated due to its cost-effective properties, which allow for effective control of operative biodegradation variables such as pH, nutrient, temperature, and aeration. The bio-pile is utilized to address low-molecular-weight contaminants that are volatile, and it may also be used to repair polluted very cold harsh situations (Gomez and Sartaj 2014; Dias et al. 2015; Whelan et al. 2015). The versatility of the bio-pile provides for a faster remediation period since a heating system can be added into the bio-pile design to promote microbial activity and pollutant availability, speeding up biodegradation (Aislabie et al. 2006).

In order to promote better bioremediation, warm air can be fed into the bio-pile design to deliver air and heat simultaneously. Bulking agents such straw sawdust, bark or wood chips, and other organic materials have been added to a biopile construct to speed up the restoration process. Although bio-pile systems are linked to other ex-situ bioremediation techniques in the field, such as land farming, bioventing, and bio-sparging, robust engineering, maintenance and operation costs, and a lack of power at remote sites, which would allow for constant air circulation in contaminated piled soil via an air pump Furthermore, high air heating can cause soil drying during bioremediation, which inhibits microbial activity and promotes volatilization rather than biodegradation (Sanscartier et al. 2009).

Land farming: Due to its low cost and low equipment requirements, land farming is one of the most basic and effective bioremediation strategies. It's most common in ex-situ bioremediation, although it can also happen in in-situ bioremediation. This factor is taken into account because of the treatment location. In land farming, which can be done ex-situ or in-situ, pollutant depth is critical. Polluted soils are excavated and tilled on a regular basis in land farming, and the form of bioremediation depends on the treatment site. Ex-situ bioremediation occurs when toxic soil is removed and treated on-site, since it has more in common with other ex-situ bioremediation processes. In general, excavated polluted soils are carefully put above the ground surface on a fixed layer support to facilitate aerobic biodegradation of pollution by autochthonous microorganisms (Silva-Castro et al. 2012). Overall, land farming bioremediation is a straightforward design and implementation technology that requires little capital investment and may be utilized to treat huge volumes of dirty soil with minimal environmental impact and energy consumption (Maila and Cloete 2004).

Windrows: Windrows are a bioremediation technique that involves rotating piled polluted soil on a regular basis to increase bioremediation by enhancing microbial degradation activities of native and transient hydro-carbonoclastic in the polluted soil. Periodic turning of polluted soil improves aeration and equal distribution of nutrients, contaminants, and microbial degradation activities, increasing the pace of bioremediation, which can be accomplished by acclimatization, biotransformation, and mineralization. Windrow treatment exhibited a higher rate of hydrocarbon removal than bio-pile treatment, but the effectiveness of the windrow for hydrocarbon removal from the soil (Coulon et al. 2010). Periodic turning in conjunction with windrow treatment, on the other hand, is not the optimum strategy for bioremediation of soil contaminated with harmful volatile chemicals. The use of windrow treatment has been associated in greenhouse gas (CH_4) release due to formation of anaerobic zone inside piled polluted soil, which frequently reduced aeration (Hobson et al. 2005).

Bio-slurping: This technology combines vacuum-assisted pumping, soil vapor extraction, and bioventing to achieve soil and ground water remediation through indirect oxygen delivery and pollutant biodegradation stimulation (Gidarakos and

Aivalioti 2007). This method will be used to recover goods from capillary remediation, light non-aqueous phase liquids, unsaturated and saturated zones. This method is used to clean up polluted soils with volatile and semi-volatile organic substances. The approach employs a "slurp" that spreads into the free product layer and draws liquids up from there. The pumping machine uses upward movement to carry light non-aqueous phase liquids to the surface, where they are separated from air and water. In this method, soil moisture reduces air permeability and oxygen transfer rate, lowering microbial activity. Despite the fact that this technique is not ideal for low permeable soil restoration, it is a cost-effective operation process since it uses less ground water and thus reduces storage, treatment, and disposal expenses.

Bioreactor: A bioreactor is a vessel that converts raw materials into particular products through a sequence of biological reactions. Batch, fed-batch, sequencing batch, continuous, and multistage bioreactors all have different operational modes. Bioreactors provide ideal conditions for bioremediation growth. For the cleanup process, a bioreactor is loaded with polluted samples. When compared to ex-situ bioremediation approaches, bioreactor-based treatment of polluted soil offers various advantages. Bioremediation time is reduced by using a bioreactor-based bioremediation process with superior control of pH, temperature, agitation and aeration, substrate and inoculum concentrations. In a bioreactor, the ability to regulate and alter process parameters indicates that biological responses may be controlled and manipulated. Bioreactor designs are versatile, allowing for maximum biological degradation while reducing abiotic losses (Mohan et al. 2004).

Bio-sparging: This method is similar to bioventing in that air is injected into the subsurface of the soil to increase microbial activity, which stimulates pollution removal from polluted areas. Bioventing, on the other hand, involves injecting air into the saturated zone, which can aid in the upward migration of volatile organic molecules to the unsaturated zone, so accelerating the biodegradation process. Bio-sparging efficiency is determined by two primary factors: soil permeability and pollutant biodegradability. Bio-sparing is a closely related approach in bioventing and soil vapor extraction (SVE) known as in-situ air sparging (IAS), which relies on high air-flow rates for pollutant volatilization, whereas bio-sparging encourages biodegradation. It has mostly been used to treat aquifers that have been contaminated with fuel and kerosene.

Permeable reactive barrier: This technique is widely used to remediate contaminated groundwater using a physical manner. Precipitation degradation and sorption of pollutant removal, on the other hand, are biological mechanisms utilized in the PRB approach. To accommodate the biotechnology and bioremediation aspects of the technique, substitute terminology such as biological PRB, bio-enhanced PRB, and passive bio-reactive barrier have been suggested. PRB is an in-situ approach for removing heavy metals and chlorinated compounds from polluted groundwater (Silva-Castro et al. 2012; Obiri-Nyarko et al. 2014).

Bioventing: In order to improve the activity of indigenous bacteria for bioremediation, bioventing techniques require regulated stimulation of airflow by supplying oxygen to the unsaturated zone. To promote bioremediation, bioventing amendments are made by adding nutrients and moisture. As a result, contaminants will be microbially transformed into a harmless condition. Among other in-situ bioremediation approaches, this one has gained traction (Höhener and Ponsin 2014).

Phytoremediation: Phytoremediation is the process of cleaning up polluted soils. This strategy uses plant interactions such as physical, chemical, biological, microbiological, and biochemical interactions to reduce the harmful characteristics of pollutants in contaminated locations. In phytoremediation, numerous methods such as extraction, degradation, filtration, accumulation, stability, and volatilization are used, depending on the amount and nature of the pollutant. Extraction, transformation, and sequestration are standard methods for removing pollutants such heavy metals and radionuclides. Degradation, rhizo-remediation, stabilization, and volatilization are the main methods for removing organic pollutants like hydrocarbons and chlorinated chemicals, with mineralization being possible when plants like willow and alfalfa are utilized (Meagher 2000; Kuiper et al. 2004).

Plant root system, which may be fibrous or tap depending on the depth of the pollutant, above ground biomass, pollutant toxicity to plant, plant existence and adaptability to predominant environmental conditions, plant growth rate, site monitoring, and above all, time required to achieve the desired level of cleanliness are all important factors to consider when using plants as phytoremediators. In addition, the plant must be disease and insect resistant (Lee 2013). Pollutant removal in phytoremediation comprises uptake and transfer from roots to shoots. Furthermore, transpiration and partitioning affect translocation and accumulation (San Miguel et al. 2013). However, depending on other aspects such as the nature of the pollutant and the plant, the method could change. Phytoremediators are usually plants that flourish in contaminated areas. As a result, any phytoremediation method's success is largely dependent on increasing the remediation potentials of native plants growing in polluted areas, either by bio-augmentation using endogenous or exogenous plants. One of the most significant benefits of utilizing plants to clean up polluted sites is that some precious metals can bio-accumulate in specific plants and be retrieved after remediation, a process known as phyto-mining.

5 Approaches of Bioremediation

Superficially, bioremediation techniques can be carried out ex-situ and in-situ site of application (Fig. 1). The type of pollutant, the depth and volume of contamination, the type of ecosystem, the location, the cost, and environmental policies are all factors to consider when choosing a bioremediation technique. The efficacy of bioremediation processes is determined by oxygen and nutrient concentrations, temperature, pH, and other abiotic variables (Frutos et al. 2012; Smith et al. 2015).

Ex-situ bioremediation techniques

It entails excavating pollutants from polluted places and delivering them to a treatment facility. Ex-situ bioremediation procedures are frequently chosen depending on the depth of contamination, the type of pollutant, the degree of pollution, the cost of treatment, and the location of the contaminated site. Ex-situ bioremediation procedures are likewise governed by performance requirements.

- *Solid-phase treatment*

Solid-phase bioremediation is an ex-situ technique that involves excavating contaminated soil and stacking it. Organic waste, such as leaves, animal manures, and farm wastes, as well as home, industrial, and municipal wastes, are included. Bacterial growth is facilitated by pipelines positioned throughout the piles. Ventilation and microbial respiration require air to flow through the pipes. When compared to slurry-phase procedures, solid-phase systems demand a lot of area and cleanup takes a long time. Bio-piles, windrows, land farming, composting, and other solid-phase treatment methods are examples (Kulshreshtha et al. 2014).

- *Slurry-phase bioremediation*

When compared to alternative treatment methods, slurry-phase bioremediation is a faster process. In the bioreactor, contaminated soil is mixed with water, nutrients, and oxygen to produce the ideal environment for microorganisms to breakdown the pollutants in the soil. Separation of stones and rubbles from polluted soil is part of this process. The amount of water added is determined by the amount of pollutants present, the rate of biodegradation, and the soil's physicochemical parameters. The soil is removed and dried when this process is completed using vacuum filters, pressure filters, and centrifuges. The next step is to dispose of the soil and treat the resulting fluids in advance.

In-situ bioremediation techniques

These methods entail treating polluted substances at the source of the pollution. It does not necessitate any excavation and causes minimal or no soil disturbance. In comparison to ex-situ bioremediation approaches, these procedures should be quite cost effective. Some in-situ bioremediation procedures, such as bioventing, biosparging, and phytoremediation, may be improved, while others, such as intrinsic bioremediation and natural attenuation, may progress without improvement. Chlorinated solvents, heavy metals, dyes, and hydrocarbons have all been successfully treated using in-situ bioremediation approaches (Folch et al. 2013; Frascari et al. 2015; Roy et al. 2015). There are two types of in-situ bioremediation: intrinsic and engineered bioremediation.

- *Intrinsic bioremediation*

Natural reduction, also known as intrinsic bioremediation, is an in-situ bioremediation process that involves the passive remediation of polluted places without the use of any external force (human intervention). The encouragement of an indigenous or naturally occurring microbial population is the goal of this technique. The biodegradation of contaminating elements, including those that are refractory, is dependent on both microbial aerobic and anaerobic processes. Because there is no external force, the process is less expensive than other in-situ techniques.

- *Engineered in-situ bioremediation*

The second method entails introducing a specific bacterium to the contaminated area. In-situ bioremediation using genetically engineered microbes accelerates the degradation process by improving the physicochemical conditions to stimulate microorganism development.

6 Recent Researches on Bioremediation

6.1 Bioinformatics Approaches in Bioremediation

From a bioinformatics standpoint, the field of bioremediation offers many undiscovered and appealing options that require a large amount of data from various sources, such as protein sequence, biology and physiology, comparative genomics, chemical structure, reactivity of organic compounds, and environmental biology. It's an interdisciplinary field of study that straddles the line between computer science and biology (Kour et al. 2021).

Computers are used in bioinformatics to store, manipulate, recover, and allocate information related to DNA, RNA, and proteins. Bioremediation technologies based on genomics Bioremediation can be studied using omics-based methods such as genomics, transcriptomics, interactomics, proteomics, and metabolomics. This approach aids in the correlation of DNA sequences with mRNA, protein, and metabolite abundance, resulting in a more accurate and complete picture of in situ bioremediation.

Genomics: For the study of microbial strains involved in bioremediation, genomics is an merging subject. This method is based on a concept that analyses all genetic information in a microbe's cell. Bioremediation has been documented using a wide spectrum of microorganisms (Khardenavis et al. 2007; Qureshi et al. 2007). Here, genomic tools are used to explain biodegradation pathways using PCR, microassay analysis, DNA hybridization, isotope distribution analysis, molecular connectivity, and metabolic engineering and metabolic foot printing, as well as metabolic engineering and metabolic foot printing to improve the biodegradation process. For microbial communities scrambled, a number of genotypic fingerprinting techniques based on the PCR are available, including amplified fragment length polymorphisms (AFLP), automated ribosomal intergenic spacer analysis (ARISA), amplified ribosomal DNA restriction analysis (ARDRA), terminal-restriction fragment length polymorphism (T-RFLP), single strand conformation polymorphism (SSCP), randomly amplified polymorphic DNA analysis RAPD could be utilised for genetic fingerprinting, structural and functional interpretation of soil microbial communities, and assessing essentially allied bacterial species (Gupta et al. 2020).

Within microbial communities, LH-PCR could be utilised to detect natural length variations of various SSU rRNA genes. T-RFLP can be used to profile microorganisms from many taxonomic groups at the same time in an environment (Singh et al. 2006). Furthermore, a combination of molecular methods such as genetic fingerprinting, FISH, microradiography, stable isotope probing, and quantitative PCR can be utilised to investigate microbial interactions with natural variables in the soil microenvironment. In the study of soil microbial communities, quantitative PCR is used to detect the abundance and expression of taxonomic and operational gene markers (Bustin et al. 2005). Using amplified PCR products, genetic fingerprinting techniques perform direct investigation of certain molecular biomarker genes. Cluster aided analysis, which analyses fingerprints from numerous samples, could be used to investigate the link between varied microbial populations.

Proteomics and metabolomics: Proteomics is concerned with the total amount of proteins expressed in a cell at a certain location and time, whereas metabolomics is concerned with the quantification and characterization of total metabolites produced by an organism at a given time or under certain conditions (Rawat and Rangarajan 2019). Proteomics-based research has proved effective in detecting the number of proteins and changes in their composition, as well as identifying important proteins implicated in microorganisms' physiological responses to anthropogenic contaminants (Desai et al. 2010). In comparison to genomics, functional study of microbial communities is more informative and has larger promise.

Metabolomics research employs two main methodologies for evaluating biological systems. The first considers a broad, untargeted investigation in which no prior knowledge of the biological system's metabolic pathways is necessary. This technique aids in the detection and recovery of a wide range of metabolites present in the sample, yielding a massive amount of data that can then be compared across samples to determine metabolic pathway interconnectedness. The second technique is to conduct a tailored investigation based on prior information to identify specific metabolic pathways or metabolites (Hussain et al. 2009). For the detection and measurement of a wide range of cellular metabolites, the microbial metabolomics toolbox comprises several approaches such as foot printing and metabolic fingerprinting, target analysis, and metabolite profiling. The combination of proteome and metabolome data will help in identification of the active molecules essential for cell-free bioremediation.

Transcriptomics and metatranscriptomics: A transcriptome is a collection of genes that are transcribed under specific conditions and at specific times, and it serves as a vital link between the cellular phenotype, genome, interactome, and proteome. Gene expression regulation is an important process for adjusting to changes in the environment and consequently for survival. Transcriptomics analyses this process across the entire genome. DNA microassay analysis is a very powerful method in transcriptomics for determining the level of mRNA expression (Dias et al. 2015). Extraction and enrichment of total mRNA, cDNA synthesis, and either whole cDNA transcriptome sequencing or microarray hybridization of cDNA are all part of transcriptomics study. DNA microarray is a useful transcriptomics technique for analysing and studying the expression of practically every gene in an organism's mRNA (Pandey et al. 2019).

Transcriptomics and metatranscriptomics are useful for gaining functional insights into the activities of environmental microbial populations by examining transcriptional mRNA patterns (McGrath et al. 2008). Metatranscriptomics, in combination with metagenomics and genome binning, has been shown to offer information on microbial associations, syntrophism, and complementing metabolic pathways during the biodegradation process (Ishii et al. 2015). Metatranscriptomics (Giovanella et al. 2020) is a powerful tool for obtaining quantitative and qualitative gene expression data.

6.2 Nano-Technological Approaches in Bioremediation

Norio Taniguchi, a professor at Tokyo University of Science, coined the term nanotechnology in 1974 (Taniguchi 1974). Nanotechnology is concerned with items with dimensions on the order of a nanometer. They are quickly recognized for removing diverse hazardous chemicals due to their specific action against various recalcitrant pollutants. Nanotechnology has brought a fresh viewpoint to technology, particularly in the realm of water treatment. Under the headings of photocatalysis and nanofiltration (Prasad et al. 2018), it is now feasible to collect ecologically favorable approaches.

Effective microorganisms (EM) technology and nanotechnology: Effective microbes (EM) technology uses effective microbes to remediate waste water, which is then recycled for irrigation (Leahy and Colwell 1990). Water contaminants can be reduced using nanotechnology and EM technologies (Shrivastava et al. 2007). The number of locations contaminated with resistant organic pollutants, such as PAHs (polycyclic aromatic hydrocarbons) containing numerous benzene rings, is enormous, posing widespread environmental issues. PAHs are mutagenic and relatively non-biodegradable.

Engineered polymeric nanoparticles for bioremediation of hydrophobic contaminants: Organic contaminants, such as PAHs and petroleum hydrocarbons, are absorbed into soil, limiting their solubility and mobility. Phenanthrene solubility is increased by polymeric nano-network particles, which improves phenanthrene release from polluted aquifer material. Poly(ethylene)glycol modified urethane acrylate (PMUA) precursor chain is used to make polymeric nanoparticles. PMUA nanoparticles are designed to maintain their characteristics in the presence of a heterogeneous active bacterial population (Bhandari 2018).

Enhanced degradation for hazardous waste treatment using cell immobilization technique: Immobilized cells have proven to be successful in the bioremediation of a variety of harmful substances. Lee and Lee (2004) used free cells and cells immobilised in Calginate gel beads to study chlorophenol breakdown. They discovered that fixed cells decomposed chlorophenols considerably faster than free cells and minimised the lag phase for the extraction of chlorophenols (Lee and Lee 2004).

7 Conclusion

Environmental contamination is the biggest challenge of the twenty-first century, and research communities are paying close attention to it. Because bacteria adapt quickly to changing and hostile surroundings, bioremediation using microbes is a powerful strategy for clearing up pollution by increasing natural biodegradation processes. For the development of ecologically stable, new, and feasible bioremediation techniques, a full understanding of microbial communities and how they respond to the natural environment and in the presence of contaminants is critical. The extraordinary significance of extremophiles in bioremediation highlights the need for more research so that new species can be discovered and the processes they utilize to survive in such harsh settings can be investigated. Multi-omics studies are still insufficient, and additional research is needed to bridge gaps in our knowledge of the ecology, gene expression, and metabolism of bacteria participating in bioremediation. Several microbes have important metabolic genes that could be transferred to other organisms. Microbes that have been genetically modified to improve their ability to degrade contaminants will undoubtedly have a bright future in this sector. Expanding our understanding of microbial genetics in order to improve our ability to breakdown contaminants and conducting field studies will undoubtedly lead to progress in this subject. It would also be fascinating if bioremediation products were developed for large-scale application.

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Exploration of Plant Growth Promoting Rhizobacteria (PGPRs) for Heavy Metal Bioremediation and Environmental Sustainability: Recent Advances and Future Prospects

Sumita Mondal, Samir Kumar Mukherjee, and Sk Tofajjen Hossain

Abstract Environmental contamination of toxic heavy metals accumulated by fast industrialization, agricultural and other anthropogenic actions, induces a drastic harmful effect on living beings, modify the soli characteristic and its biological action. Among many other existing procedures, microbial mediated remediation of heavy metals is an eco-friendly and much potent method. Diverse soil microbiomes have been perceived as a dominant tool for the sustainable agriculture and the environment, which contribute an important function in biogeochemical cycles. The rhizomicrobiome have a wide range of activities for biotic and abiotic stress tolerance, which can alter the growth and developmental rate of plants. Plant growth promoting rhizobacteria (PGPR) is a prime cluster to exhibit synergistic and antagonistic communications with the soil and involve in an array of pursuit of ecological significance. The present review emphasises current scenario and future research requirements about their role in plant growth promotion and remediation of various environmental stresses exerted pollutants for agro-environmental sustainability.

Keywords Soil microbiomes · PGPRs · Biofertilizers · Heavy metal · Bioremediation · Sustainable agriculture · Environmental sustainability · Plant–microbe interactions

1 Introduction

With the advent of the industrial revolution, rapid growth of urban areas and anthropogenic liveliness, heavy metal induced environmental pollution become a serious hazard and challenge in present days. Environmental pollution and toxicity of these heavy metal components are of considerable ecological concern because of their non-biodegradable nature and detrimental effect on accumulation. Heavy metals

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exist naturally in earth environment, however, with the advancement of industrialization throughout the world, the concentration increase beyond of their pestilential level in nature. These elements move into the ecosystem through the food chain and accumulates in every trophic level, resulting emerging health risk because of their mutagenic, carcinogenic, teratogenic effect (Ali et al. 2013; Ahamed 2019).

Heavy metals are inorganic elements with relatively high atomic weight, atomic numbers, density, and toxic even at very low concentration. Heavy metals are categorized into three classes: (a) Toxic heavy metals (Mercury, Zinc, Lead, Cobalt, Arsenic, Tin, Cadmium, Bismuth, Selenium, etc.), (b) Precious heavy metals (Silver, Gold, Palladium, Platinum, Ruthenium, etc.), and (c) Radionuclides (Uranium, Thorium, Radium, Cerium, etc.), and also includes other transition metals, metalloids, lanthanides, and actinides. Some of them like cobalt, iron, copper, molybdenum, zinc, etc. are vitally important in living organism as they act as cofactors of diverse enzyme and participating in various metabolic pathways as trace elements.

There are numerous conventional physical and chemical remediation methods like ion-exchange, chemical precipitation, chelation therapy, reverse osmosis, land filling, bio-slurries, bio-piles employed for heavy metal remediation in soil and water bodies. These methods have comprised some negative sides like low efficiency, high-cost chemicals, extended procedure, high energy consumption, soil degradation and secondary pollution. Nowadays an inclination towards execution of biological approaches has been launched and become admired due to its relative efficiency, cost effectiveness and eco-friendly nature. This emerging biotechnology has been integrated with diverse conventional physical and chemical systems for comprehensive management of heavy metal mediated pollution that seems to be a sustainable approach. Nowadays, the term 'Bioremediation' is used to describe such practice.

Bioremediation is a collective process to annihilate toxic contaminants from soil or water bodies or any other medium, induced by biological means. Various biological living organisms likes bacteria, algae, fungi, lichens and as well as plants are employed in bioremediation method. Heavy metal contamination in soil extremely affects the biodiversity of plant community of a specific location. It affects plants by alter their metabolic processes such as respiration, photosynthesis, cell division, reproduction, fruit ripening capacity, etc. Moreover, the presence of such harmful metals in the cell causes an impediment of antioxidant enzyme activity and thereby generating enormous amounts of reactive oxygen species (ROS) and drastic oxidative stress in plants. There are some plants that possess the capability to accumulate heavy metals in high percentages (Brooks 1977). The uses of such hyper-accumulators plant in the highly contaminated region is now being regular execution procedure throughout the world. A broad range of microorganisms like, *Flavobacterium*, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Corynebacterium*, *Rhodococcus*, *Methosinus*, *Mycobacterium*, *Stereum hirsutum*, *Nocardia*, Methanogens, *Pleurotus ostreatus*, *Rhizopus arrhizus*, *Azotobacter*, *Alcaligenes*, *Phormidium valderium*, *Ganoderma applanatum*, *Aspergilus niger,* etc. are used in the bioremediation technique (Verma and Kuila 2019; Shah 2020). The potency of microorganism mediated bioremediation of soil depends on various components like soil profile, the structure of microflora in the soil, the concentration of heavy metals and types of heavy metals etc.

Bioremediation carried out in two ways- In-situ process and Ex-situ process. In in-situ bioremediation methods, the removal of toxic metals takes place at the site of their origin. Whereas in ex-situ bioremediation methods the contaminated medium is excavated from the site of origin to other places and then remediation operation takes place (Paul et al. 2021). In in-situ removal process naturally inhabited microbes are used to grow in medium containing heavy metals and convert them into less toxic substance. However, occasionally either new microbial strains are being instigate within the natural microbe community or provide optimal physical and chemical environment externally for enrichment of microbial growth to improvise and accelerate the mechanism of toxic metals removal. In many instances microflora is supplemented with oxygen and nutrient materials externally to prop up the growth and development of microbes in the medium.

Bacteria play significant roles in bioremediation as they possess various resistance mechanisms against heavy metal toxicity. The morphological feature of bacteria like presence of cell wall, capsule and slime layers obstruct the entry of heavy metals within cell interior. Another mechanism of active transport of metal ions assisted by ATPase or non-ATPase types of proteins help to efflux metal ions from the cytoplasm. Diverse extracellular polymeric substances (EPS) produced by bacteria play crucial role in the adsorption of heavy metal ions like cobalt, mercury, copper, cadmium (Saraswat et al. 2020).

Plant growth promoting rhizobacteria (PGPR) are a family of plant root colonizer bacteria that enhance plant growth through diverse mechanism. These are mostly soil bacteria that capable to undergo symbiotic relationship with plants or sometime exist as free-living bacteria. PGPR can change the ROS mediated oxidative stress in plants through producing various antioxidant molecules in plants (Bumunang and Babalol 2014; Shah Maulin 2021a, b). A variety of PGPR like *Rhizobium* sp. RP5, *Pseudomonas* sp. CPSB21, etc. increase the production of antioxidant enzyme like superoxide dismutase, catalase, glutathione reductase in plants under stress condition (Gupta et al. 2018). Some PGPR can transform heavy metals into its less toxic forms by various oxidation–reduction process and accumulate them within the cell interior (Mallick et al. 2014). Additionally, through phytovolatilization process some PGPR convert less toxic heavy metal forms within the plant body (Matsui et al. 2016).

The present review article focusses on the current scenario and future research requirements about the toxic effect of heavy metals on environment and their bioremediation with a special emphasises on the role PGPR in plant growth promotion and remediation of various environmental stresses exerted pollutants for agro-environmental sustainability.

2 Heavy Metals Toxicity and Environmental Significance

Uses of heavy metal in various aspects is escalating day by day to meet the demands of increasing population. Subsequently, heavy metal mediated pollution becomes a great threat for environmentalists because these cannot be transformed into non-toxic or biodegradable substances and as a consequence deposited within the environment as hazardous waste. Most of the heavy metals present in the environment naturally and derived from pedogenetic procedures, such as erosion, volcanic activity and weathering of rocks. Though, various human anthropogenic activities such as mining, electroplating, smelting and use of pesticides, different chemical fertilizer, biosolids, intensely rising the deposition of diverse toxic heavy metals in the environment and interrupt the nature sustainability. Soil, water and air are the major parts of the environment are contaminated potently due to heavy metal deposition and as a consequence, homeostasis within biota of ecosystem is hampered significantly. Heavy metals such as, Cd, Hg, As, Cr, Ni, Cu, Pb, and Zn are the most toxic in the context of environmental pollution. Hazardous impacts of heavy metals in living organisms depend on the concentration and duration of exposure (Fig. 1).

Effects on soil: Heavy metal mediated soil pollution become a serious concern in the present days as it has direct consequences on biotic elements of the ecosystem. Unplanned anthropogenic activities cause rapid changes in soil parameters like organic matter, clay contents, pH etc. Heavy metals are emerging from mine tailings, various chemical industrial wastes, improper uses of gasoline and paints, inappropriate application of fertilizers and pesticides, sewage sludge, wastewater irrigation, etc. and get deposited in soil and alter biological and biochemical properties of soil. Sometimes such modifications in the soil have a great impact on the maturation and development of plants as well as soil microbes. Metals may exist either as individual elements or in compound form with various soil constituents. Heavy metals bioaccumulate in the living organisms and their amount increase as they transfer from creatures of bottom trophic level to upper trophic level, an episode known as biological magnification.

The primary effect of heavy metal upon deposit in the soil is to bring an enormous change in microbial community both qualitatively and quantitatively. A heavy metal can easily alter the bio-chemical properties of soil, which in turn modify the population of microorganism and their activities. Long term exposure to heavy metals can alter the naturally inhabited microbial community with heavy metal resistant strains and thereby altering the soil microbial properties like soil respiration rate and enzymatic activity. It is found that CO_2 can be released in soil exert low contamination whereas due to high metallic concentrations, soil respiration reduces significantly

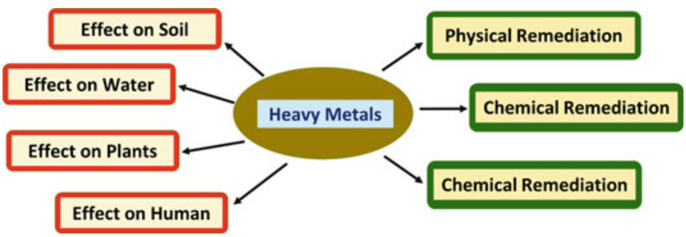

Fig. 1 Effect of heavy metals and its possible remediation strategies

by lowering microbial activity. With the increase of heavy metal concentration there is a sharp decline in enzymatic activity on soil. Heavy metals interfere enzymatic activity by the altering structure or destroying the group present on the enzyme active site. By reducing the metabolism, growth, reproduction and biomass of microbes, heavy metal contamination, also diminish the production and secretion of various microbial enzymes and metabolites. There is a very intimate connotation between soil microorganisms and soil enzymes, and most of the living microbes and microbes secreted enzymes take part in the movement of various elements of soil ecosystems.

Presence of various heavy metals like Cd, Cr, Zn, Pb, Mn has a direct detrimental effect on the function of a number of enzymes, such as protease, arylsulfatase, urease, alkaline phosphatase etc. Arsenic reduces activity of phosphatase and sulfatase whereas Pb has a direct effect on catalase, urease, invertase and acid phosphatase remarkable. Soil microbes play a significant role in recycling and storage of minerals in nature. Composition of microbial community in the soil had a large impact on the rate of decomposition and transformation of nutrients. Prolonged duration of heavy metal exposure changes the decomposition nature of microbes as well as the replaces microbial flora, which in turn effect on decomposition rate and recycling of materials in nature. Long exposure of Cr (VI) causes a huge change in microbial flora in the soil by effecting on metabolic activity of microbes. Zn and Cd also exert detrimental effects on microbial metabolism, therefore can reduce bacterial population size, diversity and activity (Huang et al. 2009; Hossain et al. 2012; Hossain and Mukherjee 2012).

Effects on Plants: Plants cannot get away from the undesirable environmental changes like other organisms due to its sessile nature. Heavy metal exposure increases an array of physiochemical and metabolic alteration in the plant body. Huge releases of toxic metals in land due to industrial waste deposition, agricultural malpractices, rapid urbanization adverse effects on plant proliferation and physical fitness in terms of reducing biomass, the rate of photosynthesis, yielding capacity, increasing chlorosis, altering water balance capacity and nutrient assimilation. Some metals such as, Fe, Co, Cu, Mn, Mo, Zn, and Ni are needed in low amount for plant growth, but at the concentration rise beyond the optimal level, can lead to toxicity and harmful effects. Absorption and accumulation of heavy metals in plant depend on temperature, water content, organic materials, soil pH and nutrient quantity. It has been reported that heavy metals like Zn, Cd, Mn and Cr accumulates in higher amounts in *Beta vulgaris* (Spinach) throughout summer time, whereas higher accumulation of Cu, Pb and Ni observed at winter time (Sharma et al. 2007).

Toxic effect of heavy metals in plant exhibited by the following mechanisms. Due to structural similarities, heavy metals compete with other essential nutrient molecules on root surfaces for being absorbed, such as, As competes the P. Different heavy metals interact with the functional groups of proteins and disrupt their structures and function. Generally, most of the heavy metals, generating reactive oxygen species and impaired the function various biomolecules. Sometime heavy metals can replace the essential ions from the specific binding site due to structural similarity (Sharma and Dietz 2006).

Root is the parts that first exposed the excess heavy metal toxicity and as a consequence the root cell division and growth are hampered, resulting altered root length and biomass. It has been reported that cadmium possesses toxic effect on the expression of regulatory protein cyclin-dependent kinase (CDK), which play an essential role in G1-to-S phase transition (Pena et al. 2012). Excess copper has an effect of auxin redistribution in meristematic zone mediated by PIN1 protein and inhibit primary root growth found in *Arabidopsis thaliana* (Yuan et al. 2013). Root deformity was found in plants like the bean (*Phaseolus vulgaris*) due to high aggregation of Cu in root (Cook et al. 1997). High accumulation of Mn, Cu, Cd, Zn, and Ni markedly affect the amount of chlorophyll and carotenoid, which reduces the photochemical activity of PSII as well. Zn has an adverse effect on Ribulose-1,5-bisphosphate carboxylase-oxygenase (RUBISCO) activity by replacing the Mg^{2+} ion in the active site of the enzyme thus regulating the rate of photosynthesis. Therefore, heavy metals have substantial impacts on pigment content, photosynthesis rate, the quantum yield of PSII system, gas exchange through stomata, CO_2 assimilation, nitrogen metabolism, etc. (Maleva et al. 2012).

Greater protease functioning in the cell has been observed due to heavy metal accumulation and thus reduced essential metabolic activity resulted from lessening the half-life of various essential enzymes, such as nitrate reductase, nitrite reductase, glutamine synthetase, Glutamine oxoglutarate aminotransferase, glutamate dehydrogenase etc. Cellular nitrogen metabolism is greatly obstructed due to Cd accumulation, which affect directly in nitrate uptake and transportation resulting inhibition in nitrogen assimilation procedure. Excess Hg^{2+} ions hinder mitochondrial activities and generate ROS, which distort bio membrane structure and cellular functioning. Due to mercury contamination in plant resulted reduce plant height, inflorescence formation, low yielding capacity on rice seedlings and tomato. Chromium can damage the chloroplast structure of therefore inhabiting the electron transport system and also has an adverse effect on the enzyme of Calvin cycle (Asati et al. 2016).

Cobalt accumulation in plant resulted in reduction of shoot and root length, low antioxidant enzyme activity and reduce the amount of sugar, amino acid and protein contents in various plants like tomato, radish, mung bean etc. Manganese accumulates in the root and shoot and thereby reduce plant growth was observed due to reduced chlorophyll a and b, carotenoid content and subsequently decrease the PSII activity. Heavy metals are able to alter hormonal activities and thereby, responsible for various physiological changes in plants. On the other hand, arsenic can alter the hormonal level like, IAA, IBA, NAA etc. as well as alter expression of about 69 microRNAs in plant body, which directly or indirectly affects on the growth and development of plants (Singh et al. 2015).

Effects on Human

Some heavy metals like iron, zinc, manganese, etc. are useful for various biological activities in plant and human body, but a high concentration accumulation of these elements causes enormous harmful consequences. Generally heavy metals are uptake by plants and then distributed among trophic levels via the food chain. After entering in biological system in excessive amount, heavy metals exert severe damages on structural integrity of cellular components like Protein, DNA and organelles like cell membrane structure, mitochondria, lysosome, endoplasmic reticulum, nucleus, etc. Heavy metals can bind with the protein active site by replacing the active metals and changes their activities, causing cellular malfunction. Heavy metals produce a number of reactive oxygen species, thereby creating oxidative stress, which leads to cell apoptosis. Prolong heavy metals exposure can lead to slowly developing of degenerative conditions in muscular, and neurological systems and ultimately that initiate the diseases like, multiple sclerosis, Alzheimer's disease, Parkinson's disease and muscular dystrophy. Regular longer duration vulnerability of some heavy metals and their combinations may even cause carcinogenic situation.

Effects on Waterbody

Heavy metals from various sources are released continuously in waterbodies creating a major problem. Manmade activity like industrial sewage effluent, mining, farming, electronic waste, etc. are major causes for heavy metal discharge. Contaminant metals are getting dissolved partially of fully in water and accumulated in the sediments of waterbodies and thus creating a devastating consequence on the ecological equilibrium of the aquatic habitat. As these metals are not completely dissolved and therefore, they react with particulate matter and precipitate into water sediments. Once macrophytes and other aquatic phytoplanktons uptake the heavy metals, these toxic contaminants enter into the food chain and affect every trophic level of an ecosystem. Organisms at the top level, including human obtain such toxicants by consuming aquatic animals specially fish. Consequently, a sharp escalate in the concentration of heavy metal in the trophic levels of the food chain is observed and the process termed as biomagnification.

Heavy metals react with diverse water component such as sulphate, carbonate, nitrate, etc. and precipitated in the bottom layer as insoluble salt complex. With the change in the pH level of water bodies due to acid rains or various other reasons, the metals can exist as their toxic form in water and do contamination. Such pollutants have great impact in altering the growth, development and reproductive ability of fishes and other water micrograms. Heavy metals have effect in the development and functioning of specific enzyme that can alter the metabolic activity in fishes and manifest cellular toxicity and ultimately leading to death. Different type of toxic metal form deposited in the sediments can vastly affect the benthic organisms and consequently lessen the food availability for bigger animals such as fish. Heavy metals develop various ROS molecules that causes oxidative damages on the organism. A toxic chemicals compound like methylmercury, produced from the organic mercury by bacterial methylation, have great hazardous effect on fish growth. These toxicants can cause gill necrosis and liver fatty acid degradation in fishes and crustaceans, as well as inhibit the activity of enzymes involved in cellular respiration, protein production (Mishra and Mohanty 2008). The high concentration of heavy metals hampers the oxygen level of water, which in turn affect the development of phytoplanktons, zooplankton, mollusks and their reproduction process.

3 Remediation Strategy of Heavy Metals

Heavy metal pollution is a world-wide concern and continuous attainment are given on this matter. Various chemical, physical and biological methods have employed to eliminate such pollutants from the environment. A few examples of physical and chemical methods are: ion exchange, coagulation and flocculation, adsorption, membrane filtration, solvent extraction, chemical precipitation, electro-chemical treatment, immobilization, etc. There are some imperfections on applying these physico-chemical approaches to combat pollutants because of high cost, inefficiency at very low concentration, and contaminant permanent changes of soil properties, etc. Nowadays, biological remediation or green remediation procedures are implemented to remove the contaminants, which is considered to be the safest, eco-friendly and cost-effective approach. In this method various microorganisms like bacteria, fungi, algae and heavy metal hyper accumulator plants are used.

Physical methods

Membrane Filtration: This technique is applied in waste water treatment. In this process an appropriate porous membrane such as cellulose acetate, polyamide, polysulfone etc. are being employed through which the solution flow out under different pressure. This process is categorized into three types: reverse osmosis under high pressure, reverse osmosis under low pressure and ultrafiltration. Membrane pore size, distribution of pores, the nature of hydrophilicity, amount of surface charge, solution flow rate and the presence of functional groups are the principal parameters applied to membrane filtration techniques. The efflux rate of solution and specificity of the membrane determines the performance of this procedure. Molecules with high molecular weight, heavy metals, suspended particles are removed by ultrafiltration method. It has been reported that reverse osmosis successfully eliminates various heavy metals and anionic toxicants from metal-plating wastewater. Although this is an effective process, but has some limitation like, membrane fouling, membrane longevity and membrane dissolution problem due to oxidizing elements, solvents and organic compounds (Hube et al. 2020).

Coagulation and flocculation: Coagulation and flocculation are an electrostatic repulsion process that can eliminate heavy metals from solution by neutralizing the surface charge of colloidal particles. In this procedure alum (aluminum sulfate), PACL (polyaluminium chloride), $MgCl_2$ (magnesium chloride), PEI (polyethyleneimine) and aluminum hydroxide oxides, etc. are required as coagulants. Flocculation involves slow mixing of destabilized particles to agglomerate into larger particles which can be easily removed by filtration, flotation, or straining (Tripathy and De 2006).

Ion Exchange: Ion exchange is a pH sensitive method in which the soluble ions are attracted from solution to solid phase. It is a cost-effective method and highly applicable for eliminating heavy metals from a liquid solution at very low concentration of pollutants. Inorganic zeolites or organic ion exchange resins can be used as solid phase ion exchanger. Once the resin bed gets saturated by ions, this can be regenerated by treating the resin beds with alkaline or acidic medium. It is a reversible process as resins absorb cations or anions in return release other ions of the same charge (Dąbrowski et al. 2004).

Adsorption: Adsorption is a very effective surface phenomenon which applied to remove heavy metals and ions from polluted water by using a suitable adsorbent. In this method the impurities get adsorbed on the surface layer of adsorbent rather enter into the internal structure. Impurities get bound tightly with the solid surfaces by van der Waals forces of attraction or hydrogen bonding. The advantages of this method are cost effective, eco-friendly, high efficiency, minimum sludge formation, reuses of adsorbent by removing deposited pollutants, etc. Activated carbon is used profusely to remove organic pollutants from waste water. Photocatalyst beads, red mud, coal, metals and metal-nanoparticles, activated sludge biomass, zeolite etc. are being used as adsorbents to remove heavy metals and ions (Chai et al. 2021).

Vitrification: It is the process of transformation of waste material into the glass like substances by applying high voltage about 3600°F. The waste present in the soil, melt down and get embedded into a glassy matrix by chemical bonding, therefore will not leak out further. The glassy matrix is chemically inert and has a low leaching characteristic. This method is used to remove hazardous radioactive and organic waste from soil (Moustakas et al. 2005).

Soil washing: Soil washing is a waste reducing technique which can be applied to the excavated soil or on-site soil. During this procedure the contaminants are removed from bulk soil by physical separation method and separated by applying aqueous chemical. Lastly the solution is recovered by chemical extraction of waste. This is a cost-effective, fast process and clean up the soil completely (Gusiatin et al. 2020).

Chemical Methods

Chemical immobilization: In this technique, pollutants are trapped by employing chemical agents and convert them into immobilize form. Through, in this process the pollutants are not physically removed from the soil, but the mobility of the pollutants through water current is blocked and thereby minimize their accumulation in plants and soil microorganisms and water bodies (Tajudin et al. 2016).

Solidification: It is a solidification technique of contaminated soils by applying various binding agents like asphalt, cement, fly ash, clay, etc. and transformed the soil into a solid block. This solid soil block is impermeable to water, thus entrapping the contaminants and stop being leached out over a long time (Tajudin et al. 2016).

Stabilization: Stabilization is an in-situ fixation process where various stabilizing agents are incorporate in the soil to initiate physiochemical interactions between contaminated heavy metals and stabilizing agents and to check their mobile nature. Various chemical substances can be used as stabilizing agents such as carbonates and phosphates groups containing material like, bone meal, hydroxyapatite, ammonium phosphate, apatite etc., alkaline agents like, calcium hydroxide, fly ash, etc., iron rich minerals and clay like, goethite, bauxite, silica gel, red mud, greensand, vermiculite, zeolites, etc., and different organic matter like, xanthate, chitosan, starch, compost, manure, biochar, activated carbon, etc. Among the various materials phosphates and carbonates containing compounds are the most propitious representative for effectively stabilizing heavy metal contaminants in polluted soils (Liu et al. 2018).

Neutralization: This is basically a precipitation reaction based neutralization process, where common alkaline reagents like lime, limestone, ferrous compound and other salt materials such as $CaCl_2$, $Mg(OH)_2$, $MgCO_3$, $BaCl_2$ etc. are used to eradicate heavy metal pollutants. The main function of these agents is to raise the pH level of water and thereby neutralize the heavy metal ions. The precipitated compounds, especially phosphate salts of various heavy metals like magnesium, iron, manganese, copper, etc. are further used as fertilizer in agricultural field. Calcium Oxide is an effective reagent than other lime because its temperature insensitive nature and CaO can neutralize cations by formation of hydroxyl ions through partial dissolution (Hermansson and Syafiie 2015).

Solvent Extraction: This is a hydrometallurgical treatment which has been employed in the operation of various metals like, gallium, cobalt, copper, uranium, nickel, zinc, molybdenum, hafnium, indium, germanium, platinum, boron, vanadium, tungsten, zirconium, niobium etc. Organic compounds which are almost insoluble in water used as extractant in this procedure. This treatment process depends on the extractant types, anions present in water and pH of water. Organophosphorus is often used in the process of metal separation. Di-(2-ethylhexyl)-phosphoric acid (D2EHPA) is used as an excellent extractant to recover zinc and copper at very low pH, as well as chromium and nickel at pH 6–7. Organophosphorus like Cyanex 272 is also employed for the removal of various metals like, Fe, Zn, Cr, Cu, Ni etc. in a solution of sulfuric and sulfate form. It was also reported that the fruitful removal of cobalt from nickel can be achieved by these compounds around 99.4% at pH 6 (Mubarok and Hanif 2016).

Chemical Precipitation: It is a widely practiced method where pH sensitive transformation of various heavy metals into sulfide, hydroxide, carbonates, or any other less water soluble compounds takes place. These transformed component compounds are then easily eliminated by sedimentation, filtration, or flotation techniques. The efficiency of this technique stands on the chemical nature, size, density and surface charge of the pollutants. Various precipitants materials such as caustic soda, soda ash, lime, sodium bicarbonate, sodium sulfide, sodium hydrosulfide, etc. are Some of the largely used for different heavy metal precipitation. Various kind of chemical precipitation have been employed such as carbonate precipitation by using soda ash or sodium carbonate, hydroxide precipitation by adding alkali or lime), sulfide precipitation by using sodium sulfide or sodium hydro sulfide, xanthate precipitation to eradicate different metals like, Cd, Cr, Hg, Zn, Ni etc. In combined precipitation treatment more than one precipitant are used in combined form such as uses of carbonate and hydroxide precipitation (Rafique et al. 2022).

Biological Methods

Biological remediation methods are also termed as phytoremediation, green remediation, botanoremediation, agroremediation, which is an eco-friendly remediation procedure, carried out by using vegetation, microbiota, soil amendments, and agricultural methods to remove environmental contaminants. Phytoremediation generally is an energy proficient procedure for heavy metal remediation in place containing little to medium levels of toxicants, and can be employed in together with other more conventional remedial systems as a complete step in the remedial procedure. The phytoremediation techniques have so much superiority in comparison with various physical or chemical remediation procedure such as, eco-friendly nature, cost-effectiveness, no need of disposal site, no need to excavate and transport the polluted media and at a time more than one pollutant can be cleaned. There are various types of technically different biological remediation methods are exploited to eradicate heavy metals from environment like, phytoextraction, phytostabilization, phytovolatilization, phytofiltration, phytodegradation, phytostimulation, etc.

Phytostabilization: In this technique heavy metals are transformed into their immobile form by metal-tolerant plant species and thereby reducing bioavailability and leaching. Phytostabilization process accelerates the reactivity and precipitation of heavy metals present in the rhizospheric soil, and thereby induce bio-adsorption on the root cell wall and compartmentalization of heavy metals within root tissue, therefore hindering the entry of metal contaminants into the food chain. Plants through their root system can prevent soil erosion and leach out of toxic metals through ground water flow and, therefore, selection of plants for phytostabilization process is very crucial. Some important features are essential for plants chosen for this technique such as, it must have a dense root system, high metal-tolerant, easy to plant and maintenance, rapid growth, longevity and easy to propagate (Alkorta et al. 2010). Various organic and inorganic materials like biosolids, litter, Zinc, phosphate, limes are used as soil amendments to enhance the phytostabilization process. These matters can alter metal solubility and reactivity by changing the soil pH. Moreover, the organic amendment acts as a nutrient source for soil microorganism and induce their flourishment. Sometime these microorganisms also assist metal stabilization process by adsorbing metals onto their cell surface, improving precipitation process and chelation by secreting chelators of heavy metals (Göhre and Paszkowski 2006).

Phytoextraction: In this process hyperaccumulator plants absorb heavy metals from rhizospheric soil and transport them to the ground above tissue. It is a permanent resolution for complete eradication of pollutants from soil and therefore ecofriendly and more commercially relevant. The phytoextraction of heavy metal contaminants using hyperaccumulator plants performed by some stepwise procedure such as, translocation of heavy metals in the rhizosphere, then uptake of heavy metals through plant roots, and after translocation from the roots to aerial parts of plant, the heavy metal ions sequester and accumulate in the plant tissues (Ali et al. 2013). The effectiveness of this procedure depends on plant category, health of the plant, concentration of heavy metals and also various physical and biochemical properties of the soil. The exploited plants must have the higher level tolerance ability, huge extraction capability, great metal accumulation capacity, considerable root and shoot growth, high root absorbance capacity, large biomass production ability, substantial disease resistance power and highly adaptive nature with changing environmental conditions (Ali et al. 2013). Mainly there are two types of phytoextraction mechanism like, natural phytoextraction and induced phytoextraction. In the natural phytoextraction process, the hyperaccumulators plants are readily extract heavy metal contaminants from the soil. Generally, hyperaccumulators are those plant species that possess the capability to accumulate heavy metals at a rate more than 100-fold greater than those of the normal plants. There are about more than 400 species belonging to 45 different families have been so far contemplate as hyperaccumulators. Plants belongs to Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae, and Scrophulariaceae families are considered as good hyperaccumulators (Ali et al. 2013). In case of induced mechanism, different chelating agents are used in soil for phytoextraction. Chelating agents like EDTA easily gets bound with the metals to form metal-chelator complexes and easily uptake by the plants.

Phytovolatilization: It is a process where toxic heavy metal contaminants, absorbed by the root system and then are converted into the less toxic volatile form, which are released by the stomatal opening and stem. Through this method various toxic organic materials and heavy metals such as As, Se and Hg can be released. A few members of the Brassicaceae family like *Brassica juncea*, tobacco plants and populus plants are capable to volatilize different heavy metals and dispersed in the atmosphere. Inorganic Se is converted into selenomethionine, which is a less toxic volatile form and released in the air. Tobacco plants are able to vaporize ionic Hg into its less toxic form. The most advantage of this mechanism is that there is no need to harvest the plant separately and no need of disposal (Terry et al. 2000; Limmer and Burken 2016).

Phytofiltration: Phytofiltration is a special type of filtration procedure for separation of pollutants from water surface by using the root (rhizofiltration), shoots (caulofiltration), or seedlings (blastofiltration). In rhizofiltration process heavy metals are adsorbed or absorbed by the root system. Some chemicals are secreted from roots that changes the pH of the rhizospheric soil environment, causes heavy metal precipitation and change the mobility of heavy metals. Mostly hyperaccumulator plants are exploited for this technique. A number of aquatic species such as water hyacinth, duckweed, *Azolla*, poplar and cattail are usually employed for eradicating toxicants in water bodies. Various terrestrial plants like Indian mustard and sunflower are the best uses in this technique as because of their dense root system (Olguín and Sánchez-Galván 2012).

Phytodegradation: Phytodegradation is a process performed by the plants, where all the steps like, transformation, breakdown, mineralization, mobilization of heavy metals happen by using various self-producing enzymes. This efficiency of this technique depends on various factors like heavy metals composition and concentration, characteristic of plant species being used, soil profile. Depending upon such factors, pollutants may escape through rhizospheric soil or may entrapped by phytosequestration or phytodegradation (Muthusaravanan et al. 2018).

Mycoremediation: In this process various fungal strains are exploited to remove heavy metal contaminants. The fungus produces various kinds of degradative enzyme which is utilized to eradicate heavy metal contaminants from water and soil. Various fungal species such as *Pleurotus ostreatus*, *Agaricus bisporus*, *Lentinus squarrosulus*, *Phanerochaete chrysosporium*, *P. ostreatus*, *P. pulmonarius, Trametes versicolor* etc. are being employed in this technique. Generally, heavy metal polluted soil profuse growth of the Ascomycetous, Basidiomycetous and Arbascular mycorrhizal fungal strain in rhizosphere have been reported. Fungal hyphae penetrate deeply through soil and secretes various chelating compounds to adsorb heavy metals. Mycorrhizal fungus can produce oxaloacetate crystal through which they can immobilize and detoxify heavy metal toxicants (Gadd et al. 2014).

Phycoremediation: Several micro- and macro-algae belonging to chlorophycean and cyanophycean algae are being employed in the bioremediation process for its eco-friendly and less expensive nature. Wide numbers of blue green algae are widely used to remove heavy metal contaminants from waterbodies. Metals are generally taken up by the adsorption process and then transported into the cell interior through chemisorption, where these pollutants get bounded by poly phosphate bodies (Dwivedi 2012). Microbial polyphosphate inclusions can easily sequester various heavy metals like Hg, Ti, Pb, Mg, Zn, Cd, Sr, Co, Ni and Cu. There are many algal species like *Lyngbya putealis*, *Sargassum myriocystum*, *Scenedesmus* sp., *Enteromorpha intestinalis*, *Cladophora glomerata*, *Ulva lactuca*, *Euglena gracilis*, *Chlorella vulgaris*, *Phormidium* sp. *Spirogyra* sp., *Oscillatoria* sp., etc., could easily remove various heavy metals such as Cu, Co, Cd, Cr, Pb, Ni, Mn, Zn, As, etc. from the medium (Singh et al. 2017).

Bioaugmentation: Bioaugmentation is an approach of using microbes indigenously or exogenously to polluted media and subsequent eradication of heavy metals. Successful application of a consortium of different strain over a single strain is found to be more effective in the removal of various metal pollutants like, Al, Cd, Cr, Fe, Ni, Pb, Zn from the medium.

4 Plant Growth Promoting Rhizobacteria (PGPR)

Rhizosphere is the soil zone immediate to root surface and is over flooded by the various nutrients. Plant roots exudate a large number of amino acids, monosaccharides, organic acids, that support the growth of microflora in the vicinity of the root. Rhizosphere is inhabited by a wide array of microorganisms that execute potential effect on plant health. Some rhizospheric soil microorganisms are pathogenic in nature and show a negative impact on the plant health, whereas some are advantageous for plant proliferation. PGPRs belong to the second type of microbes that have an immense positive impact on the health and enlargement of the plants. These are root colonizing bacterial groups which can undergo symbiotic relationship with the plant or may remain as a free-living microbe (Fig. 2). There are mainly two types of PGPRs exist in the environment such as, ePGPR or extracellular plant growth promoting rhizobacteria and iPGPR or intracellular plant growth promoting rhizobacteria. Extracellular PGPR group of bacteria reside in the outside the cell, whereas iPGPRs inhabit within the cell, formed a specialized structure form called nodules.

An ideal PGPR possess some characteristic such as, it should inhabit in rhizosphere, should be ecofriendly, should possess efficient colonizing ability with plant roots, should able to promote plant growth, should exhibit a broad spectrum of action, should demonstrate better competitive skills over the existing rhizobacterial communities and should be tolerant of physicochemical factors like heat, desiccation, radiations and oxidants. Some good examples of ePGPR include are *Azotobacter, Azospirillum*, *Bacillus*, *Caulobacter*, *Chromobacterium, Erwinia*, *Serratia, Agrobacterium*, *Flavobacterium*, *Arthrobacter*, *Micrococcus*, *Pseudomonas* and *Burkholderia*, whereas *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium* are some candidates of the iPGPR category. On the basis of functions PGPR can also be classified into some category, such as, biofertilizer, phytostimulators, rhizoremediators and biopesticides. Phytostimulators PGPR have the capability to stimulate plant growth by phytohormones production, whereas rhizoremediators PGPR are able to remediate heavy metal pollution. Biopesticides PGPR are able to control the pathogen attack by secreting various toxic metabolites and lytic enzymes (Ahemad 2019).

PGPRs also include nitrogen fixing rhizobacteria that undergo symbiotic relationship with leguminous plants and provide nitrogen to the growing plants. These bacteria either reside within the plants in a specialized structure called nodule or may present on the root surfaces. There are two mechanisms by which PGPRs effect on the plant growth as direct method or indirect method. In the direct method the PGPRs improve plant health and development by increasing nutrient uptake, nitrogen fixation, phosphate solubilization, phytohormone regulation, etc., thereby exerting

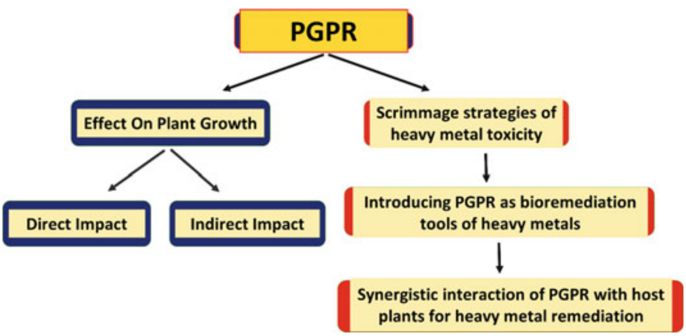

Fig. 2 Contributory roles of plant growth promoting rhizobacteria in sustainable development

effects directly on the plant growth. Where as in indirect method the PGPRs enable plants to fight against phytopathogen and induce resistance power, which are helpful to overcome stress condition. PGPRs can synthesize various kinds of phytohormones like IAA, cytokinin, gibberellin and also alter the production of these hormones in the plant body, which are a direct effect on the crop yield and biomass formation. The interaction between rhizosphere soil and microbes has a direct relation to photosynthetic yield of plants, which meet global food demand and green evolution with respect to increase the population on earth. There are several PGPRs that are reported to increase the crop yield, seedling vigor, antibiotic, salt and fungicide resistance, drought resistance, nutrient uptake capability of rice.

Biofertilizers can be described as materials that contain living microorganisms which can be spread on seeds, plant body, or soil, resulting colonization into the rhizosphere or the interior of the plant, and enhance plant proliferation by escalating the availability of various main nutrients to the host plant. Uses of PGPRs as biofertilizer not only a beneficial effect on the plants to collect the proper amount of nutrient uptake like N, K and P from soil, but also balanced the recycling of nutrients in the environment. Biostimulants are substances that stimulate plant growth and maintaining plant health without affecting nutrient supply, soil quality, pesticide resistant. Some PGPRs act as biostimulants by enhancing the phytohormone synthesis and assisting to overcome various biotic and abiotic stresses. Now a day in agricultural field various PGPRs like, *Pseudomonas*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Azobacter*, *Variovorax*, *Azosprillum*, etc. used as biostimulants. PGPRs are also involved in bioremediation process and prove to be highly effective in heavy metal eradication from contaminated soil. PGPRs can alter the bioavailability of metal-ions by affecting on their mobility in soil and also can modify toxic metals to less toxic forms. PGPRs also used as biopesticides that accelerate plant proliferation by inhibiting various phytopathogenic representatives. PGPRs can able to resist pathogenic attack by producing diverse secondary metabolite compounds as well as inducing systemic resistance in plant body (Basu et al. 2021).

5 How do PGPR Scrimmage with Heavy Metal Toxicity?

PGPRs are utilized in the bioremediation procedure because of their broad-spectrum resistance or tolerance mechanism against nonessential heavy metal. These mechanisms are mostly regulated by genes present either in chromosomes or plasmid DNA. There are several mechanisms through which PGPRs can resist heavy metal toxicity like, permeability barrier for metal exclusion, active transport of metals from microorganisms, extracellular sequestration, intracellular sequestration, altering the cellular target of metals, detoxification of heavy metals and passive transport system (Bruins et al. 2000). Permeability barrier for metal exclusion is exerted by bacterial cell wall, extracellular layers, exopolysaccharide layers, capsule, directly prevent the entry of toxic metal. Some time by genetically changing the protein channels present on cell membrane PGPRs can operate selective permeability of heavy metals within the cell interior. Heavy metals get bind non-specifically with the various extracellular polymeric substances, outer lipopolysaccharides, protein molecules present in cell membranes, outer membrane envelope and subsequently are prevented to interfere with various metal sensitive cellular biochemical processes (Bruins et al. 2000). Biosorption is a passive uptake process of metal ions, which takes place in a metabolic independent pathway by biologically dead or inactive materials. A number of bacterial genera like *Bacillus*, *Pseudomonas*, *Streptomyces* etc. can act as biosorbent and widely used in pharmaceutical and food industries (Vijayaraghavan and Yun 2008). Biosorption of heavy metals at extracellular layers and cell matrix takes place via various anionic functional groups such as, carboxyl, sulfonate, sulfhydryl, hydroxyl, amine and amide etc. and thereby inhibit the intracellular entry of diverse toxic metal ions. It has been reported that biofilm formation of microorganisms can be an efficient measure of multi metal resistance by combining the action of chemical, physical and physiological processes (Harrison et al. 2007). A consortium of microbe can help to colonize bacteria and produces biofilms in a moist environment where nutrient molecules are present sufficiently. Such colonization of microbes helps them to tolerate heavy metal toxicity. Various macromolecules present in biofilm structure can exhibit electrostatic interactions, hydrogen bonding and London dispersion forces and the extracellular polymeric substances present in the biofilm carried out an active role in biosorption of metal ions.

Active transports or efflux systems of metallic ions are either ATPase dependent or independent and responsible for the largest category of metal resistance systems in microorganisms. A large number of bacteria possess efflux transporter, which have high affinity for metal ions at very low concentration and excrete them outside the cell, thereby keep the cell interior completely free from toxicants. This resistance system can be mediated by chromosomal or plasmid encoded proteins. Chromosomal or plasmid mediated resistance to arsenate [As(V)] and arsenite [As(III)] has been observed in *E. coli*, *Staphylococcus* and various other bacteria. Arsenic resistance is solely dependent on the expression of *ars* operon, which is made up of three to five genes, namely *arsR*, *arsA*, *arsD*, *arsB*, and *arsC* which are responsible for encoding an ATPase efflux pump and detoxifying enzymes arsenate reductase and arsenic oxidase (Ben Fekih et al. 2018). In *Bacillus* sp. *S. aureus*, *cad* operon mediated and in *Alcaligenes eutrophus*, *czc* operon mediated cadmium efflux pump is found. In *E. coli,* Pb(II) resistance mediated by the gene *ZntA* (Etesami 2018). P-type ATPases, another example of an ATPase transport of metal ions is *cop* operon mediated efflux of Cu(II) ions in *Enterococcus hirae*. This operon is consisted of four genes, namely *copA*, *copB*, *copZ*, *copY*. The Cu(II) uptake mediated by *copA* gene encoded ATPase and a P-type efflux ATPase encoded by the *copB* gene (Etesami 2018). In *E. coli* a multi protein complex *CusCBA* assists in Cu efflux. A RND protein encoded by *CusA* is controlled by the proton motive force and export Cu out of the cell interior with the help of *CusB* and *CusC* gene products (Cha and Cooksey 1991). In extracellular sequestration mechanism, metal ions are accumulated in the periplasmic space or complexes with various cellular compounds as insoluble forms. Metal precipitation is an example of extracellular sequestration. Zinc ion is an essential trace element for cells, but excessive accumulation causes toxicity for cell viability. Once this ion has been excreted out from the cell, re-entry is strictly controlled by the post efflux control mechanism in several resistance strains. The mechanism involved precipitation of various metal ions, attaching to specific binding proteins within the periplasmic space (Choudhury and Srivastava 2001).

In recent years, using various biosurfactant for the removal of metals and metalloids from contaminated medium has gained huge interest in the field of bioremediation due to their low toxic nature and biodegradable properties. Biosurfactants can be defined as the surface-active biomolecules produced by diverse microorganisms, which have the property of a metaloid complex formation. It has been established that *P. aeruginosa* and *B. subtilis* can remove heavy metals Cu and Zn with the production of biosurfactant surfactin, rhamnolipid, and sophorolipid. Biosurfactant produced by bacteria possess selectivity and species-specific metal binding capacity, can efficiently remove metalloids via ion exchange, counter binding and precipitation process. The ionic character of biosurfactants may be positive or negative, which can bind strongly anionic or cationic metal-ions respectively. Another biosurfactants lipopeptide, produced by *Bacillus subtilis* has been applied to decontaminate polluted soil by removing various contaminated heavy metals and hydrocarbon compounds. Sophorolipids, a biosurfactant compound, produced by *Torulopsis bombicola*, is also able to remove more than 60% of Zn and 25% of Cu from contaminated soil (Mulligan and Yong 2004).

Intracellular sequestration is another mechanism where bacteria can accumulate toxic metals within the cell interior, so as to prevent metal ions from exposure to metal sensitive cellular components and biochemical pathways. Some PGPRs can convert some metals into innocuous form and accumulate within the cell. A metal resistant strain of *Synechococcus* sp. possess *smtA* gene, which encode metallothionein, is a class of cysteine rich protein that binds metals in cell cytoplasm and maintain the concentration of metals intracellularly, thereby regulate various metal sensitive metabolic pathways. A number of PGPRs like *P. aeruginosa*, *P. putida* and *Anabaena*, can produce metallothionein under certain stress condition and sequester diverse heavy metals. It was reported that *Rhizobium leguminosarum* exposed to Cd stress, the production of glutathione, which is a metallothionein protein, increases in several fold (Blindauer et al. 2002).

By producing siderophores, are low molecular weight iron binding chelators molecules, microbes can tolerate heavy metal stress and convert the toxicant into non available form. *P. stutzeri* KC can precipitate various metals like, Co, Cu, Ni, Pb and Zn by producing siderophores. It has also been reported that siderophores can increase chlorophyll production and thereby support plant growth under heavy metal stress. *Psychrobacter* sp. SRS8 can produce catechol and hydroxamate type siderophores and can enhance the growth and development of certain plants like, castor and sunflower under nickel and other metal stress condition (Ma et al. 2011). By the mechanism of siderophore production PGPRs can grow in multi-metal contaminated mine tailing soil. Bacterial siderophore can increase iron absorption capacity of plants and reduce toxic metal concentration in cell by binding them specifically. *P. aeruginosa* decreases the Al toxicity by producing pyoverdine and pyochelin siderophores. In *Streptomycetes*, siderophore production stimulates iron uptake simultaneously decreases Cd uptake. Therefore, evidences showed that by siderophore production PGPRs can resist metal induced toxicity and also prevents plants from being chlorotic (Bruins et al. 2000; Etesami 2018).

Methylation is another process adopted by PGPRs to eradicate metal toxicity by volatilization of metallic ions. PGPRs are able to convert various metallic ions like Se, Sn, Te, As, Pb, etc. into their methylated volatile form and thus promote their diffusion from the cell. Various bacterial species like, *Bacillus* sp., *Clostridium* sp., and *Pseudomonas* sp., are able to transform Hg (II) to gaseous methylated Hg. Various evidences have been reported for bio-methylation such as, arsenic (As) to gaseous arsines, lead (Pb) to dimethyl lead, selenium (Se) to volatile dimethyl selenide (Park et al. 2011). Several PGPRs can detoxify metal ions by enzyme assisted oxido-reduction process. Several gram positive and gram negative bacteria possess mercury resistance genes cluster *mer* operon in the chromosome. These operons mainly encode two enzymes such as, mercuric ion reductase and organomercurial lyase. The C-Hg bond can breached by organomercurial lyase and yield Hg(II) form, which is then again reduced to Hg(0) by the another enzyme mercuric ion reductase. Hg(0) is basically inert and water soluble, can easily release through the cell membrane (Bruins et al. 2000). Some other natural remedies have been adopted by PGRPs to reduce metal toxicity. Microorganisms by altering their cellular reactivity for metal ions can by-pass the deleterious effect of heavy metals, but such decreases of cellular response may not hamper the basic function of cells. Some time by increasing production of a particular cellular component to keep ahead of metal inactivation.

6 Direct and Indirect Impact of PGPR on Plant Health

PGPRs have great impacts on plant growth and development. PGPRs can improvise plant growth and yielding capacity either by direct mechanism or by indirect mechanism. PGPRs directly promote the plant growth and development by either helping in nutrient element like, nitrogen, phosphorus and essential mineral acquisition. PGPRs can also indirectly enhance the plant growth and development either by regulating diverse plant hormone levels, or by reducing the effect of various plant growth inhibitory pathogens.

Direct impact of PGPRs

Phosphate solubilization: The phosphorus (P) is a macronutrient which is found in insoluble form in the soil and soil microbes play an important role in its recycling process. PGPRs transform soil insoluble phosphorus into a soluble form and then plant can easily uptake from the soil. Phosphorus has played several crucial roles in the plant metabolism process like, respiration, photosynthesis, membrane formation, carbon metabolism and energy transfer process. Phosphate also plays a vital role in binding of heavy metals on cell wall in the form of metal-phosphate complex, thereby decreasing in heavy metal intake by the plants and subsequently increase the heavy metal tolerance. Phosphate solubilizing bacteria (PSB) secrete various enzymes that needed to convert soil insoluble phosphorus into solubilizing form. PSB can also improvise nutrient availability in soil by phosphorus mobilization via phytase enzyme secretion A number of genera, are isolated from different rhizospheric soil are found to be PSB group, like *Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Cupriavidus*, *Agrobacterium*, *Acinetobacter*, *Arthrobacter*, *Pantoea*, *Rhodococcus* etc. (Billah et al. 2019).

Sequestering Iron: Though iron is very important nutrient for plants' growth and development, but neither plants nor microbes can readily uptake iron from rhizospheric soil. Most of the iron is present in the ferric ion form in the soil but is poorly soluble in water, and therefore, the assimilation rate of plants and microbes is very low. Under these circumstances, some bacterial species can synthesize certain low molecular weight compounds designated as siderophore to trap iron efficiently from soil by forming Fe-siderophore complex. Inoculation of *P. fluorescens* C7 in *Arabidopsis thaliana* plants, leads to the formation of Fe-pyoverdine complex, which is responsible for increasing iron concentration within plant tissues (Vansuyt et al. 2007).

Potassium solubilization: In nature most of the potassium exists in three different forms viz., readily exchangeable form, slowly available form or unavailable form. Readily available potassium remains mixed with as oil, water and 1–2% present on clay surface. Potassium plays several vital roles in the plant metabolism process like, controlling cellular osmotic pressure, cell turgidity, water movement, nutrient movement, stomatal opening and closing, activation of various plant enzymes and provide protection against pathogens. Potassium solubilizing bacteria can solubilize insoluble potassium through various processes such as, acidolysis, altering pH, by organic acid production, capsule absorption and enzymolysis reactions. Bacteria by producing diverse organic acids like, tartaric acid, oxalic acid and citric acids, easily reduces the soil pH level. Acidification of soil causes dissolution of potassium from micas, illite and orthoclase, and thereafter make it available for plant uptake. In another mechanism some bacteria secrete slime or acidic polysaccharides, which can form bacterial mineral complex and assist in releasing potassium from silica. A number of microbes like, *Paenibacillus glucanolyticus*, *Agrobacterium tumefaciens*, *Rhizobium pusense*, *Burkholderia cepacia*, *Enterobacter aerogenes*, *Pseudomonas azotoformans*, *P. orientalis*, *Microbacterium foliorum*, *Myroides odoratimimus*, *Pantoea agglomerans*, *Bacillus licheniformis*, *B. subtilis*, *Pantoea agglomerans*, *Rahnella aquatilis*, etc. are reported to be potassium solubilizing bacteria (Sattar et al. 2019).

Zinc solubilization: Zinc is a micronutrient and act as cofactor of various metabolic enzymes of plant like tryptophan synthetase. Zinc is available as an insoluble form in a very low concentration in rhizospheric soil. Various bacteria have been reported to improvise zinc uptake by plants, as for example *Acinetobacter* sp., *Bacillus aryabhattai*, *B. subtilis*, *B. cereus*, *B. Megaterium*, *B. tequilensis*, *Pseudomonas aeruginosa*, *P. fragi, Pantoea dispersa, P. agglomerans*, *Agrobacterium tumefaciens*, *Rhizobium* sp., etc. Soil microbes help in zinc absorption either by lowering soil pH or by chelation process. Soil microbes solubilize zinc by secreting various organic acids like 2-ketogluconic acid, 5-ketogluconic acid etc. (Kaur et al. 2021).

Siderophores production: Siderophores are low molecular weight compound having iron binding capability. On the basis of moiety that donates oxygen ligands for Fe^{3+} coordination and mainly there are three categories of siderophores like, hydroxamates, catecholates or carboxylates and mixed type. Iron is an essential element plant cellular metabolism, which is needed to carry out electron transport chain, oxidative phosphorylation, photosynthesis, tricarboxylic acid cycle, and biosynthesis of various essential biomolecules like, nucleic acids, vitamins, antibiotics, toxins, pigments, cytochrome and porphyrins. In iron limiting condition, by secreting siderophores, microbes help to convert insoluble form Fe^{3+} form into a soluble Fe^{2+} form and make available for plants. Additionally, microbial siderophore production accelerates the heavy metal movability in contaminated rhizospheric soil by capturing them and increase the metal availability in the rhizosphere through a series of complex reaction. Siderophore producing microbes reported so far includes all known genera like, *Achromobacter* sp., *Arthrobacter* sp., *Bacillus* sp., *Streptomyces* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Curtobacterium sp.*, *Plantibacter* sp., etc. Siderophore producing bacteria can enhance the plant growth and development in heavy metal contaminated area by increasing chlorophyll contents and also reduce the other toxic heavy metals uptake by the plants. Thus, siderophore producing PGPRs can alleviate and promotes plant growth and development by increasing nutrient uptake, particularly Fe and reducing other heavy metal absorption, results reduced oxidative stress. Siderophore producing PGPRs are also playing a role in controlling the activity of different plant hormones under metal stress condition (Kaur et al. 2021).

Nitrogen fixation: Nitrogen is an important element as it is the structural components of nucleic acids, proteins, and other biomolecules. But plant cannot fix nitrogen itself from the atmosphere directly. Direct fixation of nitrogen from the atmosphere is mediated by microbes only. Nitrogen fixation takes place in two ways like, symbiotic nitrogen fixation and non-symbiotic nitrogen fixation. Atmospheric nitrogen needs to be transformed into ammonia to make it available for plants. Microbes possess an enzyme complex nitrogenase that converts nitrogen to ammonia. Bacteria can fix atmospheric nitrogen by several mechanisms like, ammonification, nitrification, denitrification and thereby complete the biological nitrogen cycle. A symbiotic association of *Rhizobium* in the legume plants can able to fix more than 50% of total biological nitrogen fixation. *Rhizobium* is species specific strain and induce nodulation on specific plant legume. Therefore, application of bacteria in soil as bio-fertilizer must be selective. It has been reported that PGPRs improves ROS scavenging activity by up taking nitrogen under heavy metal stress environment by increasing the synthesis of enzyme glutathione reductase. Diazotrophs are a class of PGPR that establish a non-obligate relationship with non-leguminous plant and fix nitrogen. The most exploited PGPRs are rhizobia including *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Azorhizobium*, *Sinorhizobium* and *Rhizobium*. There are some bacteria like *Nitrosomonas*, *Nitrococcus*, and *Nitrobacter* that contribute in nitrification procedure by converting ammonia to nitrite and then to nitrate. Free living heterotrophic diazotrophs like *Azospirillum* sp., *Azotobactor vinelandii*, *A. chroococcum*, *Pseudomonas* sp., etc. that can trap nitrogen for non-legume plants like rice, wheat and others crop. Combine application of potassium solubilizing bacteria like, *Bacillus mucilaginosus* and nitrogen fixing bacteria like, *A. chroococcum* can increase in biomass and higher nutrient uptake by various plants. *Sphingomonas trueperi*, *Psychrobacillus psychrodurans* and *Enterobacter oryzae* are found to be effective in increasing nutrient uptake like N, Ca, S, B, Cu, and Zn as well as promote plant growth. If these strains were inoculated in seedlings of maize and wheat grown under greenhouse conditions, are resulting high nutrient uptake and plant growth promotion (Xu et al. 2018).

Photosynthesis: PGPRs play an important role in enhancing photosynthesis rate by increasing photosynthetic pigment chlorophyll in plants. It has been reported that *Rhizobium* sp., and *Bradyrhizobium* sp. increases the photosynthesis activity in various plants under stress conditions. *Planomicrobium chinense*, *Bacillus cereus* and *Pseudomonas fluorescens* increases the photosynthesis efficiency of wheat grown under salt and drought conditions (Kaur et al. 2021).

Phytohormone regulation: Different types of plant growth regulators such as, auxin, cytokinin, gibberellin, abscisic acid, etc. are endogenously produced by plants which regulates the plant growth and developments. It has been documented that microbe plays a critical role in regulating the phytohormone production as well as microbe itself can able to synthesized several phytohormones under normal or stressed condition. Application of PGPRs as biofertilizers on plants under drought and other stress conditions, resulting increases stress tolerance by the plant due to modifying effect of various phytohormones like, IAA, gibberellic acid, cytokinins, ethylene etc.

Indirect impact of PGPRs

EPS production: Exopolysaccharides (EPS) are high molecular weight polymers secreted by microorganisms. EPS are consisting of homo or hetero polysaccharide and exist as a capsule or slime layer on bacterial cell surfaces providing protection to them against various stresses. EPS form complex with heavy metals and decrease their mobility, therefore, reduces the availability for plants. Microbial exopolysaccharide also plays a critical function in a different environment situation such as, act as a signal molecule during nodulation, biocontrol activity, soil particle aggregation, change the soil structure and profile, bacterial biofilm formation on root and enhance plant growth (Manoj et al. 2020).

Induction of plant production of antioxidant enzymes: Under various stress condition both biotic and abiotic, several reactive oxygen species (ROS) like, superoxide ions, peroxides, hydrogen peroxide, singlet oxygen, hydroxyl radicals are produced in plant body which causes severe damage to biological membrane and other important biomolecules. To cope up with the situation plants can produce various antioxidant enzymes like, catalase, dehydroascorbate reductase, peroxidase, superoxide dismutase, glutathione peroxidase, glutathione reductase, etc. Plant also can produce a number of non-enzymatic antioxidant component such as ascorbate, glutathione, proline, tocopherol, glycine betaine etc. for oxidative stress tolerance. It has been reported that application of PGPRs on plants under stress condition can enable plants to tackle the deleterious effects of stress by increasing the production of various antioxidant enzyme and antioxidant components. It is reported that the inoculation of Zn resistant *P. aeruginosa* with wheat seedlings under Zn stress condition, resulting increased antioxidant enzyme like SOD, POD, CAT and increase biomass (Islam et al. 2014).

Hydrogen cyanide (HCN) production: Rhizobacteria are able to produce volatile compound hydrogen cyanide (HCN) which inhibits mycelial growth of pathogenic fungus and thereby provide a defense mechanism against disease development. HCN can also induce plant growth and entrap the heavy metal movability in the soil.

Biosurfactants production: Biosurfactants are amphiphilic, low molecular weight molecules produced by microbes and found to be attached on the cell surface. A biosurfactant usually made up of glycolipids, fatty acids, phospholipids, lipoprotein, or lipopeptide and mycolic acid. Bacterial biosurfactant provide heavy metal tolerance in plants and remove toxic metals from the soil. These substances can bind pollutants with strong affinity due to their amphiphilic nature than normal cations.

Organic acids production: PGPRs secretes various kind of low molecular weight organic acids like, oxalic acid, gluconic acid, citric acid, succinic acid, etc. These organic acids help to alleviate heavy metal toxicity and promote plant growth by forming metal–acid complex like metaloxalate crystals and turn into their less toxic form or by acquisition of essential nutrients. Organic acids also play an important role in enhancing antioxidant enzyme responses of plants and solubilizing mineral phosphates.

Hydrolytic enzyme productions: PGPRs synthesize a variety of hydrolytic enzymes like chitinases, protease, lipase, cellulases, glucanases and esterases. These enzymes provide chemical defense against pathogenic fungal attack by hydrolyzing their cell wall and thus keep the plants healthy in an indirect way. These enzymes are also responsible for recycling of nutrient molecules in nature.

Antibiotic production and induced systemic resistance (ISR): PGPRs are reported to produce antimicrobial compounds, that provide plant protection against harmful fungus, bacteria, viruses, nematodes etc. via induced systematic resistance mechanism or antagonism. Various kinds of antimicrobial compounds such as, phenazines, butyrolactones, pyrole-type compounds, 2,4-diacetyl phloroglucinol, kanosamine, etc. and various peptides are produced by PGPR strains and protect crops and plants (Manoj et al. 2020).

Synthesizing ACC deaminase: Under heavy metal stress ethylene production increase drastically, which causes a reduction in root length, accumulation of hydrogen peroxide and leads to apoptosis situation. To overcome such situation, PGPR produces higher amounts of ACC deaminase enzyme to hydrolyse ACC, the immediate precursor of ethylene. Therefore, enable plants to reduce ethylene level.

7 Synergistic Interconnection of Plants and PGPR in Heavy Metal Remediation

Plants and microbes interact with each other to get benefited. Sometime plants and microbes form a symbiotic association with each other. In some circumstances, both live as independently free-living organism, but interact with each other. The plant provides nutrients and space to the microbes, and in return of which microbe increase bioavailability of nutrients from the soil surface, detoxify the toxic heavy metals, provide resistance against pathogenic attack, thereby vastly influence on the growth and health of the plants. Thus, plants and microbe establish a synergistic interaction between them, which has been exploited nowadays as a successful bioremediation technique of heavy metals from media (Fig. 3). The efficiency of such dynamic interaction depends on various factors like the plant species, microbial flora type, type of pollutants, physical and chemical characters of soil. PGPRs are group of soil residing bacteria that interact with plants to promote their biomass increase as well as also assist them to cope up with the heavy metal stress. Co-culture of PGPRs and plants provide a new path in the field of phytoremediation of heavy metals. Plants will store diverse contaminants in rhizosphere that may later be harvested, whereas microbial accumulation can even transform contaminants like heavy metals to stable and less harmful type. Plants secrete various substances like enzymes, amino acids, sugars, aromatics, aliphatic that stimulate root associated microbial community expansion in return microbes reduces toxicity of metals and enhance the capability of plants to degrade and sequester the heavy metals. The process of phyto-extraction by hyper-accumulators plants i.e., absorption of heavy metals and their translocation to above ground tissue is accelerated by PGPRs inoculation. PGPRs improvise the mobility of heavy metals and transportation by changing soil pH, demineralization, biosurfactant production, siderophore secretion and chelator production. PGPRs strain produces oxalic acid, citric acid and other organic acids, which increases metal solubility and mobility by reduction process. Phytovolatilization process is also increased by PGPRs inoculation. It has been well documented that inoculation with PGPRs can able to increase heavy metal resistance of plants by switching on different transcription factors, various protein molecules related to stress and thereby provoking the stress signal pathways and genes to overcome the stressed situation. Under heavy metal stress a number of signaling pathway like MAPK pathway, ROS signaling pathways, Ca-dependent pathway, hormone dependent pathway gets activated which in turn activates stress related transcription factors and genes (Manoj et al. 2020).

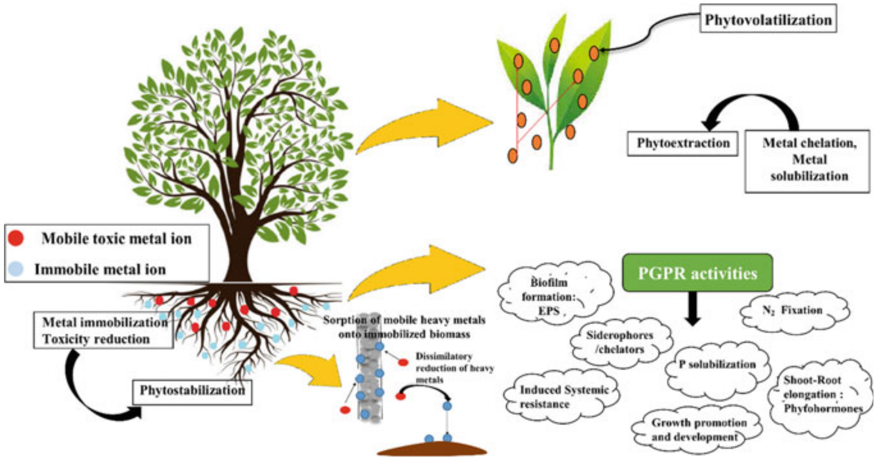

Fig. 3 Plant-PGPR interacting network model

8 Conclusions and Future Prospective

This review revealed that uses of PGPRs become an integral component in the modern bioremediation technique of heavy metals and subsequently describe the scientific gradual progress in the application of this procedure. There are various advance types of bioremediation approaches that has been started to implement in worldwide to mitigate the hazardous effect of heavy metals on environment. Though, it is well established that combining application of PGPRs and plants is the best approach for metal contaminants bioremediation due to a healthy synergistic interconnection between microbes and plants. The details molecular mechanism of such synergistic interaction is yet to be explored more vividly. Although few limited researches have revealed roles of metal transporter genes, regulatory genes and transcription factors in some microbial assisted phytoremediation technique, but in future, there is need for further details study on the regulation process in miRNA and siRNA level, which may provide us the concrete picture of multicomponent system of PGPR assisted phytoremediation. The focus should be given on the application of genetically engineered microorganism on the demand of plants type and the type of metal contaminant in different medium and also carefully visualize any negative impact of the engineered microbes on the ecosystem.

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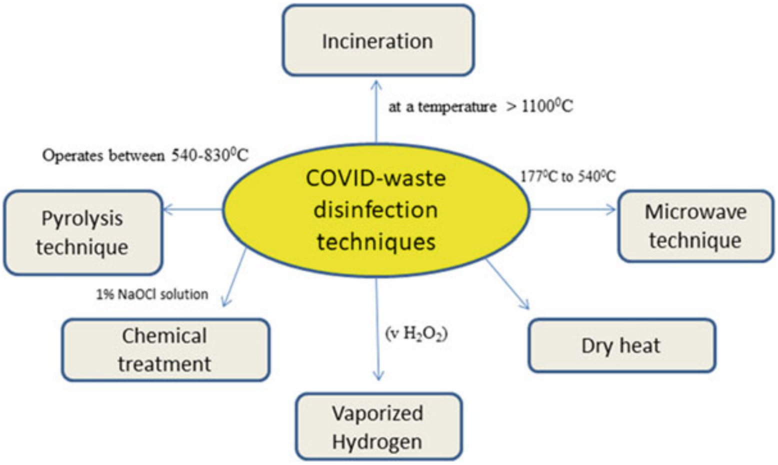
Decontamination Strategies and Technologies for Tackling COVID-19 Hospitals and Related Biomedical Waste

Rishav Sharma, Pinakiranjan Chakraborty, and Shraman Roy Barman

Abstract



The complexities of waste management have been enhanced by the arrival of the novel coronavirus disease-2019. Since the outbreak of COVID-19, biomedical waste (BMW) is being generating in huge amount worldwide by the isolation ward, institutional quarantine centres, COVID testing facilities and even household quarantine. The major contributors to the waste volume include personal protective equipment (PPE), testing kits, surgical facemasks and nitrile gloves. Discharge of new category of BMW (COVID-waste) is of great global concern to public health and environmental sustainability if handled inappropriately. It has been established that COVID

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virus survives up to 7 days on waste like facemasks, which may cause exponential spreading of this fatal disease with the waste acing as a vector. As vaccines were not prepared at early phase, to lower the threat of pandemic spread, many protective gears have been used and discarded. Thus, for sustainable environmental management, addressing the issue of proper disposal of COVID-19 related waste is an immediate requirement. Henceforth, in the present article, disinfectant technologies for handling COVID-waste from its separate disposal to collection to various physical and chemical treatment steps have been reviewed. Furthermore, policy briefs on the global initiatives for COVID-waste management has been including. The application of different disinfection techniques has also been discussed with some potential examples effectively applied to reduce both health and environmental risks.

Keywords Biomedical waste · Biomedical waste management · COVID-19 · Infectious waste · Waste treatment

1 Intrduction

Biomedical waste (BMW) is the by-product of the process including sampling, testing, diagnosis, treatment, vaccination and surgical treatment of humans, animals, and in research (Quadar et al. 2018). They are a group of hazardous waste that can spread infectious disease if not handled and treated properly (Shammi et al. 2021). The management of BMW is of prime importance to lower public health hazard especially to sanitation and healthcare personae (Chakraborty and Maity 2020). The overall wellbeing of a society depends on efficient BMW management and this need has prompted various legislation and planning (Singh et al. 2018).

Source segregation has undoubtedly been an indispensable step in managing any type of solid waste (Saraiva et al. 2018). Common Biomedical Waste Treatment Facility (CBWTF) is another vital course of action towards sustainable BMW management (Devi et al. 2019; Shah 2020). The treated waste is utilized in landfill sites and recycled accordingly (Arun and Priya 2020). The concern of medical administrators regarding handling of this group of waste has now turn into a statuary necessity with the affirmation of the India governance. The biomedical waste that are produced from medical care units' inhabitancies, specialization unit of medical care, reusable things and their proportion being used, infrastructure and resources and their accessibility (Dutta et al. 2018).

For execution of industrial plan for medical waste several trial projects have been done to improve the segregation of hazardous as well as non-hazardous waste that can be cause decrease of waste that are hazardous in this sector (Dahchour et al. 2020). Different kind of containers (for clinical hazardous wastes) disposal have been used by different workers of the hospitals to arrange off different kinds of waste that are harmless (Chakraborty and Maity 2020). The biomedical waste management concerned has now extended and turned into a humanitarian topic around the world human and animal health along with the environment are affected by the hazardous and poor management of biomedical waste which turned into a concerning matter. The topic of Biomedical Waste Management and its maintenance has been gaining expanding importance for as far back as couple of years and uniquely in this situation of worldwide pandemic Covid-19 (Yousefi et al. 2021).

1.1 Definition

Biomedical waste (BMW) is defined as any waste, generated during the discovery, treatment or vaccination of humans or animals, or in related research activities, or in the production of biological experiments and animal waste in slaughterhouses or other similar construction (Rules for -Biomedical waste (Management and Handling) Rules 1998 India).

Biomedical waste Management (BMW) has kept on being a cause of arising toxins essentially brought about by health care practices like medical diagnostics, therapy and immunization, and/or biological research in animals (Datta et al. 2018). Drug withdrawal of patients where the dynamic part of the medication and metabolite, substance and drug deposits, similar media containing iodine, etc. As per the World Health Organisation, healthcare-waste 2018 around 85% of BMW's total volume is viewed as non-hazardous waste, and the remaining volume falls under hazardous waste. Improper disposal of hazardous biomedical waste (HBMW) poses significant risks to public and environmental wellbeing as it goes about as a variation of pathogenic microorganisms. Bacteria organisms present in HBMW if not treated appropriately can enter into human's body through scratching, scratching or cutting on the skin, mucous layers, smell, and sting. Respiratory contaminations, gastrointestinal diseases, skin contaminations, fever, microscopic organisms, viral hepatitis, flu are probably the most widely recognized contaminations carried almost by HBMW openness (WHO 2020). The most common instance of biomedical/solid waste collectors can be traced directly to pathogens in contaminated waste revealed in study directed in Brazil, Greece, India (Singh et al. 2020). Consequently, the actual administration of HBMW is vital to control the transmission of disease. In this context, the World Health Organisation (WHO) has set key focuses for June 2017 by accentuating the speculation of proper assets and a total obligation to lessening wellbeing dangers and contamination (Datta et al. 2018).

1.2 Biomedical-Waste Classifications

There are various categories of Biomedical Waste, some of which are important and relevant. According to the World Health Organization (WHO), natural waste can be divided into eight categories, each indicating a different risk of diffusion of contagious diseases or adverse health effects due to human contact with this waste.

- **General Waste**: General waste represents less or less hazardous to human health as it is mainly composed of household or household waste i.e., kitchens, packaging materials, laundry and other non-communicable materials that do not require special treatment.
- **Disease waste**: Disease waste comprises tissues, organs, organs, human embryos and animal corpses; and especially blood and body fluids. Apart from the infectious nature of this waste, its proper disposal is necessary for ethical reasons.
- **Radiation**: Radiation waste comprises solid, liquid and gaseous pollutants infested with radionuclides produced by in vitro study of body tissues and fluids, in vivo body organ imaging and tumour localization processes, as well as procedures. Its significance lies in the statistics that several nuclear accidents resulting from improper disposal of nuclear material are known to have occurred, with a large number of people suffering from the effects of exposure.
- **Chemical Management**: Chemical waste comprises of solid, liquid and airborne disposable chemicals, from diagnostic and testing, cleaning, housekeeping and disinfection work. These wastes can be either harmful or not.

  (i) It is toxic,
  (ii) Disabling (acids with pH 2.0 and a base pH 12.0),
  (iii) It burns,
  (iv) It works (explosive, water works, scared),
  (v) Genotoxic (carcinogenic, mutagenic, teratogenic or else) capable of altering materials, eg, cytotoxic drugs, non-hazardous chemical waste contains chemicals different from those described above eg, sugar, amino acids, certain salts of organic and inorganic substances.

- **Infectious Waste**: Infectious waste is that waste which contains germs with sufficient density or mass exposure to it can cause disease. This category of cultures and stocks of contagious substances from workshops, waste from surgical operations and autopsy, wastes from an infective patient, segregated wards, wastes connected to an infected patient using haemodialysis (eg, gloves and lab coats); wastes that has been in interaction with animals that have been injected with an infectious agent or that are suffering from transmittable diseases.
- **Sharps**: Sharps is a term used to describe waste, which can cause the user to cut, cut or pierce the skin. This category includes needles, syringes, saws, blades, broken glass (slides), nails, etc. which can cause cuts or piercings.
- **Medical Handling**: Medicinal waste includes pharmaceutical products, drugs and chemicals recovered from wards, disposed of, expired (expired) or soiled, or must be discarded as they are no longer needed.
- **Pressure containers**: Pressure containers include those used for display or teaching purposes, containing hazardous or intrusive gas, and aerosol cans that may explode or explode accidentally.

1.3 Health Care Institutions Generating Waste

World Health organization convened a working group at Bergen in 1983 which has identified the following types of health care institutions as generating biomedical wastes (WHO, Bergen 1983).

Primary sources

i. Hospitals
ii. Nursing home
iii. Dispensaries
iv. Maternity home
v. Dialysis centre
vi. Research laboratories
vii. Medical colleges
viii. Immunization centre
ix. Nursing home
x. Blood bank
xi. Industries.

Secondary sources

i. Clinic
ii. Ambulance services
iii. Home treatment
iv. Slaughterhouse
v. Funeral service
vi. Educational institute.

1.4 Categories of Biomedical Waste

According to Biomedical Waste Management and handling rule, 1998, there were 10 categories, but in 2011 Amendment comes with 8 categories (Table 1).

1.5 Covid-19 Pandemic and Increase in Biomedical Waste

A novel virus, similar to Severe Acute Respiratory Syndrome known as SARS-Cov-2 and later called COVID-19 by World Health Organisation (WHO), was discovered in Hubei province in Wuhan, China, in December 2019. SARS-CoV-2 is a single-line RNA virus with virion sizes ranging from 50 to 200 nm (He et al. 2020). SARS-CoV-2 has a 10 to 20-fold higher binding compound than previous respiratory syndromes (Chan et al. 2020). The rapid spread of SARS CoV-2 to human populations may be due to high cell-ACE2 receptor cell exposure (Wan et al. 2020).

Table 1 Categories of Biomedical wastes (Ministry of Environment, Forest and Climate Change Government of India 2016)

Waste category	Waste class and description
Category No. 1	Human Anatomical Wastes
Category No. 2	Animal Wastes Animal flesh, organs, corpses, bleeding parts, fluid, blood and trial animals used in research, waste generated by veterinary hospitals, colleges, discharge from hospitals, animal houses
Category No. 3	Microbiology and Biotechnology Waste., Wastes from research laboratory culture, stocks or specimens of micro-organisms, live or attenuated vaccines, human and animal cell culture used in research and infectious agents from research and infectious agents from research and industrial laboratories, wastes from production of biologicals, toxins, dishes and equipment's used for transfer of cultures
Category No. 4	Waste Sharps Needles, syringes, scalpels, blades, glass etc. that are capable of causing puncture and cuts. This includes both used and unused sharps
Category No. 5	Rejected Medicine and Cytotoxic Drugs
Category No. 6	Soiled Wastes Items contaminated with blood and body fluids including cotton, dressings, soiled plaster casts, beddings and other material contaminated with blood
Category No. 7	Infectious Solid Wastes Wastes generated from disposable items other than the waste sharps, such as tubing, catheters, intra venous sets etc
Category No. 8	Chemical Wastes Chemical used in production of biologicals, chemicals used in disinfection, as insecticides etc

COVID-19 transmission was detected through human contact shortly after a pneumonia outbreak in Wuhan (Hubei province, China), and the disease was proclaimed a global pandemic by the WHO. COVID-19 becomes a global concern, to date (01/09/2020) affecting 213 countries and territories and there are 30,350,821 Covid-19 cases with more than 900,000 registered people worldwide. With the exception of the income group (low, medium and high income), the COVID-19 epidemic has highlighted several shortcomings in the socio-economic, health, and environmental sectors of the world (Owusu et al. 2020). To date, no drugs or vaccines have been registered to treat COVID-19 patients directly. Mass sampling in rapid trials, segregation of suspects/patient use of preventive measures, social retardation and health-supporting treatments are well-known ways to combat/prevent this deadly epidemic. According to the WHO, COVID-19 virus is transmitting mainly through saliva drops or from the nose and mouth when an infected person coughs or sneezes (Otter et al. 2020). After these droplets fall into items and the regions surrounding a person, some persons become infected when they touch their eyes, nose, or mouth after coming into contact with contaminated regions. As a result, the WHO and the Ministry of

Health and Family Welfare of the Government of India recommended that individuals and paramedics (doctors, nurses, cleaners, and police officers) should wear personal protective equipment (PPE), surgical (protective) equipment, aprons/gowns, and nitrile gloves when exposed to germs and pollution (Singh et al. 2020).

In addition to guidelines on the rational usage of COVID-19 protective gears, healthcare sectors are facing greater demand for PPE across all levels of health workers for fear of infection. Fear often leads to the misuse of PPE which often exacerbates the problem by producing large quantities of BMWs that are difficult to maintain and transport with limited resources and staff available during a disaster (Garg et al. 2020). The amount of biomedical waste (BMW) produced by COVID-19 patients is steadily growing. Due to the stockpile of gloves, gowns, masks and other protective equipment, there appears to be an emergency waste due to the unusual form of families and health facilities (Ma et al. 2020). Also, with increasing number of tests for COVID-19, and widespread vaccination drives, infectious waste are further increasing with time. India produces approx. 2,00,000 tons of biomedical waste (BMW) per year are treated by 198 Common Biomedical Waste Treatment Facilities (CBMWs) and 225 burn captives, so the daily generation of BMW is imminent. 550 tons. It is estimated that hospitals produced six times as much medical waste during the COVID-19 outbreak.

COVID waste can be controlled by regulation but there are other concerns that the disease is spreading beyond hospitals. Some people with minor symptoms have asymptomatic, who may not know that the waste they dispose of can be contaminated. This means that people are more likely to produce more contaminated waste and that this will contaminate sanitation workers as the virus is said to persist for days on cardboard, metal and plastic (Ranjan et al. 2020). The enormous growth in the number of persons infected with COVID-19 has demonstrated that the world will be inundated by COVID waste in the future years, and the impact of this glut will have a profound influence on the on-going waste process (Cutler 2020).

SARS-CoV-2 can survive in COVID-waste like needles and syringes used to draw blood samples, surgical masks, and personal protective equipment (PPE) kits. Exposure to COVID-Waste has the potential to boost the virus's transmission by raising the birth rate (R0) from its predetermined range of 2.2–3.58. (Li et al. 2020; Zhao et al. 2020). Without antibiotics, improper discharge of COVID-Waste can put ordinary people and health personnel at danger of infection. To limit the spread of the infection, proper COVID-Waste management, including suitable disinfection and disposal procedures, is required.

2 Biomedical Waste Treatment Methodologies

Phases in Waste Management for the effective management of biological wastes, various steps must be followed from collection through disposal, which are summarised below.

2.1 Waste Segregation

Segregation is the main factor in a waste management framework. Reliant upon the treatment alternative and the elimination of different waste things, compartments of specific tones should be isolated and put away in a brief stockpiling region for removal inside 48 h. Trash that should be covered further should be gathered in a plastic bag or yellow bin. Waste should be collected in a red or blue container or bag for autoclaving, microwaving, chemical treatment, and finally disposal or recycling. Trash used to sterilise and destroy or cut off needles, sharp edges, and other sharp objects should be gathered in a white container that will be inserted or thrown for reuse as a final disposal. Chemical waste (strong), expired pharmaceuticals, and cytotoxic drugs that need to be stored safely should be gathered in a black container and labelled as such. Other than a black cabin or bag in which a cytotoxic label should be marked, all containers and bins should have biohazard labels.

This steps also includes waste the board of different sorts for a few recycling bins (reduce, recycling and minimization). Reuse of chemicals, medical equipment etc. Recycling certain items such as sterile and shredded plastic helps the second industry to reduce waste production which decreases the price of waste disposal. The transmission of infection is detected by segregation thus decreasing the chances of infection by health care workers. Scratching or piercing damage that causes waste needs to be discarded as "sharp" and should be separated from other waste. A mixture of sharp metals and broken mirrors is allowed but not with uncomplicated debris. Blending of glass or plastic waste containing flammable waste, organic waste or other laboratory waste should avoided.

2.2 Waste Storage

Waste must be stored as required in according with the Biomedical Waste (Management and Handling) Rules, 1988. Landfill sites takes place between the land disposals. Before safe disposal natural waste can be temporarily sealed under the refrigerator without creating a problem with nature. A storage area is located near the garbage dumps. There should no drain pipes containing the spill and must be reverted to the liquid storage elevator. Stiffness of the liquid is required on the floor and walls, in simple compliance with cleaning procedures. Regular disinfection is mandatory. Refrigerator is required to have rot and other contaminants alive for a period. There must be a post in the last place displaying signs of 'EXPLICIT' (Da Silva et al. 2005).

2.3 Containers and Their Label

Non-invasive containers must be used in conjunction with good labelling and integrity, as long as the treatment is performed chemically and physically. The bio hazardous material content should be properly closed. The usage of leaking containers that are strong against heat and chemical treatments ought to be used chemically. Sturdy containers that are resistant to piercing and not tightly closed should be used to sharpen the metal for proper disposal and should be able to withstand a pressure of 40 psi without cracking. For non-hazardous materials heavy plastic bags or other such containers should be used in conjunction with the Biohazard logo. Biohazard bags in red or orange should not be used for non-hazardous materials. Sturdy and perforated containers would be used for Pasteur pipes and broken glass (plastic, heavy cardboard or metal) and sealing should be done in "Biohazard bags" made of heavy plastic and these are easily accessible. No label is required unless there is a possibility of waste recycling and in such cases the container should be marked 'Do not recycle'. Bags should not used for open injections or other forms of injection. Each bag or container should have a label based on adherence to the generator information included in the bags containing medical waste. Construction services should provide special labels with location for recording dates and contact persons and those labels should be used in all containers packaged inside medical trash cans. A clear diagnosis of all containers of untreated bio-hazardous waste and their appropriate label and biohazard label should be made.

2.4 Organic Waste Management

Organic waste should be collected and transported in such a way that it poses no damage to human health or the environment. Only highly qualified technical employees should manage or dispose of bio hazardous material that remains unprocessed. Following the manufacturing of rubbish separators in containers or bags designated in a bright colour should be done as soon as possible. During the treatment of this waste, it is vital to reduce the risk of needle wounds and infection. Biomedical waste must not be combined with any other sort of garbage. If biological waste is left unprocessed on site, it must be transferred from the manufacturing plant to a treatment and disposal facility.

The following points need to be follow for the transportation of BMW:

1. The carrier must be provided with separate chambers and containers of natural waste.
2. Ensuring the foundation of the recyclable quality waste disposal cabinet.
3. The waste disposal room should be designed in such, that it can be simply kills the germs, and help to keep containers.

2.5 Treatment and Disposal Method

The basic criterion governing the disposal of anatomical wastes is that mutilation or shredding must be capable of preventing unauthorised recycling. Chemical treatment with 1% hypochlorite is recommended in the basic form. On the other hand, there is no pre–treatment in the incineration process. The process of deep burial is only required in cities with populations of less than five lakhs. Waste management should also be performed as close to the site of origin as possible. With all of these considerations in mind, the treatment and disposal of various types of wastes must be carried out with caution and precision.

1. Animal carcasses and body parts: Incineration, biodegradation or landfill.
2. Solid waste (bedding, manure, etc.,) from animals:

   i. Animal waste (Biohazards): Thermal or chemical treatment for incineration and disinfection.
   ii. Animal waste (Non–hazardous): Using as compost or fertilizer.

3. Chemical waste: It should be disinfected using a 1% sodium hypochlorite solution or another chemical agent of the same type. Following the treatment of fluids and the acquisition of landfills for solids, sewage discharge is required.
4. Materials containing recombinant DNA or organism that have been genetically altered must be disposed of according to National Institutes of Health (NIH) rules.
5. Human pathological waste:

   i. Cremation or burial for a deceased corpse with identifiable body parts.
   ii. Incineration or disinfection for disposal of other solids.
   iii. Body fluids—thermal or chemical disinfection before to discharge into the drain system.

6. Metal sharps: To avoid laboratory, storage, and landfill worker injuries, metal sharps should be disposed together with encapsulation. After cleaning or capping in the original container, needles, knives, and other sharp objects pose a biohazard. If autoclaving is required, an autoclave marking tape strip should be placed in the container prior to the autoclaving process. Gas chromatography needles should be rinsed to remove chemicals (hazardous) and disposed of with broken glassware (non–contaminated).
7. Microbiological waste: It must be thermally or chemically processed before being discharged into the sewage system.
8. Non–hazardous biological waste:

   i. Even if the materials are non-hazardous, all microbiological components must be autoclaved or thermally treated for improved laboratory procedures.
   ii. Solid–solid waste should be placed in trash dumpsters.
   iii. Liquid–liquid pollutant should discharge into large sewer system directly.

9. Plastic waste, Pasteur pipets; glassware (broken): If the equipment's got contaminated by biohazards materials, then it should be Sterilized thermally or chemically or encapsulation. And in case of non-contaminated wastes, they should be trash in dumpster. And plastic and glassware should always be incinerated (Ravi Kant et al. 2002).

Schedule I of Biomedical Waste (Rule 6) (Table 2).

Schedule II of Biomedical Waste (Rule 6) (Table 3).

According to Biomedical waste management and handling rule, 2016, MoEFCC. Government of India:

Table 2 The classification of biomedical wastes, as well as their treatment and disposal (Rule 6, Biomedical waste management and handling rule, 2016., Ministry of environment, forest and climate change, Government of India)

Waste category no	Waste category (type)	Treatment and disposal optiont
Category No. 1	Waste from the human body (human tissues, organs, body parts)	Incineration**/deep burial*
Category No. 2	Waste from animals (waste of veterinary clinics and practical's)	Incineration**/deep burial*
Category No. 3	Microbiology and Biotechnology waste and extra laboratory wastes	Disinfection at the site of origin by chemical treatment**, autoclaving, or microwaving, followed by mutilation/shredding***, and ultimate disposal in secure landfill or recycling through authorized recyclers
Category No. 4	Waste sharps (needles, scalpel, scissors etc.)	Chemical treatment** or needle and tip cutter distribution, autoclaving or microwaving, followed by mutilation or shredding***
Category No. 5	Rejected medicines and cytotoxic drugs	Disposal is seemed landfill and Incineration**
Category No. 6	Soiled waste	Incineration**
Category No. 7	Contagious solid waste (disposal materials other than waste sharps) (IV tubes, oxygen masks, saline bottle, surgical gloves)	Chemical treatment**, autoclaving, or microwaving, followed by mutilation and shredding***
Category No. 8	Chemical waste (chemical access in making medicines)	Chemical treatment** then release it into drains

**Chemical treatment with a 1% hypo chloride solution or any other chemical reagent that is similar. It is critical to guarantee that treatment results in disinfection

***Mutilation or shredding should be done in such a way that unauthorised re-use is prevented

**Before incineration, there is no chemical pre-treatment. Incineration of chlorinated plastics is prohibited

*Only municipalities with populations of less than five lakhs and rural regions would have the option of deep burial

Table 3 Colour coding and type of bins for discarding of Biomedical Waste (Rule-6, Biomedical waste management and handling rule, 2016., Ministry of environment, forest and climate change (MoEFCC), Government of India)

Colour coding	Type of container	Waste category no
Yellow	Non-Chlorinated plastic bags	Category NO.1, 2, 5, 6
Red	Non-Chlorinated puncture proof plastic bags	Category No. 3, 4, 7
Blue	Non-Chlorinated plastic bags	Category No. 8
Black	Non-Chlorinated plastic bags	Municipal Solid Waste

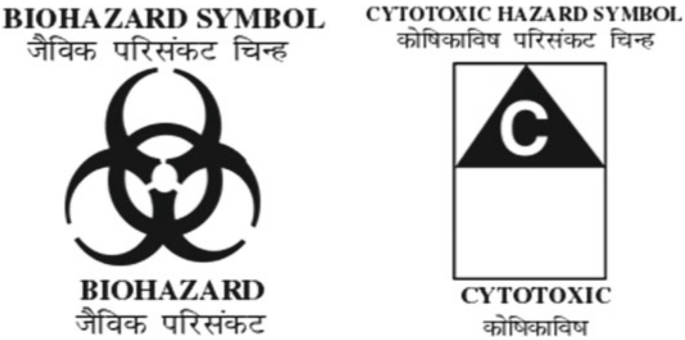

Fig. 1 Label for Biomedical Waste Containers/Bags (Rule 6, Biomedical waste management and handling rule, 2016, Ministry of environment, forest and climate change, Government of India)

1. Depending on the treatment option chosen, which must be as stated in Schedule I, colour coding of waste categories with numerous treatment choices as established in Schedule I shall be selected.
2. Chlorinated plastics should not be used in the trash collecting bags for incineration waste.

Schedule III of Biomedical Waste (Rule 6) (Fig. 1).

3 Disinfection and Reprocessing Techniques of Covid-19 Waste

3.1 Disinfection Using Incineration

This is described as a thermal process that requires a high temperature under regulated waste burning circumstances in order to convert waste into non-reactive products and gases. Worldwide, three types of incinerators are utilised to dispose of biomedical waste: multiple hearth types, rotary kilns, and air types (controlled). Each main and secondary combustion chamber is available in three different configurations, providing optimal combustion (Chan et al. 2020). It is a frequently utilised disposal method that is safe, simple, and effective, particularly in industrialised countries (Ghodrat et al. 2018). The incinerator's temperature must be more than 1000 °C. This high temperature could not only entirely kill microorganisms, but also incinerate and not only burn the greatest amount of microorganisms, but also incinerate and burn the majority of organic matter, converting it to inorganic dusts. Once the material is incinerated, the amount of waste produced might be decreased by 80–85% (Wang et al. 2020). Other biomedical wastes, aside from radioactive and explosive wastes, can be incinerated.

The majority of COVID waste is transported to incinerate at a temperature > 11000C, according to BGL private limited (a typical biomedical waste treatment facility recognised by Jharkhand Pollution Control Board, India). Depending on the volume reduction of COVID waste, the leftover mass is sometimes re-incinerated with fresh charge. However, pollutants such as product of incomplete combustion (PIC) and dioxins are created during the process (Singh et al. 2020). Waste components dissolve and recombine during incineration and post-combustion cooling, generating new hazardous particles known as PIC (Vilavert et al. 2020). Dioxins are an accidental by-product of trash combustion that occurs in incinerators. This is a collection of 75 chemicals that live alongside another group of poisons known as furans. Toxins have a proclivity for accumulating in fatty tissues and travelling up the food chain. The most significant dioxin producer in the environment is the burning of medical equipment manufactured of Polyvinyl Chloride (PVC). Furthermore, metals found in medical waste act as a catalyst for the synthesis of dioxins. These are very dangerous, having been identified as carcinogenic, and cause harm to the human immune and nervous systems. In January 2017, recognising the importance of dioxins in the environment, the Council of Scientific and Industrial Analysis and the National Institute for Interdisciplinary Science and Technology launched a cooperative research to investigate the presence of dioxins in Thiruvananthapuram (Subramaniam et al. 2020). Furthermore, incinerator ash is dangerous and must be tested for toxin levels before being transferred to a secure landfill. As a result, most nations are turning to alternate ecologically acceptable BMW disposal procedures, taking these factors into account.

3.2 Disinfection Using Alternative Thermal Techniques

To deal with COVID-waste, there are primarily two types of thermal technologies available and in use: (i) high temperature pyrolysis and (ii) medium temperature microwave technology.

(i) **High temperature pyrolysis technique**

Pyrolysis, also known as controlled air incineration or double-chamber incineration, is the most reliable and often used treatment process for health-care waste. In comparison to incineration, pyrolysis is a more technically sound technique. It has a pyrolytic chamber and a post-combustion chamber, and it performs the following activities (WHO, biomedical waste treatment):

- The wastes were thermally destroyed in a pyrolytic chamber, creating solid ashes and gases through an oxygen-deficient, medium-temperature burning process (500–850 °C). A fuel burner is included in the pyrolytic chamber, which is used to initiate the process. The garbage is deposited in a dumpster or a rubbish container.
- In the post-combustion chamber, gases generated by a fuel burner are burnt at high temperatures (900–1200 °C) with an abundance of air to reduce smoke and smells.

Plasma pyrolysis technique is state–of–the–art for medical waste disposal. It's an environmentally beneficial technique that converts organic waste into economically useful byproducts. Disposal of solid wastes, such as biomedical and hazardous wastes, produces a lot of heat (Surjit et al. 2020). It has pyrolysis-oxidation, plasma transformation, induction-based transformation, and laser-based transformation and operates at a temperature range of 540–8300 C (Datta et al. 2018). The air measured below the theoretical chemical reaction is equipped to a fixed level of the first chamber in pyrolysis-oxidation The organic solid and liquid wastes are vapourised at a temperature of 600 °C in the presence of air turbulence, leaving ash, glass, and metallic pieces behind. The flammable gaseous vapour is combusted at high temperatures between 982° to 1093 °C in the second phase of combustion, completely destroying harmful substances such as dioxins and generating clean exhaust steam. Given the fast spread of SARS-CoV-2, it is advised that plasma energy be used to quickly decompose COVID-waste rather than the typical laser/gaseous combustion (Wang et al. 2020). This process produces a low emission rate, an inert residue, a volume reduction of up to 95%, and a mass reduction of up to 90% (Ilyas et al. 2020). To get plasma energy, plasma pyrolysis uses plasma torches. The ionising gas will carry electrical current in the plasma form, however the electrical energy will be born-again to energy due to its high resistance. Carbon, black, vitrified glass aggregates, and metallic residues are among the residues produced. Infectious waste, sharps, plastics, dialysis waste, hazardous trash, chemotherapeutical waste, chemotherapy waste, and low-level radioactive waste are all often eliminated in plasma-based technologies (with the exception of mercury, which plasma systems do not handle) (Datta et al. 2020) (Fig. 2).

(ii) **Medium temperature microwave technique**

Microwaves are electromagnetic waves with wavelengths ranging from 1 to 1000 mm and frequencies ranging from hundreds to 3000 MHz. 2450 ± 50 MHz and 915 ± 25 MHz are the most common microwave frequencies used for sterilisation. Microwaves move through the medium and are absorbed by it, causing heat to be generated. The heat was created by the material molecule vibrating and rubbing billions of times per second, creating the effect of high temperature disinfection.

Fig. 2 From BMW/hospital waste creation through disinfection and disposal processes, here's a schematic. Adopted from Ilyas et al. (2020)

When disinfected, this method is known for its energy efficiency, low action temperature, slow heat loss, quick action, light damage, and minimal environmental pollution, with no residues or harmful waste product. It has a broad bacterial disinfection spectrum and may destroy a variety of pathogens. In terms of pathogen destruction, there is now strong confirmation that specifically designed microwave systems may effectively kill inert bacteria; nevertheless, the process must be closely regulated by particular microwave equipment. Though certain improved microwave technologies with precise measurements are now employed primarily for the treatment of biohazardous wastes, it must be determined if they would be beneficial for methods requiring the management of water content, such as drying bio-therapeutic products.

This technique works at temperatures ranging from 177° to 540 °C and comprises reverse polymerisation using high-energy microwaves in an inert environment to break down organic materials. As a result of the vibration and rubbing of molecules caused by the absorption of electromagnetic waves (with a wavelength of 1 mm to 1 m and a frequency of several hundreds of megahertz to 3000 MHz). Although an inert atmosphere created by nitrogen prevents combustion with oxygen, high-temperature disinfection is demonstrated. Microwave technology has a number of advantages, including lower energy and action temperatures, less heat loss, and less environmental load with no hazardous residue after disinfecting. SARS-CoV-2 will be rendered inactive by specifically constructed microwave devices used in a tightly regulated manner. According to a report by the Chinese Ministry of Ecology and Environment, this disinfection technology may achieve logarithmic values of killing hydrophilic viruses (Wang et al. 2020) and is recognised to be highly effective for COVID-waste transportation on-site disinfection that saves time (Resilient Environmental Solutions

2020). In the instance of COVID-waste disinfection, the microwave approach is employed in conjunction with autoclaving where steam is used for sterilisation (in temperature vary from 93 to 177 °C).

3.3 Chemical Disinfection Technique

Chemical disinfection, which is often used in health care to kill bacteria on medical equipment, floors, and walls, is now being extended to the treatment of medical waste. Chemicals applied to pathogens deactivate or kill them, thus this treatment results in disinfection rather than sterilisation. This method is effective for dealing with liquid wastes such as blood, urine, faeces, and hospital sewage. Although solid and even severely hazardous health-care wastes, as well as microbiological cultures, sharps, and other items, may be chemically disinfected (WHO, medicine waste management 2018). Chemical disinfection of human waste and animal carcasses is not recommended. If no other options for disposal are available, they will be shredded and subsequently subjected to chemical disinfection (B.B.R Panel 2012). Chemical disinfectants should be disposed of in the proper manner. And, if the procedures are not correctly implemented, they will result in serious environmental issues.

Chemical disinfection is commonly used to pre-treat COVID waste after it has been mechanically shred. To prevent the generation of aerosols during shredding, the exhaust air is routed through a high efficiency particle absolute filter. The bulk of the crushed trash is combined with chemical disinfectants and maintained in a closed system or under negative pressure for a set period of time. The contagious germs are destroyed or inactivated by the orezarganic degraded material in this procedure. Chemical disinfectants have several advantages, including low effective concentrations, steady performance, quick action, and a broad sterilising spectrum, as well as minimal residual risks. They not only successfully kill germs but also render bacterial spores inert (Wang et al. 2020). COVID-waste treatment is frequently separated into chlorine and non-chlorine-based systems. The disinfection medium in a chlorine-based treatment system is NaOCl or ClO_2, where the tendency of chlorine aids in oxidising peptide linkages and denaturing proteins that follow penetration of cell layers even at neutral pH. Although NaOCl is one of the principal chemical disinfectants that releases halo acetic acid, dioxins, and chlorinated biocide, it is only employed on-site due to its unstable nature. It also decomposes to produce salt and less-toxic compounds that aren't affected by alcohol or ammonia (Ilyas et al. 2020; Shah 2021a, b). On the other hand, in non-chlorine-based treatment systems, H_2O_2 is commonly employed as a disinfection medium. It oxidises and denatures proteins and lipids, resulting in membrane disorganisation due to the inflammation of saturated H+-ions. This approach is favourable because of its high reactivity and lack of toxicity associated with chlorinated systems.

3.4 Disinfection Technique for Processing of Personal Protective

Surprisingly, the potential application of disinfection technology will not be restricted to the degree of safety; rather, due to the global scarcity of personal protective equipment, its value will be huge (Mallapur 2020; Singh et al. 2020). Gloves, aprons, long-sleeved gloves, goggles, fluid repellents, eyes, nose, and mouth protection, face visor, and breathing mask are among the COVID-19 pandemic protective equipment. As a result, several governments are pursuing unplanned ways to recycle discarded personal protective equipment, despite the severe health hazards involved with incorrect decontamination (Barcelo 2020). There has been some attention on airborne particles emitted by COVID-19 infected patients' breathing or sneezing, which may travel several metres, remain suspended for approximately 30 min, and survive on the environment for many days. Because the coronavirus does not puncture materials, disinfection or PPE sterilisation of the surface or contact area will be sufficient (Rowan and Laffy 2020). The most difficult task, however, is the re-use of personal protective equipment that is employed at the same time as ensuring materials are functional after treatment. As a result, in addition to the replacement of personal protective equipment, an effective disinfection method is also necessary. The above-mentioned disinfection temperature techniques are not acceptable due to thermal-sensitive qualities, resulting in reprocessing; nevertheless, the chemical disinfectant spray is found to damage personal protective equipment (Rowan and Laffey 2020). The use of vaporised hydrogen peroxide (vH_2O_2) instead of an aqueous disinfectant solution has demonstrated encouraging results in the sterilisation of bacteria, prion, and viruses (Barcelo 2020). The key benefit of low temperature vH_2O_2 is polymetric significant compatibility, while processing time is reduced (from 10 to 15 h using ethylene oxide to less than six hours in the conventional vH_2O_2 process) under both atmospheric and vacuum settings. However, due to the reduction in H_2O_2 energy in the presence of cellulose, compatibility with cellulose-based compounds and the capacity to penetrate into specific regions are reduced, limiting the application of vH_2O_2 (McEvoy and Rowan. 2019). The N95 mask was recently disinfected using two methods: I dry heat (using hot air at 750 C for 30 min) and (ii) ultraviolet germicidal irradiation (UVGI, at 254 nm and 8 W for 30 min) (Price et al. 2020). Several studies have found that hot air treated N95 masks used for more than 5 cycles do not degrade mask fit (change equally, 1.5%; p-value, 0.67), whereas UVGI treated N95 masks used for more than 10 cycles significantly degrade mask fit and do not pass quantitative fit testing in a human model (change in fit factor, −77.4%; p-value, 0.0002). However, even if decontamination applies to all layers of the trapped virus I particles, there remain outstanding questions that must be addressed before COVID waste may be reprocessed.

3.5 Common Safety Measures on Disinfection Process

COVID-waste has the potential to transmit hospital-acquired infections. However, as part of overall COVID-waste management, certain safety issues must be adhered to (Yang et al. 2020).

- For household cleaning and disinfection: Use household cleansers and disinfectant containing 2 g/L chlorine for 30 min on regularly handled surfaces such as tables, doorknobs, lights, switches, handles, desks, and toilets. For cleaning and disinfection of electronic gadgets, follow the manufacturer's instructions. When it comes to devices, consider utilising a washable cover. If the manufacturer's instructions are not available, alcohol-based wipes or sprays containing at least 70% alcohol can be used to disinfect the touch screen. To avoid liquid pooling, make sure all surfaces are completely dry.
- Cleaning and disinfection of homes with persons isolated in home care (e.g., COVID-19 suspected or confirmed): COVID-19 symptoms and how to prevent the spread of COVID-19 in the home should be educated and understood by all members of the household. Spray high-touch surfaces four times a day with a disinfectant containing 75% ethyl alcohol to clean and disinfect them (C_2H_5OH). Personal cleaning materials may be provided by the caregiver for a sick person who has been occupied by a kid or another individual for whom such supplies are not acceptable. Tissues, paper towels, cleansers, and disinfectants are among these items. After collecting COVID-waste in double-layered firmly zipped yellow bags, the same cleaning process must be used on the waste released by patients.
- White-coats, N95 facial masks, surgical masks, surgical hats, protective goggles, shoe covers, isolation gowns, gloves, protective suits, and another pair of gloves, protective hoods, and boot covers should all be worn in the following order: white-coats, N95 facial masks, surgical masks, surgical hats, protective goggles, shoe covers, isolation gowns, gloves, protective suits, and another pair of gloves, protective hoods, and boot covers. The main disinfection procedure while removing protective clothing is to spray ethyl alcohol in the buffer room, and then they can be permitted to take off the hood, PPE, goggles, and surgical mask successively in another room. After that, in a semi-contaminated room, remove the isolation gown, surgical cap, N95 face mask, and gloves, as needed. After hand washing and donning a clean-surgical mask, the healthcare worker can be authorised to enter the clean area.

All used objects must be collected as COVID-waste (even after disinfection spraying) and processed for the next level of treatment, such as incineration or microwave (as defined in above section) (Table 4).

Table 4 Each disinfection technology's overall Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis (ACR Plus 2020)

Disinfection technology	Strengths	Weakness	Opportunities	Threats	References
Incineration technique	Simple operation, complete destruction of BMW/COVID-waste	Energy-intensive, high capex, release of toxins and solid residual waste	−90% reduction of waste volume	Release of secondary pollutants like dioxin, furans and bottom ash	Dutta et al. (2018), Wang et al. (2020)
Pyrolysis technique	Complete destruction of toxins like furan and dioxins	High investments costs and strict demand for heat value	Energy saving and complete decomposition of waste volume	Not known and taken as a safe technology	Dutta et al. (2018), Wang et al. (2020)
Microwave technique	Low action temperature conserves energy and reduces pollutant discharge while avoiding gaseous emissions	Because of the rather narrow disinfection spectrum, autoclaving is occasionally required	On-site trash treatment is facilitated by the construction of a transportable microwave treatment unit	Disinfection has a number of complex effect elements	Datta et al. (2018), Wang et al. (2020)
Chemical technique	Stable and rapid performance, as well as a wide range of sterilizing options	BMW's volume and bulk are not reduced	Disinfectants used in-house/on-site have the capacity to kill viral spores, effectively controlling virus transmission	Inhalation of anthropogenic aerosols can reach alveoli, whereas skin absorption of atomized disinfectants promotes cancer	Mallapur (2020), Rowan and Laeffy et al. (2020), Singh et al. (2020)
Vaporized hydrogen	Low-temperature heat-sensitive application	In the presence of cellulose molecules, concentration decreases	After a thorough cleansing, protective objects can be reprocessed and reused	Fogging creates atomized aerosols to inflict serious health harm to alveoli, skins, and mucosa	Barcello (2020), McEvoy and Rowan (2019)
Dry heat technique	Compatibility of polymeric materials with the ability to be reprocessed	How decontamination works across all of the layers of virus trapped in the particles remains unresolved	Reuse of N95 masks and PPE are possible that can mitigate the risk of supply-chain	It's debatable if all layers of trapped virus in particles can be decontaminated	Price et al. (2020)

4 Present Scenario of COVID Waste Management Worldwide

With COVID-19's expanding worldwide reach, the world is confronted with new difficulties that will put leadership and community resolve to the test. Despite the fact that governments accept stay-at-home orders and varied degrees of state crises, households continue to generate garbage. As a result, waste management services must guarantee that urban base services promote residents' health and virus containment to the greatest extent feasible. In this regard, a set of new rules for dealing with COVID-19-related trash has been issued by a number of organisations and governments. The new recommendations are precautionary measures to ensure that no additional health hazards arise as a result of the epidemic. In addition to the standards governing biological waste disposal, these guidelines must be followed. Although it is the responsibility to update this guidance as needed, it is based on existing knowledge and COVID-19 protocols for the management of infectious waste created in hospitals when treating viral and other infectious disease (Fig. 3 and Tables 5, 6).

4.1 On-Site Treatment of COVID-Waste in China

The COVID-19 pandemic and legislative approaches to end the outbreak of the infection have prompted a worldwide monetary downturn and furthermore produced

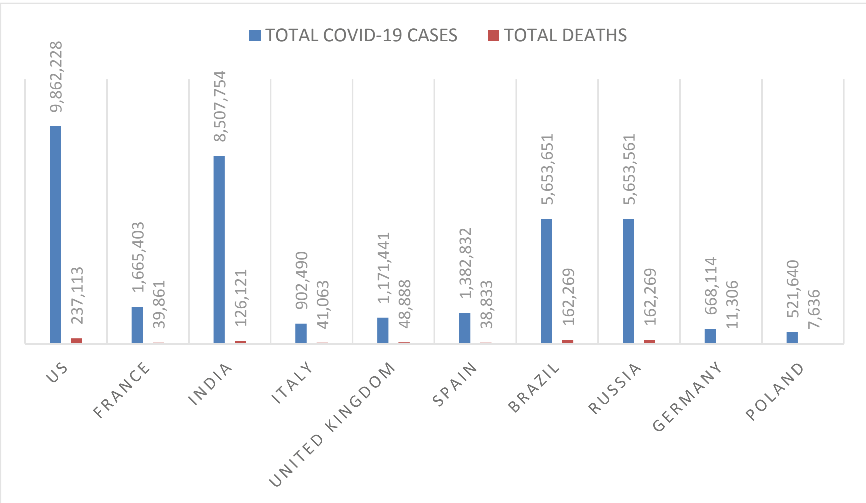

Fig. 3 Graph showing the countries where COVID-19 effected most and number of individuals deceased due to this (Gutierrez and Clarke 2020)

Table 5 Global distribution of confirmed COVID-19(Top 10 countries) (Gutierrez and Clarke 2020)

Country	Total COVID-19 Cases	Total deaths
US	98,62,228	2,37,113
France	16,65,403	39,861
India	85,07,754	1,26,121
Italy	9,02,490	41,063
United Kingdom	11,71,441	48,888
Spain	13,82,832	38,833
Brazil	5,653,651	162,269
Russia	56,53,561	1,62,269
Germany	6,68,114	11,306
Poland	5,21,640	7,636

a lot of clinical waste. This segment was impacted by dispensable plastic-based individual defensive hardware (PPEs) and single-utilized plastics by buying shopping on the web for most essential requirements. PPE during pandemics and the utilization of single-use plastics builds the volume of clinical waste as well as changes the normal thickness of clinical waste (Xuo 2020). Coronavirus is an ecological and general wellbeing risk, particularly among waste creating PPEs and single-use plastics, predominantly in creating economies and experiencing significant change nations. Safe strong waste administration is as of now a significant worry for nations where there is an absence of protected and supportable practices and medical services waste isn't sufficiently controlled (Singh et al. 2020). The current fast development of medical care waste because of the COVD-19 pandemic is worsening the issue and there's an impending risk that the impacts of risky removal of medical care waste will spread to the danger of ecological contamination (Jiangtao and Zheng 2020). Risky removal of medical services waste contaminates the climate, but at the same time is related to the spread of transmittable illnesses similar to hepatitis, HIV/AIDS, cholera, typhoid and respiratory intricacies, fundamentally through the reuse of removal clinical hardware. Given the unexpected ascent in medical services waste age, powerful encounters and exercises of COVID-19-drove waste the board in Wuhan, China, those are possibly useful procedures for some agricultural nations where COVID-19 is as yet growing and influencing a lot of medical services waste age. By and large, dismissed medical care wastes and different types of clinical waste are arranged off in clean land—or burned as waste energy recuperation. Furthermore, wellbeing waste alongside city strong waste is unloaded in the open or in inadequately oversaw land in many agricultural nations—where the development of waste pickers and animals like canines, goats and cows is regularly noticed (Nezedigu and Chang 2020). A few nations have likewise utilized cutting edge innovation to treat their clinical waste by steam-sanitized or compound sanitizers, yet this is extraordinary. Albeit many created nations have exhibited great administration of COVID-19-drove clinical waste administration, China, like other developing economies and countries undergoing major transformation, has demonstrated compelling and successful strategies to combat COVID-19-driven clinical waste administration. More than 30 administrative orders and crisis board orders on ecologically solid administration of clinical waste have been executed in China since 2003, after the outbreak of the Severe Acute Respiratory Syndrome (SARS) in the area (Wee and Wang 2020). Exercises and powerful measures in Wuhan and different districts of China might be important for significant data for some non-industrial nations despite an unforeseen flood in

Table 6 Different countries and agencies have updated their COVID-19-related waste rules. (WHO 2020; EU 2020; SNPA 2020; US OSHA 2020)

Countries/agencies	New guidelines to handle biomedical waste at the time OF COVID-19
WHO	All biomedical waste generated during the care of COVID19 patients should be collected in designated containers and bags, processed, and then securely disposed of or treated, or both, preferably on site, according to the WHO. Waste can be transferred off-site with only suitable treatment and disposal facilities in place. It has also been emphasised that all personnel participating in health care waste management should wear adequate PPE (boots, apron, long-sleeved gown, thick gloves, mask, and goggles or a face shield) and wash their hands after each usage
European Union (EU)	The EU has released the following guidelines/recommendations for household waste management: (EU 2020). Paper tissues and face masks should be thrown away right away in the trash bag that was provided in the patient's room specifically for that reason. A second bag should be kept separate from the first to store the caretaker's and cleaner's gloves and face masks. The collected bag must be kept closed at all times and should never be dumped into another bag. All of these bags should be gathered and kept in a clean garbage bag (doing so makes the waste to be collected in a double-layered bag). If the aforementioned actions are carefully performed, however, these bags can be placed immediately in the unsorted garbage, with no further collection or disposal methods required
ITALY	In Italy, a government agency has attempted to distinguish between two types of municipal garbage created by homeowners. T1: Households with COVID-19 positive persons in isolation or under forced quarantine create municipal garbage. T2: Municipal garbage produced by families that do not have COVID-19 positive persons in isolation or who are required to be quarantined. T1 type waste should be categorised as contagious medical waste and treated as such, in accordance with applicable laws. In most cases, just a few firms deal with this type of garbage, and they collect it in uniform bags before sterilising it. T2 waste, on the other hand, is collected in accordance with the existing separate collection system. Tissues, masks, and single-use gloves should all be part of the residual waste stream, which must be transported in two sealed bags. Personal protective equipment (PPE) should be worn by personnel who handle such trash. The rules suggest that the elderly should not deal with T1 waste, but they can deal with T2 waste if they take the required measures

(continued)

Table 6 (continued)

Countries/agencies	New guidelines to handle biomedical waste at the time OF COVID-19
USA	The Occupational Safety and Health Administration (OSHA) of the United States has stated that waste that is suspected or known to contain or be contaminated with COVID-19 does not require any additional safeguards beyond those already in place to protect workers from the hazards they face in their daily work in solid waste and waste water management. Furthermore, they have stated that municipal solid waste with possible or known SARS CoV-2 contamination should be managed similarly to non-contaminated municipal solid waste, with strict engineering and administrative controls, safe work practises, and personal protective equipment (PPE), such as puncture-resistant gloves and face and eye protection, in place to protect workers from being exposed to recyclable materials they manage, including any contaminants in the materials

clinical waste acquired through SARS and COVID-19-drove clinical waste administration. At the peak of the plague, roughly 247 tonnes of clinical waste are generated every day in the Chinese city of Wuhan, which is multiple times higher than the pandemic. The peak came in between 15 February and 15 March (Singh et al. 2020). Before the Covid-19 flare-up, the city had a clinical garbage removal limit of 50 tons each day with a normal yield of 45 tons (Zuo 2020). This limit depended entirely on a protection plant that worked typically day in and day out without the capacity to discard any extra saves or capacity for clinical waste administration. With the rise of COVID-19 cases in the city, clinical waste production increased by 110–150 tonnes per day in mid-February and continued to rise to 247 tonnes per day by the conclusion of the episode on March 15. Later it slowly got back to business as usual in mid-May (Singh et al. 2020). At the point when neighbourhood specialists understood that clinical waste was running out of existing ability to securely discard the developing measure of clinical waste after third seven day stretch of January, they chose to discover master direct techniques and remember four exceptional organizations for Solid Waste Management including Giant, which professes to need assembled a crisis therapy plant with a limit of 30 tons/day by February 22, treated about 25% of the absolute clinical waste created in the city during the COVID-19 plague (Wei 2020). From January 23 to March 8, 2020, the neighbourhood government authoritatively recorded lockdown and actual distance approaches in the city. During this time, the creation of clinical waste has surpassed the current therapy/removal plant limit in the city, there was an enormous amount of clinical waste accumulated in wellbeing establishments and put away for a couple of days for guaranteed removal. This drove the nearby waste administration position to convey versatile incinerators in the city of 11 million individuals to discard disposed of PPEs, for example, face covers, gloves and other tainted single-utilize defensive stuff. Moreover, neighbourhood specialists took some intense measures to securely discard the developing measure of clinical waste as per public laws and guidelines, and if any demonstration was ignored by the administration framework, the comparing join was fined likewise

(Chen et al. 2020). As per government reports, around 50,333 instances of COVID-19 have been accounted for in Wuhan city. Information delivered by organizations engaged with the city's clinical waste administration shows that there are around 90,000 beds in medical clinics and centres in the city, remembering 54,000 beds for significant clinics, 14,000 beds utilized by COVID19 patients alone and 20,000 beds in recently constructed impermanent emergency clinics (Wei 2020). Waste created from isolate focuses and self-separation zones was not treated as clinical waste yet waste produced from possibly dubious family and isolate regions was securely gathered and discarded appropriately as clinical waste. The report shows that China's public clinical garbage removal limit expanded to 6066.8 tons/day on March 21, 2020, contrasted with 4902.8 tons/day before the pandemic. In the city of Wuhan, it arrived at 265.6 tons/day from the initial 50 tons every day prior to the pandemic. (Chen et al. 2020). A significant proportion of involvement and exercises learned in clinical waste administration during COVID-19 flare-ups in Wuhan is: (i) All the regions of the city can utilize different crisis removal hardware like protection gadget, portable therapy gadgets, family incinerators, and mechanical furnaces for removal of clinical waste. Notwithstanding satisfactory capacity and hold limit of clinical waste treatment offices is vital, which can forestall the stripping up of waste created during crises, for example, COVID-19.

(ii) Changes in clinical garbage removal procedures. Three key shifts were observed during the flare-up in Wuhan: from decentralisation to centralization, from occasional to regular activity, and, for the most part, from cremation enlistment removal innovation. Therapy offices for clinical waste ought to be more mechanized and with negligible specialist association, the Internet ought to be founded on the innovation of the Internet of Things (IoT). Through IoT innovation, the whole cycle of removal of clinical waste was made in the city of Wuhan constant following and control measure. IoT's innovation additionally understood the objectives of computerizing cycles and utilizing insignificant specialists for irresistible waste, remembering data for detecting gadgets, area frameworks, filtering gadgets and video reconnaissance, and Internet access with every gadget. Enormous limit of versatile offices ought to be kept up, particularly during pestilences, which can be vital for agricultural nations where clinical garbage removal offices are restricted. Portable highlights are appropriate for crisis circumstances, yet can likewise be utilized as essential reinforcement ability for the state later on.

4.2 COVID-Waste Management in South Korea

On January 28, 2020, shortly after COVID-19 was discovered in South Korea, the environment ministry strengthened the current "Waste Management Act" by declaring "Extraordinary Measure Way for Safe Waste Management against COVID-19" (Ministry of Environment, Republic of Korea (MoE-RoK) 2020). COVID waste may no longer be retained for more than 24 h and must be destroyed on the same day of collection, as opposed to the previous legislation, which allowed for seven days for the last time to be burned within two days after delivery. COVID garbage will also include indoor domestic waste, which will be subject to conventional COVID waste disposal procedures. (United Nations Economic and Social Commission for Asia and the Pacific (UNESCAP 2020). After elevating health warnings to level 4, the guidelines were revised to state that garbage disposed of by home-based patients would be placed in bags and containers provided after spraying. If COVID-19 positivity is discovered, stored waste should be kept near to the resin box. Local trash disposal facilities are ordered to address medical waste created by the patient's isolation from home and other municipal garbage first, in order to treat waste collected on an urgent basis (within 24 h). The help of the desired waste management organisations is aided in this effort by the distribution of almost 84,000 units of PPE and masks to project participants (UNESCAP 2020).

Until mid-July, more than 2600 tonnes of medical waste were gathered from 91 COVID hospitals, 8 residential centres, 24 temporary centres, and isolation houses that were set on fire. COVID waste is generated not only by hospitals, health facilities, and isolation, but also during disinfection in a public place or when an infected person is visited, and such waste is instructed to be treated as medical waste, with recycling being made twice as mandatory before being sent to the hot spot. COVID-19, a waste generated by health personnel and medical waste examiners, has also been chosen as an alternate treatments.

The South Korean government's prompt, flexible, and transparent steps to identify COVID waste can help avoid dangerous circumstances and diseases caused by medical waste. Closing COVID waste output at home quarantine, designated hospitals, and health care institutions has been discovered to assist track their disposal and treatment within a 24-h period. Local environmental officials can conduct a thorough investigation to guarantee that disposal requirements in bags and containers are strictly followed. Furthermore, as illustrated in Fig. 4a, b collaborative approach for COVID waste management might be useful in developing a straightforward decision-making process (MoE-RoK 2020). In order to solve the issues, organised and prioritised collaborations between the agencies engaged under the direction of the central disaster headquarters and the control of the Ministry of Environmental Affairs and local government have been proven to be successful.

4.3 Using Resources to Handle COVID Waste Efficiently in Spain

Spain is one of the countries that has been the most affected by the SARS-CoV-2 pandemic. Spain had registered 255,953 COVID-19 patients as of July 14, 2020, with 28,406 passing. The nation was afflicted with 8271 new instances on March 26, 2020, resulting in the recovery of 1648 people. As a result, the country has seen an increase in COVID usage, with treatment being a particularly difficult task due to a scarcity of treatment facilities. Clinical waste, such as protective gloves, face veils, and personal

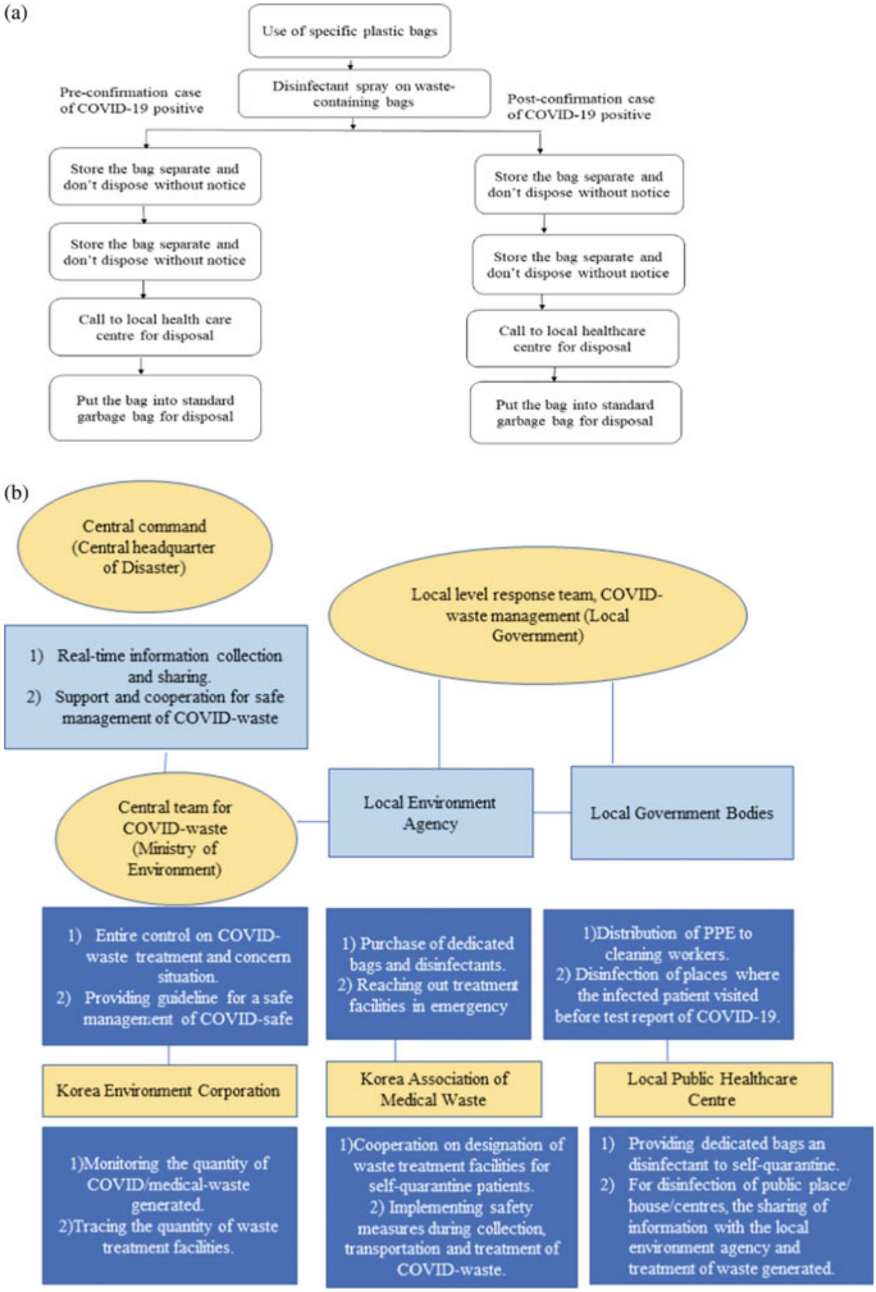

Fig. 4 In South Korea, public standards for the treatment of trash for self-quarantine and COVID-19 patients have been created, as a cooperative framework for the proper management of COVID-waste (UNESCAP 2020)

protective equipment (PPE), increased by 350% in mid-March 2020, according to the Catalonia Waste Agency. On average days, a regular clinical container of 275 tons/month produced almost 1200 tonnes of COVID (Ilyas et al. 2020).

To help the quick and powerful treatment of gigantic measures of infectious waste, the organization has used to reinforce the overall administration of three approved plants. The burning of a bit of clinical waste (the one considered as generally safe) is approved by some treatment places getting waste from wellbeing offices and changed inns. Existing civil waste incinerators have placed work in any case for clinical garbage removal. Despite the fact that it needed some expenditure, around 700 tonnes of clinical waste from a total of 1200 tonnes was handled in Catalonia begin on April 15, 2020, using metropolitan rubbish removal agencies (Ilyas et al. 2020). According to the Association of Cities and Regions for Sustainable Resource Management's assessment, the most difficult issue is separating COVID waste from strong city/nearby garbage, particularly family-owned waste. Given the intricacy and restricted assets that don't have clear rules during the main flare-up, the present circumstance has been seen as not comparable to South Korea. Notwithstanding, taking into account the abrupt attack of COVID-19 and the energizing European nations, for example, Spain their reaction to the COVID embarrassment is to a great extent disregarded.

It very well may be seen that the COVID waste assortment is controlled related to MSW, notwithstanding, the guidelines are obvious to twofold close waste compartments and keep them separate from people who are independent and don't blend them in with standard family garbage removal (Association of urban communities and Region for Sustainable Waste Management 2020). The primary disadvantage of the framework was found in the hotly anticipated conveyance of the last removal. Because of the long-term industriousness of SARS-CoV-2 in diverse places (Chin et al. 2020; Doremalen et al. 2020), there is a risk of contamination dropping into the COVID waste creation hole and being sent to a mechanised cremation office (Fig. 5).

4.4 COVID-19 Waste Management Strategies in Kenya

In April 2020, Kenya's Ministry of Health (MOH) issued a special process titled "Safety management and disposal of safety items in the prevention of COVID-19 transmission," which applies to communities, public places, healthcare institutions, and COVID-19 isolation centres. COVID-19-related waste handling is strictly regulated. All garbage generated by houses where COVID-19 cases are suspected or confirmed must be separated and placed in leak-proof liner bags/containers labelled "infectious waste." The leak-proof liner bags/container will be provided by the public health officer from the nearest health facility to the above-mentioned families. The public health officer is responsible for ensuring that infectious trash from suspected or confirmed COVID-19 households is properly managed. According to Ministry of Health (MOH) guidelines, infectious waste from suspected or proven COVID-19 must be cleaned daily by the household. When the waste liner bag is two-thirds

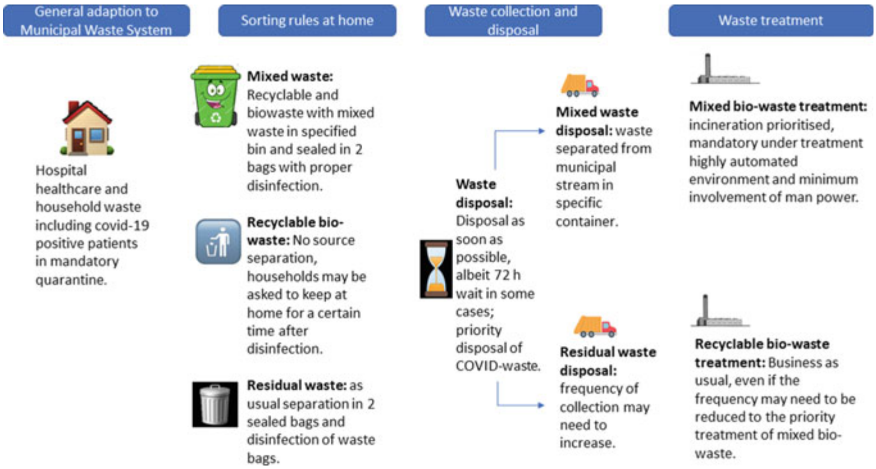

Fig. 5 After the breakout of COVID-19 in March 2020, a schematic of waste management in European nations is shown (Ilyas et al. 2020)

(2/3) full, disinfect it according to MOH guidelines, tie it up correctly, mark it as infectious trash, and deposit it in a designated area for collection (Olukanni et al. 2018). According to National Environmental Management Authority (NEMA) regulations, infectious trash from suspected or proven COVID-19 households must be transferred to the nearest public health centre. The public health officer is responsible for receiving garbage and managing it in accordance with MOH guidelines and NEMA regulations.

Commercial, office, factory, and industrial waste, as well as waste from other public places: All trash created by the aforementioned entities is considered potentially contagious (Wainaina 2020). According to MOH guidelines, the owner/occupier/manager/caretaker provided suitable coloured leak-proof liner bags/containers to such public spaces. In conjunction with appropriate actors, the public health officer oversees the safe treatment of infectious waste generated in such public spaces, as well as ensuring that garbage is handled by registered BMW handlers in accordance with NEMA regulations. According to MOH guidelines, the infectious waste was cleaned everyday by the owner/occupier/manager/caretaker. Infectious waste was potentially transported, handled, and disposed of in accordance with NEMA regulations. In accordance with MOH guidelines, all trucks, conveyors, containers, and receptacles used in the holding, storage, transportation, treatment, and disposal of potentially contagious material were disinfected.

In terms of garbage created by quarantine centres, all waste generated by these facilities was considered as contagious waste. To quarantine centres, administrators and supervisors were given enough correctly coloured leak-proof liner bags/containers. The Ministry of Health also oversees the proper disposal of infectious material from quarantine centres. The administrators and managers were assured that, in accordance with NEMA regulations, garbage would be handled by licenced BMW handlers. When the bags were filled to two-thirds (2/3), the garbage was disinfected according to MOH guidelines, correctly knotted, labelled as infectious waste, and put in a designated collecting area. Infectious waste from quarantine centres was collected, transported, processed, and disposed of in accordance with NEMA regulations.

4.5 COVID-19 Waste Management Strategies in Australia

The National Biohazard Waste Industry (BWI) advisory group, a division of the Waste Management and Resource Recovery Association of Australia (WMRR), has developed table direction to assist in providing guidance to medical clinics, matured, and medical services suppliers overseeing Coronavirus-influenced materials, as well as to assist those who are in charge of waste management, both inside and outside of these offices. In the wake of the revelation of Coronavirus as a pandemic by the World Wellbeing Association (WHO), numerous partners including emergency clinics, matured and medical care suppliers, are thinking about the extra measures to guarantee the suitable administration of waste from patients, affirmed or suspected, contaminated with Coronavirus. WMRR said that the articulation looks to offer general direction to partners. BWI likewise suggests that associations contact and work with their waste administration suppliers in the event that they have particular inquiries or require additional data. The BWI added that its assertion is adjusted from the 3 march 2020 WHO directing record—Water, sterilization, cleanliness and waste administration for Coronavirus. The WHO's specialist brief's content is based on the data now available for SARS-CoV-2 and the ingenuity of previous Coronavirus diseases. It incorporates information and advice from microbiologists and virologists, contamination control experts, and others with practical knowledge of water, sanitation, and hygiene (WASH) and infection prevention and control (IPC) in crisis and sickness situations.

Nobody knew of any proof that Coronavirus was not transmitted through unprotected human contact during the treatment of biomedical waste until recently, and Coronavirus is not considered a 'category A' infectious illness (World Healthcare Organisation 2020). BWI likewise comprehends that the WHO and same Australian healthcare authorities have indeed, proclaimed that clinical waste from tainted patients ought to be treated as would be expected clinical waste with no extra measures, BWI feels it is reasonable to propose the reception of extra measures.

It was suggested from BWI that those proposed measures ought to be received close by current PPE and other significant practices. Individuals dealing with clinical waste should wear appropriate PPE (boots, covers, long-sleeved suits, thick gloves, covers, and goggles) and complete proper hand hygiene following waste removal. Once more, BWI would rehash the significance of all offices proceeding to work and draw in with their waste administration suppliers on the suggested extra measures, which incorporates Execution of "twofold sacking" of waste from patients affirmed as Contaminated with Coronavirus. This was effectively be accomplished by first covering all clinical waste. Mobile Garbage Bins (MGBs) with clinical waste canister liners. For canisters or compartments that have been utilized in segregation rooms or in nearness to patients affirmed as contaminated with Covid-19, the outside surface were cleaned off as per WHO rules preceding assortment.

4.6 Strategies for Management of COVID-19 Waste in Sri Lanka

In March 2020, the Sri Lankan government plans to issue a "Interim rule for the board of solid garbage formed by families and spots under self-isolation due to the COVID-19 occurrence." The interval regulation is set up in accordance with the existing strong waste management strategy, guideline, and standards; nevertheless, provisions have been made to accommodate the specific needs of the present health crisis situation, with local specialists in Sri Lanka overseeing garbage. Neighbourhood specialists and partners should follow these general rules. The first phase in the COVID-19 waste management strategy is to identify families, places, and individuals who are at risk of self-isolation, and to provide extraordinary assistance to such places and families. Local experts allocated after assets to provide varying rubbish collection services. They arranged for a different truck to transfer and arrange the rubbish. Having a sufficient number of waste management groups and a supply of appropriate trash collection bags. General wellbeing officials were relegated to exhort family units on location removal and furthermore to prepare and oversee exercises of waste taking care of by occupants and assortment groups in the neighbourhood authority. Guidelines were given to family units undergoing self-isolation not to practise illegal open unloading (stressing the possibility of making a legal move against such cases) and the acceptance of waste for reusing centres or shops was temporarily halted until the COVID-19 pandemic ended if a patient or defilement was recorded in the assigned region. Furthermore, until the COVID-19 pandemic ended, the preparatory programmes, site inspections, and study trips were temporarily suspended to put the executive destinations that were being worked on by local authorities to waste. Individuals and beasts were not allowed to go to dumpsites or rummage through waste. Capable officials were selected to actualize waste the board exercises.

Guidance for the family unit garbage removal incorporates age of insignificant waste and restricted use of pressing material, instruments utensils and so forth Further these family units have additionally been told to carefully submit to the waste decrease direction and guidelines given by the nearby position and general wellbeing official (Durate and Santana 2020). MSW from families, as well as one's own isolating locations with COVID-19 positive cases or those suspected of being tainted, was divided into at least three kinds. side-effects and room (natural waste)—Waste produced from food measure, extra once utilization, ruined/disposed of food sources, Non-biodegradable waste, Special waste—Waste and without a doubt polluted things like face veils, covers, gloves, cloths, tissues, cleanly cushions, diapers, and elective materials debased by body liquids of inhabitants.

For word related wellbeing and security of waste controllers: Local specialists will supply proper PPE for all people engaged with waste assortment, transport, and removal. The PPE incorporates unique work wear (overalls), veils, gloves, shoes/boots and a cover or dispensable work dress. Clean specialists ought to be told to utilize PPE appropriately and will be checked. The waste overseers and managers should be appropriately informed and upheld with respect to the idea of the waste and the essential security strategies to help them to complete their errands. They should wear adequate PPE, and a new set of PPE should be worn every day of unusual help. After thorough cleaning and sanitation, overalls can be reused. It is the power's responsibility to provide basic PPE. Waste managers should be instructed to maintain a social distance of at least one metre when performing door-to-door combination exercises. After emptying the trash, the unique waste collection vehicle will be sanitised at the final disposal location (showering a sanitizer arrangement, i.e. 70% alcoholic sanitizer/clothing cleanser arrangement/fluid/bathroom cleaners). For each assortment trip, the clearing will be accomplished. Rubbish collection workers will be given a sufficient supply of hand sanitizers and cleaning professionals (cleaner/sanitizers) to wash their hands every time they collect distinct waste from a separate house/location. During the collection of rare wastes, assortment groups will be encouraged to minimise their interaction with the outside environment (carefully instructing them not to drink tea/water, bite betel nut, smoke, or visit stores). They should be reminded not to touch their faces or the veil when collecting various wastes.

4.7 COVID-19 Waste Management from Bangladesh's Perspective

Biomedical waste poses a concern to worldwide public health, particularly in low- and middle-income nations like Bangladesh. Each year, at least 5.2 million people, including 4 million children, die from illnesses linked to uncontrolled medical waste throughout the world. Human health can be harmed by sharp items, infectious illnesses can be spread to humans, and the environment can be contaminated by poisonous and dangerous substances if medical waste is not properly disposed of. Bangladesh has 460 Upazilla level hospitals, 9722 community level clinics, and 1449 outdoor health facilities that are covered by the DGHS at the national level. According to the research of Biomedical Waste Management in Covid-19 (Rahman et al. 2020), there are approximately 117 hospitals operating at the district level. There are around 2501 recognised private hospitals and 5122 registered diagnostic centres throughout the country. There are also several clinics, including over 5000 government and non-governmental organization-run clinics, as well as doctor's chambers, where medical waste is created. There are over 1200 hospitals, clinics, and diagnostic centres in Dhaka City alone. According to a Bangladeshi research (Haque et al. 2020), medical waste is not correctly managed in most Bangladeshi hospitals due to a lack of proper guidelines, commitment, and training of healthcare professionals. The biomedical waste management and disposal guidelines were enacted by Bangladesh's Ministry of Environment and Forest in 2008. The development of various boards with their responsibilities, the order of various biomedical waste and their specific removal techniques, the use of various shading coding frameworks and images in waste administration, directions for organisations engaged in conclusive removal of biomedical waste while ensuring natural safety and security, and finally punishments for breaking these standards are all detailed in these guidelines. The Ministry of Health and Family Welfare (MoHFW) is collaborating closely with the Ministry of Local Government to ensure that medical clinic trash is properly disposed of. BMW's executive exercises were first remembered for the MoHFW's 5-yearly HPSP in 1998 for medical clinics providing optional services. In HPNSDP 2011–16, the Health Service organised an Environmental Assessment and Action Plan. DGHS and DGFP devised a plan to form an Infection Prevention and Control Committee that would bring all of the emergency clinics together under the leadership of the association's president to care for medical clinic trash across the board. Other than the government, a few NGOs are currently working on this issue. For the time being, the government is just providing coordination to public clinics for the BMW board. To obtain their operations authorization, private clinics must follow to government runs on BMW executives. Bangladesh's government is implementing BMWM in collaboration with non-governmental organisations. Currently, five non-governmental organisations (NGOs) are operating in various parts of the nation to collect and dispose of medical clinic trash. Only PRISM is using all of the tactics for removing BMW for good, including burning, autoclaving, and unloading. Various organisations rely only on unloading. Before the COVID-19 epidemic, Bangladesh was dealing with hapless clinical waste administration, and it has now been impacted severely by a rapid increase in the volume of clinical waste. In Dhaka, Bangladesh's capital, the usual clinical waste age rate is 1·63–1·99 kg per bed per day (Miah and Rashid 2020). Because of Coronavirus, at least 14,500 tonnes of trash from clinical consideration were created across the nation in April 2020 (Chartier et al. 2020), which has probably increased as a result of the increasing tainting rate. Every day, 206 tonnes of clinical waste are generated in Dhaka alone due to Coronavirus (Business standard report, Dhaka 2020). This incapably directed waste addresses a colossal regular risk and may make a drawn out and unwanted general wellbeing peril and be a possible wellspring of returning illness. Clinical benefits workplaces in Bangladesh present gigantic peril to prosperity and environment by uprightness of insufficient waste administration. Appropriate waste administration strategy is needed to guarantee wellbeing and ecological security. Basic changes in arrangement and arranging and backing from government and coordinated effort among public and private areas would acquire significant changes medical care wastes the executives. Notwithstanding, the medical care waste the executives rule, arranging and strategy ought to be under the shadow of enactment, accentuation ought to be given in the advancement of instructive preparing program, record continuing, checking, audit of existing circumstance and there ought to be cooperation between various services, emergency clinic specialists, and dynamic investment from the local area.

4.8 Identification and Isolation of COVID-Waste for a Safer Treatment in India

Although no specific rules for dealing with COVID-waste were provided till the middle of March 2020, as COVID-19 expanded across India, an acceptable COVID-waste management framework was supplied. The major notable step ahead was the passage of the Epidemic Disease Act of 1897, which allowed the Indian Central Government to directly impose its policies on state governments (Somani et al., 2020). On March 18, 2020, the Central Pollution Control Board (CPCB), which is part of the Ministry of Environment, Forestry, and Climate Change, issued the specific rules (CPCB 2020). The "Rules for taking care of, treating, and removing waste produced during treatment/finding/isolation of COVID-19 patients" were issued to reduce COVID-garbage removal at medical facilities, which included isolate camps, home-care, test collection centres, testing labs, state contamination control sheets, and bio-waste therapy offices (CPCB 2020). Despite the fact that the Bio-clinical Waste Management Rule 2016 was passed, the requirements were maintained clear to ensure that COVID-waste was removed in a sensible manner (Aggarwal 2020).

As of late, the two most influential urban centres in India are Delhi and Mumbai. In Delhi, more than 40 sanitation workers have tested positive for the disease, with 15 of them dying. In Mumbai, 10 experts and two security screens were infected with COVID-19 and retrieved from the city's two landfills, Deonar and Kanjurmarg. These are only two of the most heavily hit cities in the country today (Mallapur 2020). India is on the verge of a COVID-sanctioned trash emergency, and experts are concerned. Similarly, used veils, tissues, head covers, shoe covers, expanding material outfits, non-plastic, and semi-plastic overalls were to be disposed of in a yellow bag recommended for burning at a standard biological waste treatment facility (CBWTF).

There are 200 biomedical waste therapy facilities in the country, two of which are in Delhi and one in Mumbai. Furthermore, according to CPCB data, these work settings are currently operating at 60% capacity, a 15% increase since March. In light of the several occurrences that have occurred in Delhi and Mumbai, the public normal is low. According to the CPCB and the Maharashtra Pollution Control Board, the CBTWFs in these two metropolitan areas are operating at 70–75% and 70% cut-off limits, respectively. Prior to the COVID-19 incident, a charity or a private calamity centre would provide 500 g of biological waste each bed, every day. According to SMS Water Grace BMW Private Limited, one of Delhi's CBWTFs, which collects waste from labs, segregation centres, and crisis facilities, including one of the city's COVID-19 government workplaces, the Lok-Nayak Jai Prakash Hospital, that number has risen to between 2.5 and 4 kg per bed per day. Every day, a massive COVID-19 government office may generate between 1800 and 2200 kg of biological waste. At the moment, double this by the number of Covid-19 crisis facilities in the nation, which is 2900. When you add in biomedical waste generated by 20,700 isolate centres, 1,540 example assortment centres, and biomedical trash collected by districts (Delhi alone has 12,000 home seclusion offices), you get a sense of the magnitude of the problem (ICMR 2020). Per day, Delhi generates 11 tonnes of COVID-related garbage (CPCB 2020); Mumbai has supplied 9 tonnes of COVID-related rubbish every day (assessed by Brihanmumbai Municipal Corporation). If Covid-19 instances continue to climb in the coming months and testing limits remain skewed, several urban zones, including Delhi, may be forced to ship their Covid-waste to neighbouring states for disposal, according to CPCB experts. Not only in Delhi and Mumbai, but also in Kerala, the progress in biomedical waste is focusing on specialists. Kerala has remunerated over 100 tonnes of garbage from Coronavirus treatment centres within two months after the state began regulating Coronavirus biomedical waste based on new CPCB standards (Ramteke and Sahu 2020).

Due to the Coronavirus pandemic, India produces around 600 metric tonnes of biomedical waste each day, which is approximately 10% more garbage than before the outbreak. Because Covid-waste is transported at such a high rate and volume, it requires considerably greater scrutiny to ensure that it is managed without generating further clinical concerns (ICMR 2020). It is estimated that 2 tonnes of Covid-waste is generated in each state as a result of the inspection, separation, and treatment of sickness. This is exorbitantly low when compared to the 240 tonnes of garbage produced each day in Wuhan, the pandemic's confluence point (CPCB 2020).

On March 18, 2020, the Central Pollution Control Board, Government of India, issued a set of guidelines for supervising, treating, and expelling waste communicated throughout treatment, as well as determining and isolating Coronavirus sufferers. Disengagement wards in medical clinics must maintain segregated concealing coded canisters for waste detachment under these conditions (Datta et al. 2020). A provided holder labelled 'Coronavirus' should have been stored in a separate, small extra area and should only be considered by authorised personnel. In these wards, a separate sanitization worker strategy for biological waste organisation was presented. The CPCB educated collecting of biological waste in yellow packs and the canisters holding this should be sent over to certified professionals for seclude camps and home thinking of the predicted patients. According to the requirements, people handling such garbage should be provided with suitable preparation and personal protective equipment (PPE), such as three-layered coverings, sprinkle confirmation covers, gloves, gumboots, and safety goggles (CPCB 2020; Ramteke and Sahu 2020) (Table 7).

The National Green Tribunal's (NGT) Status Report documents an increase in improper biomedical waste segregation from COVID-19 isolation units, quarantine centres, and quarantine houses (Singh et al. 2020). According to the BMWM Rules 2016, all isolation wards for the diagnosis and treatment of COVID-19 patients must separate their trash into various color-coded containers or bags. While Urban Local Bodies (ULBs) may give color-coded yellow bags for hazardous COVID-waste and manage it from quarantine houses, Indian families do not follow the segregation process. For example, an uninfected mask or glove should be shredded and wrapped in paper for 72 h before being discarded as trash (CPCB 2020). In this case, ULBs serve a critical role in sanitation worker training and monitoring of BMW disposal in a safe and scientific manner. While some governments have chosen private garbage collection organisations and deployed cars until CBTWFs are ready, others have given CBTWFs the responsibility for waste collection. ULBs must confirm that biological waste is removed from hospitals and isolation centres as soon as possible by an authorised CBTWF. They must give yellow color-coded bags to quarantine households and guarantee that household medical waste is collected door-to-door by trained waste workers or CBTWFs directly. According to the recommendations and the BMWM Rules 2016, home hazardous waste might be deposited at collection

Table 7 Guidelines for COVID waste management in India from the Central Pollution Control Board (CPCB 2020; Ilyas et al. 2020)

Directions to	Guidelines for the handling of COVID-waste
Isolation ward of COVID-19	• Use of different coloured bins/bags in wards and proper waste segregation as per BMWM Rules 2016 • In the case of COVID-waste, use double-layer yellow-colored waste • After disinfecting the inside and exterior surfaces of the bags with a 1% NaOCl solution, the COVID-waste is stored in a special collecting container labeled "COVID-19". COVID-trash must also be labeled as "COVID-19 waste" to guarantee priority disposal at treatment facilities • General garbage, excluding COVID waste, should not be combined and should be disposed of as common solid waste • Maintain a separate record for COVID waste generated in isolation wards • Separate collection staffs for COVID-waste and other solid trash to enable timely garbage collection and disposal • SPCBs recording waste creation, collection, and treatment data
Testing labs and sample collecting centers	• State pollution control boards are reporting the opening of collection facilities and testing labs to monitor COVID-waste records • All isolation ward guidelines should be followed at sample collecting facilities and testing labs
COVID-19 patients' quarantine camps and home care	• Solid waste treatment of ordinary collected garbage (non-medical) • Separately collect BMWs in yellow colour bags/bins if any are present • As soon as the BMW is created, the quarantine camps must notify the CBWTF operator so that COVID-waste may be collected on time • Waste generated by self/home-quarantine suspects/patients should be collected separately in yellow bags and given over to the local bodies authorized collectors

(continued)

Table 7 (continued)

Directions to	Guidelines for the handling of COVID-waste
Common biomedical waste treatment facility	• Notifying the appropriate SPCBs when COVID-waste is received from isolation wards, quarantine facilities and households, and testing centres • Sanitation of waste collectors on a regular basis • Providing personal protective equipment (PPE) such as nitrile gloves, three-layer masks, splash proof aprons, safety boots, and goggles • Use a designated vehicle for COVID waste collection, with vehicle branding and sanitization with 1% sodium hypochlorite as needed. • COVID-waste must be disposed of as quickly as possible after receipt, and the facility's operator must keep separate records for COVID-waste collection, treatment, and disposal • If a worker exhibits sickness signs, he or she should be given enough absence without being paid less

centres by the generator, however due to the lack of such centres, real implementation is hampered. The rules also allow ULBs to appoint a nodal person to be in charge of data feeds on a daily basis in the 'COVID-19 BMW Tracking App'.

India now has 200 CBTWFs operating for the treatment and disposal of BMW (usually for BMW generated in HCFs), with the exception of five states/UTs (Andaman and Nicobar Island, Arunachal Pradesh, Goa, Mizoram, Nagaland, and Sikkim), which have none. Aside from that, there are 15,281 HCFs with captive treatment facilities (installed in the absence of CBTWFs within 75 km of the HCFs). According to the NGT Status Report, the states have already used 55% of the total incineration capacity. Even before the pandemic, India's infrastructure treatment capacity was inadequate. BMW disposal has grown much more difficult now that the number of coronavirus infections has surpassed four million. The number of instances registered and the available disposal capacity appear to be proportionate. Around half of the states and UTs have insufficient disposal facilities. States that use 70% of total incineration capacity have the most positive examples. Because of a lack of waste segregation methods and technical advancements in machinery, the majority of garbage is burned in incinerators, resulting in the production of poisonous fumes that are dangerous to employees and residents living near these facilities.

The CPCB standards allow the use of at least 100 feet-deep burying trenches as a last option in the case of BMW generation if the required disposal facility is lacking. Furthermore, according to the WHO data cited earlier, 56% of BMW is discarded publicly, and there have been reports of unlawful dumping in agricultural fields. Such practises harm soil health and contaminate groundwater, rendering it unsuitable for human consumption. According to the WWF, if 1% of all masks are disposed of wrongly, 10 million masks might be spread in the environment each month. Environmentalists have warned that the pandemic would result in a new environmental disaster (Table 8).

Table 8 Details on generation of COVID-19 waste and Facilities for Disposal (NGT Status report 2020)

State/UT	COVID waste generated (in tonnes per day)	Details of facilities for disposal of covid-19 waste			Adequacy of existing traetment facility
		No. of CBTWFs Engaged	Captive fcailities (Yes/No)	Deep burial pits (Yes/No)	
Maharashtra	17.494	29	No	Yes	Adequate, Stand-by arrangement also made with TSDFs in Mumbai, Pune, Nagpur cities
Gujarat	11.693	20	No	No	Adequate
Delhi	11.114	2	No	No	70% of existing capacity of 2 incineration utilised. Need to ensure proper segregation
Tamil Nadu	10.41	8	No	No	91% of incinerator capacity utilised. Need to ensure proper segregation and identify alternate incinerator/disposal options
Madhya Pradesh	7.486	11	No	No	Adequate
Uttar Pradesh	7	18	No	No	Adequate
West Bengal	6.5	6	No	No	Adequate
Rajasthan	5.9	8	No	No	Adequate information not submitted
Andhra Pradesh	5.516	0	Yes	No	Adequate with captive facilities
Kerala	4.71	1	Yes	No	All COVID-waste sent to CBTWF. Capacity of CBTWF not adequate for total BMW. Hence captive facilities need to be operated

5 Recommendation

COVID-waste is clearly at the top of the BMW priority list. The only way to cope with this seductive abuse of high danger is to collect, transport, and remove COVID-waste in a timely and efficient manner while adhering to safety precautions. The segregation of wards/emergency clinics/isolate focuses/home-isolates should practise separate variety in twofold seal allocated sacks/receptacles. For a convenient assortment and removal, the job of metropolitan local bodies is somewhat fundamental regardless of the way that inside the interment sum a few waste treatment offices are confronting the workers emergency. Along these lines, the specialists associated with this work should be taken as a piece of a fundamental assistance (Prata et al. 2020). Local governments and CBWTF officials should be in charge of authorised medical services and wellness assessments. It is recommended that no COVID-waste be dumped by mixing it with household garbage and stored in closed compartments/containers. The human-to-human contact as well as openings with the other potential transporters like cell phones, consoles, and so on ought to be kept away from. Additionally, following each cycle of COVID-waste assortments, the cars should be cleaned with a 1% Sodium hypochlorite arrangement. After putting on the evacuation PPE and facemasks, the personnel should avoid touching their faces, noses, mouths, and eyes, especially after using a 70% liquor sanitizer (Borg et al. 2020). Individual mindfulness may be a panacea for more secure COVID-waste treatment, thus the government, local governments, and waste treatment facilities should spearhead the mindfulness programme, using a variety of media to directly reach people. The regular usage of protective facemasks and hand gloves is often consumed to a large amount, and because of their small size and light weight, there is a chance that these wastes will be disposed with strong trash. It is recommended that such trash be handled with care, since they might be highly contagious for up to 7 days. As a result, the rules to keep waste covers falling for at least 72 h must be observed, as "prevention is better than treatment". The large number of applied analysts that must address the successful control of pandemic flare-up because of the encompassed novel Coronavirus highlights the large number of the applied analyst that must address the successful control of pandemic flare-up because of the encompassed novel Coronavirus (Wigginton and Boehm 2020). As a result, environmental engineers, medical professionals, healthcare workers, and researchers may use their extraordinary talents and expertise with multidisciplinary research to answer the urgent demand. For example, disinfectant spray (H_2O_2/NaOCl) is frequently advised to render the encapsulated virus inactive on the waste surface (Gallandet et al. 2017), however this does not always work. Blood on fomites would necessitate much higher sanitising assessments (Wood et al. 2020), which can be better sensed for a few distinct scenarios through multidisciplinary studies.

Because of the global shortage of personal protective equipment, reprocessing, rather than uncontrolled recycling methods, can help to alleviate the shortage. VH_2O_2 and hot air disinfection measures can potentially apply for the reprocessing of COVID-waste, notwithstanding, convenient defeat from existing constraints like decrease in oxidant concentration in the present, as reprocessing technology will not only help to reduce infection spread and environmental health but also increase the accessibility of personal protectives by their conceivable re-use. On this premise, it is suggested that an integrated strategy including environmental engineers, healthcare professionals, and researchers be used to overcome the challenges of COVID-waste management.

Apart from that, there are a few policy proposals for policymakers that might aid in the planning of a framework to deal with pandemics in the future.

i. Sanitation employees must be safeguarded, and all governments must acknowledge their importance. For example, the UK government has awarded trash specialists 'Keyworker' status, which implies that education and care arrangements for their children and families would be maintained throughout the COVID-19 emergency in order for them to continue with their services.
ii. Waste management should be included in disaster preparation, which is now focused on debris. To cope with and respond to the dynamics of waste generated during a future pandemic, response measures and guidelines should be calculated. While bringing states and centres together, such a charter should be entrenched alongside the regulation for catastrophe waste management planning.
iii. While solid waste management is incorporated into disaster waste management planning, it is necessary to guarantee that those involved are well-prepared to deal with hazardous biological waste, which should be accomplished by establishing a global standard information sharing platform.
iv. For an efficient and practical biological waste management system, a national policy framework with guidelines and specialised norms is essential. Most nations, whether developed or developing, use color-coded BMW segregation according on their legal structure. Universal coding based on the kind and nature of trash, as well as training for healthcare workers, would aid in the accurate classification of infectious waste and reduce waste creation.
v. New and sustainable improvements in the reuse of blended and other complicated types of plastics should be prioritised. Incorporating AI into the reuse process's organising and handling phases might result in greater recyclability rates and subjective goods. Complex and financially unviable to reuse objects, as well as fancier and multi-layer plastic packing, should be handled. Incentives for homogeneous plastics, environmentally friendly bio-plastics, and round developments should also be developed and implemented effectively.
vi. A mechanism should be put in place to distribute additional monies in order to educate people about circular economy concepts, which should be achievable through a combination of public and private investments. Greener goods, such as bio plastics and biodegradable materials with increased recyclability, should be rewarded and promoted. It should be obvious that the devastation caused by the COVID-19 emergency should not be addressed at the expense of solving the longer-term issue of environmental emergency (Climate Action Tracker 2020). Outflows might rebound if monetary upgrade packages responding to

the COVID-19 pandemic recovery include low-carbon improvement strategies and arrangements, such as for greener and more practical items. According to a handful of recent environmental change reports, the chances are that it will even exceed lately expanded levels by 2030, notwithstanding decreased monetary growth (Climate Action Tracker 2020). In this vein, a frameworks level technique on a global size would be required in the post-COVID-19 world to handle the issue of robust waste administration and maintain our existing situation.

6 Conclusion

Handling COVID waste has a larger risk than handling regular BMW. To deal with this extremely infectious waste, it is critical to collect, handle, and dispose of COVID waste in a timely manner while adhering to thorough safety procedures. Wards, hospitals, detachment centres, and home quarantines should all have their own collections of double-seal designated bags/containers. Even though many waste treatment institutions are experiencing personnel challenges during the shutdown period, the involvement of urban bodies is critical for timely collection and disposal. As a result, workers who perform this activity should be viewed as part of a vital service. Local organisations and operators of the Common Biomedical Waste Treatment Facility shall be responsible for taking appropriate health and safety precautions (CBWTF). It is not suggested to dispose of COVID waste by combining it with household garbage and storing it in a sealed container or bin. Personal touch, as well as exposure to other potentially harmful things such as cell phones, keyboards, and other electronic devices, should be avoided. In addition, vehicles used in COVID waste collection should be disinfected after each collection by spraying a 1% solution of sodium hypochlorite on them. Employees should avoid contacting their face, nose, mouth, and eyes after using 70% alcohol sanitizer shortly after removing PPE and face work. Because public awareness can be a safe alternative to COVID waste treatment, the government, local groups, and waste treatment facilities should undertake an awareness campaign utilising various media means to reach out to the general public. Face masks and gloves are commonly used, and due to their small size and low weight, there is a significant likelihood that this trash will be discarded in solid garbage. It is suggested that such garbage be handled with caution because it might be infectious for up to 7 days. As a result, "prevention is better than cure," and standards for keeping paper waste disposal for at least 72 h should be observed. The need for applied researchers to deal with an effective pandemic outbreak as a result of a coronavirus-covered novel by reducing the threat of SARS-CoV-2 using COVID-waste forms, its release by the host, and persistence in carrying areas highlights the number of applied researchers who need to deal with an effective pandemic outbreak as a result of a coronavirus-covered novel (Wigginton and Boehm 2020). Environmental engineers, medical physicians, health care professionals, and scientists may now combine their distinct abilities and expertise with a range of studies to solve the demand for an hour through an integrated strategy. For example, it is recommended to use an antiseptic spray (H_2O_2/NAOCI) to inhibit the surface-sprayed virus (Gallandat et al. 2017), however this does not always succeed. Blood in fomites may necessitate a very high antibiotic dosage (Wood et al. 2020), which can be better understood in a variety of different situations through multidisciplinary study.

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Bioremediation of Chlorinated Compounds

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Abstract Environmental contamination by various emerging organic and inorganic pollutants has become an issue of global concern in recent times. Amongst the various emerging contaminants known, chlorinated compounds constitute a major group of interest due to their deleterious effect on humans and the environment. Chlorinated compounds are widely utilized for different purposes in various areas of modern society. These contaminants are present in pesticides, petroleum derivatives and solvents that are used on daily basis in different sectors. Some of these compounds tend to be persistent in the environment and have the tendencies to bio accumulate as they are passed across the food chain. Remediation of chlorinated compounds in contaminated areas from various environmental matrices is therefore of significant priority. Various approaches have been employed for the remediation of this group of compounds some of which have their inherent limitations. Bioremediation technology is a promising approach in this regards due to its cost effectiveness, ease of execution and environmental friendly nature. This chapter critically reviews the bioremediation of chlorinated compounds. It discusses the source, distribution and types of chlorinated compounds found in the environment, their effects on humans and the environment, the role of aerobic and anaerobic microbes in biodegradation

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and the various classes of enzymes that are involved in the bioremediation of chlorinated compounds. An attempt is also made in highlighting the future trends in this regards.

Keywords Bioremediation · Chlorinated compounds · Contamination · Enzymes and solvents

1 Introduction

Organic and inorganic pollutants present in the environment are threat to drinking water and other natural resources such as soil and atmosphere. Amongst the various organic contaminants, chlorinated compounds mostly in the form of their solvents constitute a major group of concern due to their deleterious health impacts on the environment and humans. They are therefore the primary pollutants of ground water in most countries of the world. Different physical and chemical processes have been employed for the removal of these contaminants from various environmental matrices. Some of these methods have their inherent limitations in that they also generate undesired by products, are highly expensive and non feasibility for large scale applications (Ali et al. 2019).

One of the most recent approaches that have been widely considered to be efficient with various promising modifications for better results is the use of bioremediation. Bioremediation is a complex phenomenon that involves various biological processes that utilize bio-systems for the restoration and clean-up of polluted areas. Studies have documented that about 400,000 sites around the United States of America are polluted with chlorinated compounds mostly in the form of solvents. Chlorinated compounds have been employed widely as degreasing agents in most industrial processes for a long time. There are various methods of disposal that have been employed and these have contributed to the broad spread of such compounds in ground water and soils. One of the most common practices was the returning of such used chlorinated solvents back to a drum. In such a case, immediately the drum was filled, it was then covered and buried underground with other drums. Such drums corroded eventually and the contents inside escaped into the soil and eventually end up in the underground water (Sharma 2020).

The microbial communities play an indispensable role in bioremediation technologies. There are various groups of indigenous microbes that are indispensable in the process of environmental restoration. The microbial population achieves this through processes such as immobilizing, oxidizing and transformation of the contaminants. The primary role of the microbes is to bring about a reduction in the levels of the contaminants to non-toxic, undetectable and within permissible range set by various regulating agencies. Most recent approaches to bioremediation involve the search for novel microbial groups from the polluted site. The microorganisms isolated are considered to have a unique ability to induce the remediation of the contaminants (Mishra et al. 2021, Shah 2020). Several studies have reported the potential of bioremediation in the restoration of environments contaminated with chlorinated compounds. In one of the most recent studies, Cichocka et al. (2010) assed the reduction removal of the chlorine substituents present in tetrachloroethene to ethane. The study involved the use of culture containing Dehalococcoides, which dechlorinated the compounds to ethane. In their investigation, the microcosms were made from ground water and then modified using hydrogen or lactate as electron acceptor and donor respectively.

Poritz et al. (2013) showed the potential of two Dehalococcoides strains in coupling all the reductive dehalogenation processes for the conversion into ethane. They revealed that the genome of Dehalococcoides spp was capable of encoding for over 20 reductive dehalogenases and this was documented to be the first case of genome that has three different enzymes that are vital for the coupling of the contaminant to ethane. Kranzioch et al. (2015) observed a rise in the gene copy of the various intermediates during the process of dechlorination. Matteucci et al. (2015) observed that trichloroethene and perchloroethene and other chlorinated compounds are widely distributed in ground water contaminants. They used a microcosm anaerobic approach for the assessment of the potential for enhanced or in situ bioremediation. Positive results were obtained for most of the microcosm with regards to dechlorination, most especially those that were inoculated with mineral medium. This showed the presence of an active indigenous dechlorinating group within the ground water. Butyrate and lactate were shown to enhance the process of dechlorination among the electron donors investigated. Mundle et al. (2012) documented that chlorinated ethenes are one of the most common contaminants in ground water. He also opined that there are limited studies details on the isotopic enrichment factors connected to the various processes. Hence in their study, they determined the enrichment factor connected with microbial degradation of ethane under anaerobic microcosms using cultures obtained from River Side in Georgia.

This chapter critically reviews the bioremediation of chlorinated compounds. It discusses the source, distribution and types of chlorinated compounds found in the environment, their effects on humans and the environment, the role of aerobic and anaerobic microbes in biodegradation and the various classes of enzymes that are involved in the bioremediation of chlorinated compounds. An attempt is also made in highlighting the future trends in this regards.

2 Concept of Bioremediation

This is a fast emerging technological process which can be utilized alongside together with various chemical and physical processes of treatment for the effective removal of various classes of organic and inorganic contaminants in the environment. This is a remarkable and promising technique for efficient environmental management of contaminants. Bioremediation is a remediation technology that involves the utilization of various microorganisms, fungi or plants for the degradation of contaminants thereby enhancing the transformation or utilization of substances. There are various groups of microorganisms that play role in the process of bioremediation with bacteria being one of the most prominent. Bacteria are ubiquitous and are found in various environmental media such as soil, water and air. Processes that involve the utilization of localized bacteria for the degradation of contaminants under existing conditions of subsurface are known as the natural attenuation or passive bioremediation process (Yan et al. 2020, Shah 2021a, b). Natural attenuation processes are most common within the subsurfaces where the population of the bacteria responsible for the degradation is high. The enhanced process of bioremediation is the type in which the indigenous bacteria community is stimulated through the addition of electron donors or substrates so as to bring about an increase in the growth of the bacteria enhancing faster rates of biodegradation. The substrate introduced is depended on the nature of the bacteria that are being stimulated for the degradation of the contaminants (Xu et al. 2018).

Various agencies, industries and researchers have utilized some organic substrates for the promotion of anaerobic reductive dechlorination of numerous chlorinated compounds into their final innocuous end results. Various remarkable and promising outcomes have been documented in field anaerobic bioremediation applications. Phytoremediation and bioremediation have advanced progressively and proved efficient most especially in the area of treatment of contaminated areas. Sites polluted with recalcitrant and chlorinated compounds have been shown to prove more resistances to these techniques, but there are however remarkable progress in the field and laboratory. Some of the most recent breakthroughs in the area of bioremediation of chlorinated compounds include advancement in various anaerobic and aerobic processes, bio reductive dechlorination processes, biomonitoring, bioaugmentation and phytoremediation. Various advanced processes of bioremediation which involve destructive and non destructive approaches are being employed in the process of bioremediation. Some of the approaches include phytoremediation, biodegradation, thermal incineration, reductive dechlorination and advanced oxidation processes (Sharma 2020).

3 Sources and Distribution of Chlorinated Compounds in the Environment

Chlorinated compounds have been utilized in various industries as solvents for degreasing machinery. Common chlorinated compounds are derivatives of ethanes, methanes and ethane. Most of the known chlorinated compounds show minimal solubilities in water, variable vapor pressure, and are also denser than water. Hence some of these chlorinated solvents are commonly described as dense non aqueous state solvents. There are various chlorinated pollutants that are discharged into the environment with the prominent being carbon tetrachloride (CT), trichloromethane (TCM), methylene chloride (MC), dichloromethane, tetrachloroethene (PCE) and trichloroethane (TCA). Some other chlorinated compounds include: chlorinated pesticides such as chlordane, chlorinated cyclic compounds, polychlorinated biphenyls (PCBs) and others (Lin et al. 2021). These chlorinated compounds are of serious concern because of their deleterious impact on the environment and human health as well as their remarkable resistance to biological processes of degradation. Since most of these compounds are usually found in their oxidized form, there are there not vulnerable to aerobic processes of oxidation, except in the case of co-metabolism. Chlorinated compounds such as polychloroethylenes, polychloromethanes and polychloroethane are commonly employed as degreasing agents and solvents in various commercial and industrial products. They are ubiquitous hence found in water, soil and atmosphere. These compounds have serious health implications as they also affect the ecosystem at large. There are various sources of chlorinated compounds which primarily include the use of volatile chlorinated solvents products, the process of disinfection using chlorinated compounds, emission from various industrial processes as well as improper disposal and storage methods (Marcon et al. 2021).

There are various groups of chlorinated compounds such as trichlorethene and perchloroethene as well as other chlorinated solvents which are very common as underground contaminants. They constitute a dense liquid phase that is non aqueous and sink through ground water aquifers that are permeable until a zone of nonpermeability is reached. Chlorinated compounds are known to be persistent in the environment and one of the well known contaminants at various industrial sites. The ubiquitous nature of these compounds is as a result of their wide applications in dry cleaning, metal degreasing and other production processes (Matteucci et al. 2015).

4 Types of Bioremediation Techniques for Chlorinated Compounds

There are various strategies involved in the process of bioremediation of chlorinated compounds.

Two major approaches for the bioremediation of chlorinated solvents present in ground water have been identified.

In the first approach, the water is pumped to the surface and treatment is carried out above the ground inside a bioreactor. In another approach, the aquifer is remediated within (in situ).

i. In situ bioremediation of chlorinated compounds

In situ approach of bioremediation is a better and more reliable approach when compared to such traditionally employed methods. In situ bioremediation was first utilized as a natural approach for petroleum based compounds in contaminated underground aquifers. Further studies later reported the potential of microbial population in the degradation of chlorinated compounds as well as other organic and inorganic contaminants.

The in situ bioremediation approach is further classified into two. The first is intrinsic in situ bioremediation while the other is engineered in situ bioremediation.

In intrinsic in situ bioremediation, the primary interest is monitoring the process of degradation which is already on going so as to ensure that the contamination plume does not expand.

For the engineered in situ bioremediation, natural degradation is not taking place in the environment and in some other cases it occurrence is very slow, hence the subsurface environment is first manipulated so as to stimulate the process of biodegradation and the rate of the process is enhanced. In the engineered approach, the primary strategies involve the supply of nutrients such as electron acceptors, phosphorus and nitrogen to the subsurface. One of the most prominent acceptor of electron employed is oxygen. However as a result of the low solubility of oxygen gas in water and the accompanied high biomass production, anaerobic processes have been applied recently (Philp 2015).

This approach involves the treatment of contaminated substances at the area of contamination. Hence it does not need the process of excavation; therefore, it is usually followed by small or no disturbance to the structural composition of the soil. Normally this approach is supposed to be relatively cheaper in comparison to the ex situ technique of bioremediation since there is no extra cost required for the excavation of the soil around the site. However the economic constrains of this approach is the additional cost that is required for the onsite installation and design of some of the complex equipment required for the technique. Some of the in situ approaches could be aided (phytoremediation, biosparging and bioventing) while some others could proceed without the need for any form of enhancement (natural attenuation and intrinsic bioremediation). Intrinsic bioremediation technique has been efficiently employed for the treatment of site contaminated by chlorinated compounds. However an effective and successful application of various conditions such as pH, moisture content, temperature and availability of nutrients must be suitable for the process (Ashraf et al. 2013).

ii. Ex situ bioremediation of chlorinated compounds

In this approach, the contaminants are removed through excavation from the site of pollution and then conveyed to another area for the purpose of treatment. This approach is commonly considered on the basis of extent of pollution, economic implication of treatment, performance criteria as well as the geographical location.

4.1 Biopile

The biopile aided bioremediation involves the pilling of excavated contaminated soil above ground which is then accompanied by the amended of nutrients and in some cases aeration to aid bioremediation through the improvement of microbial activities. This technique includes some primary components which are irrigation, aeration, collection systems for leachate and nutrients and bed for treatment. The application of ex situ technique for chlorinated compounds is considered increasingly as a result of its constructive characteristics together with cost efficiency, which aid effective breakdown on the condition that temperature, nutrients and aeration are controlled effectively (Wuana and Okieimen 2011).

4.2 Windrows

Windrows is an ex situ technique that depends on the periodic turning of the piled contaminated soil so as to aid bioremediation through the increase of degradation processes of the indigenous hydrocarbonoclastic microbes that are present within the soil. The continuous turning of the contaminated soil, alongside the addition of water gives rise to an increase in the uniformity of the contaminants, and aeration hence favoring the process of degradation.

4.3 Bioreactors

As the name suggests, it involves the use of a vessel where the raw material conversion take place. Several biological reactions are involved. There are various modes of operation for bioreactors some of which include: fed batch, continuous, multistage and batch. The selection of a specific mode of operation is depended on cost and market economy. The conditions of the bioreactor are capable of supporting natural processes of cells through mimicking and maintenance of their natural environmental conditions (Das and Chandran 2011) (see Fig. 1).

5 The Role of Microorganisms in the Bioremediation of Chlorinated Compounds

Subsurface microbes have the potential for the degradation of most of chlorinated contaminants. In some instances, the process of biodegradation takes place naturally without any necessary engineering process, in some others, electron acceptors and nutrients need to be introduced into the subsurface, or some specific microbial groups have to be stimulated for the purpose of creating some suitable conditions. Bacteria involved in the process of bioremediation are grouped based on their usage of oxygen: anaerobic, aerobic and facultative anaerobes. The aerobes need oxygen while the anaerobes do not require oxygen in their environment while the

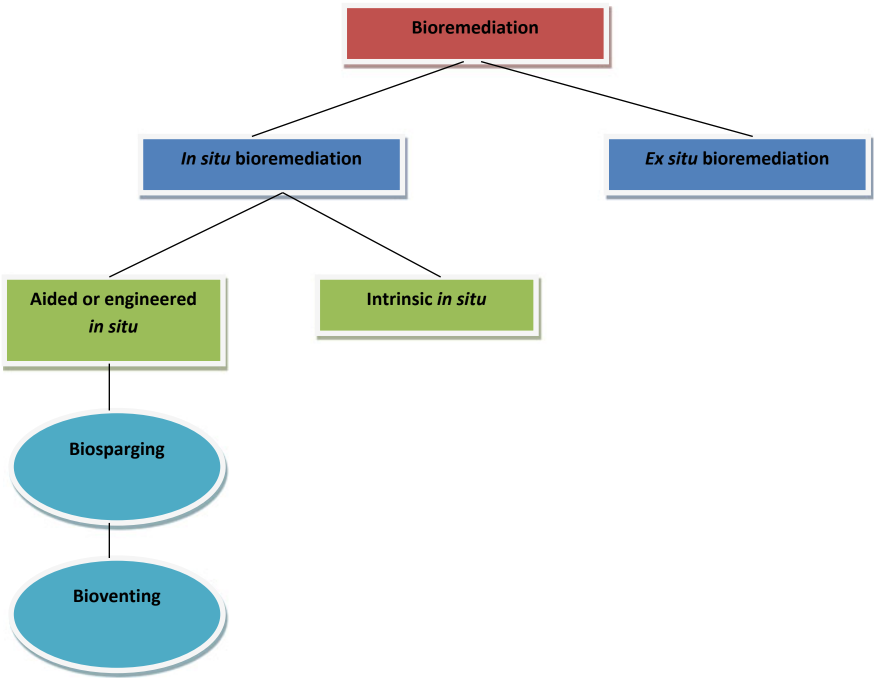

Fig. 1 Types of bioremediation technologies

facultative anaerobes are able to survive in both anaerobic and aerobic environments. Enhanced bioremediation involves the addition of exogenous microbes to a polluted site. Bioaugmentation basically is employed together with nutrient and substrate injection approach during the remediation of an aquifer that is polluted with chlorinated compounds (Nikolova and Gutierrez 2020).

Although several aerobic bacteria posses the ability to degrade chlorinated compounds, aerobic processes has been shown to be less promising for the remediation of the subsurface. Composting, which is an aerobic treatment has been employed for field scale treatment of polluted soils. The application of this approach is however restricted by a rise in the volume of the waste substance, the end products that are not well characterized and the potential of residual toxicity which is still possible after treatment makes it less suitable.

Bioremediation under anaerobic environment was found to be favorable. The creation of an anaerobic condition in a slurry of soil through the addition of byproducts of potatoes processing was effective for the stimulation of an anaerobic bacteria population capable of degrading the chlorinated solvent contaminants (Jing et al. 2018).

6 Human Exposure and Health Impact of Chlorinated Compounds

There are various routes of human exposure to these compounds which inhalation, dermal contact and ingestion. Various studies have documented the toxicological impacts of these compounds and the findings from the study show the possibility of association of these with cancer incidence in humans. Most chlorinated compounds of environmental concern have been enlisted as pollutants of priority by various environmental agencies.

Humans and other organisms within the environment are exposed to broadly complex mixtures of toxic chlorinated compounds which also include various derivatives of organic and inorganic chlorinated compounds. The various routes for exposure differ depending on the compounds and the nature of the organism under consideration. The absorption of volatile organic compounds could take place mainly through inhalation. There are cases were these chlorinated contaminants are absorbed directly from the environment. For instance, the flatfish commonly found lying on polluted sediments may absorb a high amount of chlorinated compounds. For classes of chlorinated compounds that are highly volatile, their intake is usually through inhalation. Plants could absorb soluble derivatives of these compounds when dissolve and washed into the environment. Some of the most investigated chlorinated compounds due to their inherent toxicity and environmental impact are the organochlorines, most especially pesticides. The most vital pathway for biological exposure to some of these volatile chemicals is through the consumption of food. There is also occupational exposure of humans to these chemicals at work places (Olutona et al. 2016).

7 Processes and Mechanisms in Bioremediation of Chlorinated Compounds

The bioremediation of chlorinated compounds occurs through aerobic and anaerobic processes. Among the class of chlorinated compounds, the chlorinated ethenes are the most commonly detected within the ground water contaminants and soil. Tetrachloroethane (PCE) is capable of resisting aerobic process of degradation and it's the only member with this specific property. Vinyl chloride and the three isomers of dichloroethene are mineralized by phenol-oxidising bacteria and methanotrophic microbes through aerobic co-metabolic processes. The oxygenases with wide group of substrates are responsible for the metabolic oxidation. Some other bacteria also use vinyl chloride as a source of electrons and carbon for their growth. All the chlorinated derivatives of ethane are dechlorinated reductively under anaerobic environment giving rise to harmless compounds as by products such as ethane and ethane. There are existing evidences which show that the oxidation of vinyl chloride takes place in the presence of iron (III) reducing conditions. Perchloroethane is dechlorinated to tetrachloroethane through the action of methanogens, homoacetogens, sulfate reducers amongst others in a co-metabolic process. The dechlorination process is catalyzed by various enzymes which contain tetrapyrrole cofactors such as porphyrins, corrinoids and factor F430. Also, the tetra chloroethane in most bacteria acts as end electron acceptor during respiration process. Most of these isolates dechlorinate these compounds into *cis*-1, 2-dichloroethene, although there are also instances where there is complete dechlorination into ethane (Ferguson and Pietari 2000).

There are various electron donors for the dechlorination of tetrachloroethene and perchroethene which include formate, pyruvate, acetate, lactate, ethanol and molecular hydrogen. The Dehalospirillum multivorans posses a rather wide substrate spectrum, whereas Dehalobacter restrictus only uses hydrogen.

Chlorinated compounds when discharged into the environment are vulnerable to natural processes of microbial breakdown. They commonly act as acceptor of electrons due to the presence of substituent that are highly electronegative. The redox characteristics of the organo halogenated compounds are determined by the nature of the halogen present and the conditions of the reaction. On a general note, the greater the number of halogen present as substituent with higher oxidation state, the more the ease with which reduction occurs. Particularly, the reductive process of dehalogenation is the pathway through which an halogen atom present is reduced and there is replacement of a chlorine atom with an hydrogen atom. This occurs mainly in compounds which have a large number of halogens as substitutes and are totally not affected by aerobic microbes. In the natural route of microbial breakdown, they produce 1,2-cis dichloroethene which is then converted to vinyl chloride, a compound known to be highly carcinogenic. Some of the available studies have reported the potential of Dehalococcoides spp, a bacteria genus in the complete dechlorination of the chlorinated compounds to terminal products (Weigold et al. 2016).

There are various reaction mechanisms and routes such as bioaccumulation, routes of biodegradation as well as various modes of adsorption using plants and microbes that have been reported for the elimination of contaminants.

There are several reactions that are connected with the degradation of chlorinated compounds found in the subsurface under both anaerobic and aerobic conditions. However not all the chlorinated compounds are amenable to the process of degradation through each of these processes. However, the processes of anaerobic biodegradation may have the potential of inducing the degradation of most common chloroethanes, chloroethenes and chloromethanes (Peng and Shih 2013).

i. Anaerobic reductive dechlorination process

This is a process of degradation that is targeted by induced anaerobic bioremediation. The introduction of organic substrate to the subsurface brings about the enhancement of the process converting mildly anoxic aquifer areas to anaerobic zones that are reactive, making them suitable for the anaerobic degradation of chlorinated compounds.

Some of the processes that have been identified to be connected with the degradation of chlorinated contaminants include abiotic transformation, aerobic oxidation, cometabolic anaerobic reduction, aerobic co-metabolism and anaerobic oxidation (He and Su 2015).

ii. Direct anaerobic dechlorination

It is a process in which the bacteria gain energy resulting to their growth since one or more atoms of chlorine are replaced by hydrogen in an anaerobic atmosphere. In this process the chlorinated compounds act as the acceptor of electrons while hydrogen atoms act as direct donor of electrons. The hydrogen that is used up during this reaction comes from the fermentation of the organic substrate.

iii. Cometabolic reductive dechlorination

This reaction involves the reduction of chlorinated contaminants through the action of a co-factor or an enzyme that is non specific. The cometabolic process for chlorinated contaminants does not give rise to growth benefits or energy the microorganisms that mediated the reaction process.

Abiotic reductive dechlorination is a chemical process of degradation, it is not connected with biological activity, the reduction of a chlorinated compound takes place through the action of reactive compounds. Basically, biotic anaerobic processes take place through the stepwise removal of chloride ions.

Findings from existing studies have shown that under natural settings, some chlorinated compounds can be degraded anaerobically into other compounds. For example, trichloroethylene can be broken down into ethylene, vinyl chloride and dichloroethylene. Studies in small scale of in situ anaerobic and aerobic co-metabolic processes of transformation have revealed that local indigenous microorganisms that have been grown on phenol are more efficient for the breakdown of cis-1,2-dichloroethylene when compared to other microorganisms that are grown on methane. Results from modeling investigations also show that the elimination of dichloroethylene is as a result of the biostimulation of the local microbial population. Modeling studies and field tests show that under certain conditions, the degradation process becomes limited stoichiometrically (Atashgahi et al. 2018) (see Fig. 2).

8 Advantages and Limitations of Bioremediation for Chlorinated Compounds

Bioremediation has the advantage for its ease in treating the contaminants in place without need for removal of large amount of sediments, soil or water do not need to be removed before treatment for the removal of the compounds. Bioremediation promising for chlorinated compounds in that it is an environmentally friendly approach for pollution management.

Bioremediation is however limited in its application for chlorinated compounds in situation where there is high concentration of the contaminants within the site hence the need for combination approaches with other processes of remediation. Like some other existing technologies for the remediation of various chlorinated

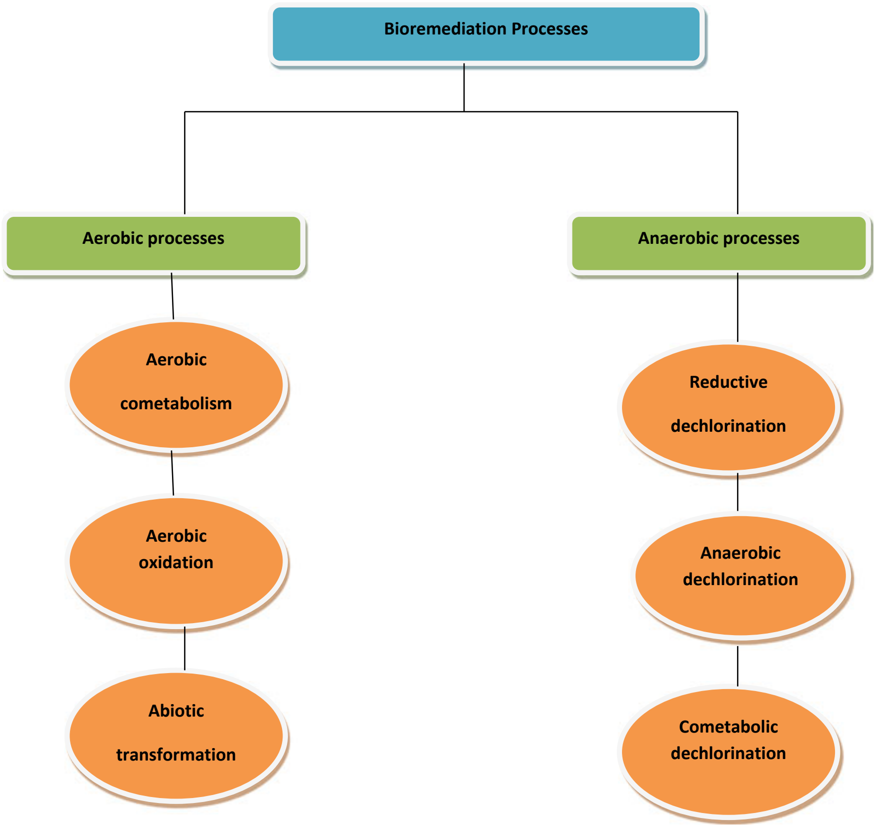

Fig. 2 Bioremediation processes for chlorinated compounds

compounds, bioremediation also has some other inherent limitations in this regards. Some of the contaminants that are highly chlorinated with a high molecular mass such as the chlorinated polycyclic compounds are not easily amenable to degradation by microorganisms. Also in the microbial breakdown of some of these chlorinated compounds, the intermediates and end products in some cases tend to be also toxic and in some cases even more toxic than the parent compounds. A typical example is the reductive dehalogenation of some chlorinated compounds which could result in the accumulation of toxic by product which is the vinyl chloride which has been reported to be carcinogenic. Thus bioremediation approach for some groups of compounds such as the chlorinated compounds requires a comprehensive understanding of the microbial processes as well as the intermediate compounds and byproducts. Otherwise the outcome could be deleterious to the ecosystem (Ghattas et al. 2017) (see Table 1).

Table 1 Advantages and limitations of bioremediation for treated of chlorinated compounds

Bioremediation	Advantages	Limitations
	Ease of treatment	Not suitable for treating highly contaminated sites
	Environmentally friendly for most contaminants	Some intermediates products e.g. vinlyl chloride are more toxic than starting contaminants
	Suitable for different groups of organic contaminants	Not efficient for highly chlorinated compounds with a large molecular mass
In situ bioremediation	Does not require excavation Structural composition of affected site is maintained	Additional cost for the onsite installation of required equipment
Engineered in situ bioremediation	It is a fast process	Requires the supply of nutrients which act as electron acceptors
Ex situ bioremediation	Recommendable for small polluted sites	Cost for conveying and treatment away from the contaminated sites

9 Conclusion and Future Trend

Bioremediation is a fast growing technology that has proven to be highly reliable and promising for the remediation of chlorinated compounds. Its unique potential of being utilized alongside other chemical and physical treatment process makes it further promising. Its sustainability as an approach for the management of chlorinated compounds has been well documented. There is however need for further investigations most especially on the intermediates formed during their degradation and their impact on the immediate environment.

There is need for further investigations in the area of bioremediation applications for the treatment of chlorinated contaminants. It is also paramount in this regard to proffer a synergistic relationship between the environmental effect on behavior and fate of these pollutants as well as the efficiencies of the various bioremediation technologies. Other approaches such as biofiltration could also be incorporated further into bioremediation and adapted for industrial scale application for the treatment of chlorinated compounds. There is also need for multidisciplinary technologies for the efficient treatment of various chlorinated compounds using bioremediation. The need for further studies into the bioremediation of chlorinated compounds is focusing on the methods that have been known to bring about alteration of various environmental parameters and conditions as well as improving the mechanisms through which co metabolic pathway to bioremediation functions. There is also need for evaluating nutritional needs, suitable environments, degradation rate and lag time for various classes of chlorinated compounds. Also there should be more studies with focus on optimization of various environmental conditions, and enhancement of essential growth conditions within site specific differences. Finally there is also need for up to date and reliable studies into bioaugmentation together with the specific microorganism responsible for the degradation of particular chlorinated compounds as well as the detailed mechanism involved.

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Removal of Heavy Metals Using Bio-remedial Techniques

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Abstract Heavy metal pollution has become one of the most significant environmental problems globally leading to ecological imbalance. Different techniques like physical, chemical and biological have been used for removal of heavy metal contaminants from the environment. Some of these have limitations such as cost, time consumption, logistical problems, and mechanical involvedness. Biological strategies, unlike other methods of remediation, are unique in that biological strategies are environmentally friendly and acceptable, the diversity of organisms involved is wide and of diverse capabilities that have not yet been exhaustively exploited and also amenable to genetic modification for accelerated bioremediation. There are several techniques entails with the removal of heavy metals. Therefore, this chapter proposes to present thorough information on some techniques that might be applied for the removal of heavy metals using bioremediation. The encouraging evidence as to the usefulness of microorganisms and their constituents for the remediation of heavy metals from contaminated environment is reviewed in detailed. Recent advances in the application of removal of heavy metals through bioremediation also were highlighted.

Keywords Bioremediation · Remediation · Heavy metals · Techniques

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1 Introduction

The soil contamination through metalloids and heavy metals is a worldwide problem as a result of the accumulation of these compounds in the environment, endangering plants, human health as well as animals. Metalloids and heavy metals are usually there in nature, although the increase of industrialization has cause concentrations to rise compare to the acceptable ones. However, they are toxic and non-biodegradable, this happen at lower concentrations. Deposits accumulate in living beings in addition be converted into dangerous each point in time they are incorporated and pile up more rapidly compared to when they are metabolized. Consequently, the potentially dangerous effects are as a result of persistence in the surroundings, toxicity as well as bioaccumulation in the organisms (Tchounwou et al. 2012; Briffa et al. 2020) (see Fig. 1).

The rigorousness effects depend on the kinds of metalloid or heavy metal. Certainly, several of the heavy metals (such as, Fe, Mn, Ni and Co) at concentrations very low are vital for living organisms, despite the fact that others (includes Pb, Hg and Cd) are nonessential, moreover they are toxic even in trace quantity. It is significant to scrutinize the concentration of metalloids and heavy metals in the ecological system and approve techniques to eliminate them. For this rationale, diverse methods have been created in some years back, these includes: chemical remediation (which includes catalysis, adsorption, solubilization/precipitation, electrokinetic techniques), physical remediation (such as thermal desorption, washing, solidification), biological remediation (viz: phytoremediation,

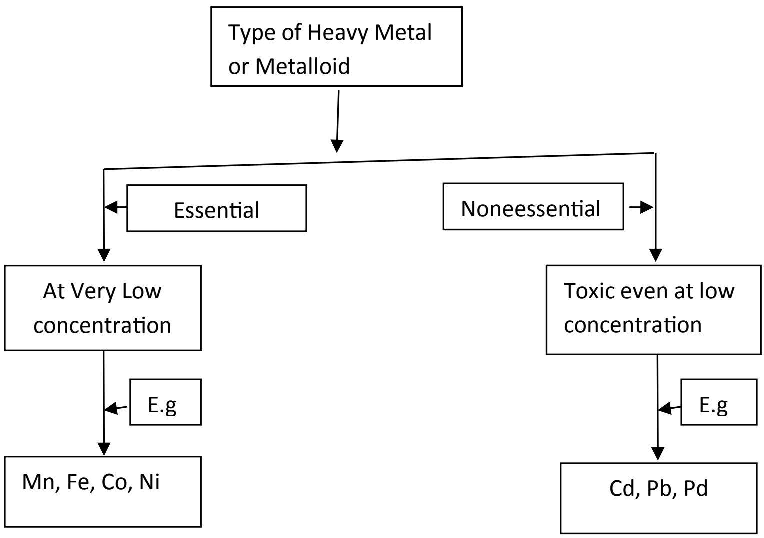

Fig. 1 Type of heavy metal or metalloids concentration

biodegradation, bioventing), and combined remediation (including washing–microbial degradation and electrokinetic–microbial remediation;) (Fig. 2) (Raffa et al. 2021).

In the study of Dhaliwal et al. (2019) revealed the pollution obtained from heavy metal which is part of the severe problems and infects the environment through diverse ways amid the blow of manufacturing in numerous nations. Many techniques such as chemical physical, as well as biological have been utilised for elimination of heavy metal pollution from the environs. However, several of these techniques have constraints which include time consumption, cost, mechanical involvedness along with logistical problems. At the present time, phytoremediation, in situ immobilization of metals as well as biological techniques seem to be to the best way out for removal of metal/metalloids from the soil. They targeted contaminant site for remediation which is to restrict the heavy metal to go through into the soil, food-chain, along with the introduction to human beings. In the other hand, the sort of technique applied for a given location depends on the features such as usual developments take place at the polluted location, type of chemicals, soil type, and the intensity of polluted site.

Typically, heavy metals are channeled to environment through the disposal and processing of heavy metal containing manufactured goods. Pollution of the environment caused through the heavy metal enhances awareness globally as a result of their toxicity in animal, human beings as well as plant along with their inadequate of biodegradability. As soon as the metals are polluting the ecosystem, they possibly will continue for some period depending on the kids of metal that that is present in the site. The process of remediation for heavy metal polluted sites might be ex-situ or in-situ, biological and off-site or on-site, chemical as well as physical. Furthermore, many of these methods applied in mixture through each other intended for more cost-effective and proficient remediation of a heavy metal polluted environment. Remediation using biological means in biotransformation of heavy metals into non-harmful type was look into, in the study. The molecular mechanism of heavy

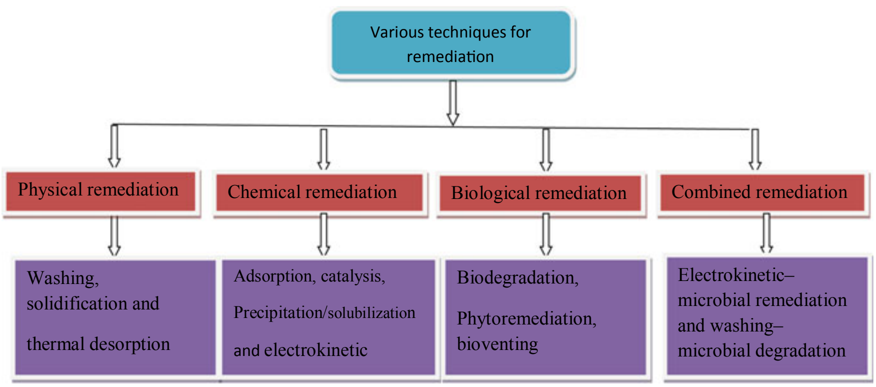

Fig. 2 Types of techniques in bioremediation

metal buildup has often biotechnological insinuations for bioremediation of polluted metal sites (Madhuppriya et al. 2020).

Also, Akhtar et al., (2020) in their study, opined that contamination of heavy metal has turn out to be one of the major momentous environmental problems worldwide leading to environmental disproportion. Several of the techniques such as biological and physicochemical are consider for the elimination of heavy metals. A good number of the physical and chemical techniques are less cost-effective and less eco-friendly, at the same time the biological techniques are not fast in reactions. Nanoparticles, in recent times, have been recommended as proficient substitutes to obtainable treatment techniques, in altogether supply maintenance as well as ecological remediation of compounds generated from anthropogenic activities. Nanotechnologies are persistent way out vectors in our monetary ecosystem. Synthesis of biological nanoparticles has developed noticeably to generate novel resources that are cost-effective, eco-friendly as well as established with enormous significance in wider use in the regions of medicine, agriculture and electronics. Consequently, they focus on a proportional remediation of heavy metals by means of chemical, biological and physical techniques. The nano-structured copper iodide is applied as an adsorbent in the process of eliminating zinc and chromium. The techniques used in the removal of heavy metal in the study are; chemical (UV photocatalysis by the application of CuI), physical (UV light irradiation and adsorption studies by means of CuI) and biological techniques (by means of co-culture bacteria strains). A grouping of biological and chemical techniques was in addition investigated by means of CuI–polyvinyl alcohol nano-composite consists of bacterial co-cultures.

Liu et al. (2018) in a related study, used techniques of ex-situ and in-situ remediation to remedy the heavy metal polluted sites, such as encapsulation, surface capping, soil flushing, landfilling, electrokinetic extraction, soil washing, solidification, stabilization, phytoremediation, bioremediation and vitrification. These remediation methods make use of restraint, removal/ extraction, and immobilization schemes to decrease the pollution effects by chemical, physical, electrical, thermal as well as biological remedy developments. These methods display precise disadvantages, applicability and advantages. In addition, the technique of in-situ soil remediation tends to be more cost-effective compared to ex-situ treatment, and pollutant extraction/removal is more encouraging than containment and immobilization. Among the accessible soil remediation methods, chemical stabilization, phytoremediation and electrokinetic extraction are at the advance phase, whereas some of the others have been adept at complete, field scales. Comprehensive evaluation point out that chemical stabilization proves a momentary soil remediation method, phytoremediation requires enhancement in effectiveness, serious-contamination sites, landfilling and surface capping are related to small, while vitrification and solidification are the most recent remediation alternative. However, treatability studies are vital to decide on practicable techniques for a soil remediation scheme, with considerations of the degree and type of pollution, site characteristics, remediation goals, implementation time, public acceptability and cost effectiveness.

Awasthi et al. (2022) study the penalty of heavy metal pollution progressively mortifying soil eminence in this current era of industry. As a result of this reason, enhancement of the soil eminence is essential. The application of plants to eliminate toxins from the soil, like trace elements, heavy metals, radioactive substances, and organic chemicals, is said to be bioremediation. Fly ash and Biochar techniques are evaluated for efficiency in enhancing the quality of polluted soil. They compiles amelioration methods and how they are applied in the field.

With their toxic effluent, municipal wastewater, or slurry comprising a divers of heavy metals, anthropogenic and industries activities all around us pollute our mineral resources. These hazardous metals, in turn, are posing new health concerns to humans, including allergies, infections, deformities, and diseases. As a result, there is a growing demand for environmentally friendly, systematic, and creative approaches of removing these harmful heavy metals. In dealing with a polluted ecosystem, chemical, biological and physical techniques have not shown to be very effective. These traditional methods have drawbacks in terms of energy consumption, efficiency and cost. Overcoming their limitations, adsorption, a chemical and physical surface phenomena, has emerged as a far more cost-effective, reactive, flexible, and efficient method of removing heavy metals such as chromium, cobalt, nickel, lead, arsenic, mercury, cadmium, copper and uranium. Microbes, industrial waste biomass, lignocellulosic material, metal organic frameworks (MOFs), nanotubes, and nanocomposite substance are all used to fabricate a spectacular adsorbent by modifying their chemical and physical features (Mahendra et al. 2021).

Heavy metal contamination has been detected in soils and rivers as a result of anthropogenic and rapid industrialization activities like the uncontrolled use of fossil fuel burning, wastewater sludge dumping and agrochemicals. Heavy metals are non-biodegradable and have a long shelf life. As a result, remediation is needed to prevent heavy metal mobilization or leaching into the ecosystem, as well as to make heavy metal removal easier. Microbes are used in bioremediation to remove heavy metals. Microbes use a variety of bioremediation processes. These mechanisms are one-of-a-kind in terms of their specialized requirements, benefits, and drawbacks, because their success is largely determined by the types of organisms and toxins associated with the process. plants, Heavy metal contamination causes stress to humans, animals, plants, and other species in the ecosystem. To ensure cost-effective and effective processes, a thorough understanding of the process along with various remediation options at several stages is required (Kapahi and Sachdeva 2019).

2 Physicochemical and Biological Methods for the Removal of Heavy Metals

Since the dawn of time, humanity has used plants and natural materials to combat the threat of heavy metal toxicity in both human health and the ecosystem around them. Affected by exposure to about thirty-five metals have been reported as a result of accidental or occupational exposure. Twenty-three of them are heavy metal bands.

The rising use of heavy metals, especially radionuclides, is causing health problems. The presence of heavy metals in the environment, as well as their impacts on humans further down the food supply chain, poses a health risk. As a result, the abolition of heavy metal has become a top priority. Their study shows systematic of books, patents, and scientific material from widely recognized scholarly databases and search engines on plant-based and natural chemicals against heavy metal contamination are presented. It is thought that a variety of phytoconstituents agents, along with microorganisms, could operate as heavy metal removers in both humans and the environment. Bacteria, algae, fungi, and yeast are among the microorganisms that are utilized to remove heavy metals out aquatic environment (Sharma et al. 2016).

Heavy metal contamination has already been recognized as a global problem since the beginning of the industrial revolution. Because of its poisonous nature, heavy metal pollution poses major environmental and health problems. Heavy metal remediation using traditional techniques is inefficient and results in a huge amount of secondary trash. Biological process, on the other hand, including microorganisms and plants, provide simple and environmentally friendly methods for removal of metal ions, and are thus regarded cost effective and substitute metal removal tools. Reduction, adsorption, or removal of pollutants from the ecosystem using biological resources is referred to as bioremediation (both plants and microorganisms). Microorganisms' heavy metal remediation abilities are derived from self-defense strategies including enzyme production, cellular morphological alterations, and so on. These defense systems include the active participation of microbial enzymes which including oxygenases, oxidoreductases, and other enzymes that impact bioremediation efficiencies. Immobilization methods are also enhancing the technique on a large scale (Jacob et al. 2018).

Toxic heavy metal pollution is one of the most serious environmental challenges, and it has accelerated considerably as a result of shifting industrial activities. This review focuses on the most popular heavy metal phytoremediation techniques, approaches, including biological techniques. It also gives a broad review of the role of microbes in heavy metal environmental remediation in damaged ecosystems. Biological and Physicochemical approaches of heavy metals removal are effective measures, with the latter divided into ex situ and in situ bioremediation. The in situ practice such as biosparging, bioventing, bioaugmentation, phytoremediation and biostimulation. Ex situ bioremediation which includes composting, land farming, bioreactors and biopiles. Bioremediation make use of microorganisms that naturally occur such as *Sphingomonas*, *Pseudomonas*, *Alcaligenes*, *Mycobacterium* and *Rhodococcus*. Bioremediation, in general, requires very little work, is labor-intensive, sustainable, inexpensive, environmentally friendly, long-term, and relatively simple to apply. The majority of bioremediation's drawbacks are related to its slowness and time-consuming nature; also, biodegradation metabolites can occasionally be more harmful than the initial substance. Bioremediation summative assessment may be difficult due to the lack of an acceptable end - point. More research is needed to develop phytoremediation innovations and discover more biological remedies for the bioremediation of heavy metals pollution in various ecosystems (Sayqal and Ahmed 2021).

Nanotechnology has engulfed all aspects of life, encompassing industry, medicine and health, agriculture, environmental challenges, and bioengineering, to name a few. Nanostructure materials have transformed every industry. Environmental contamination is a major worry in today's world, affecting both industrialized and advance nations. To address this issue, a variety of techniques have been implemented. The use of nanotechnology in environmental pollution bioremediation is an approach beyond revolution. Several in-situ (bioslurping, bioventing, phytoremediation, biosparging and permeable reactive barrier) and ex-situ (windrows, biopile, land farming and bioreactors) methods are used to accomplish this. Nanoparticles are appropriate for natural applications due to improved qualities such as reduced time utilization, nanoscale size, high adaptability for Ex-situ and In-situ use, indisputable amount of surface-region to-volume percentage for probable sensitivity, and protection from environment components. To cure contaminants, many nanomaterials and nanotools are available. The qualities of foreign compounds and the pollution location influence each of these approaches and nanotools (Hussain et al. 2022).

Aquaglyceroporins, phosphate transporters, and the effective extruded scheme all take in arsenic, which is then decreased through arsenate reductases via a dissimilatory reduction pathway. Arsenic oxyanions are used by some autotrophic and heterotrophic bacteria for energy renewal. Arsenate can be used as a nutrient by some microbes during the process of cellular respiration. In bacteria, decontamination operons are a prevalent form of arsenic resilience. As a result, bioremediation may be a viable and cost-effective method of removing this contaminant from the ecosystem (Lim et al. 2014).

Adsorption on novel adsorbents, membrane filtration, on exchange, reverse osmosis, electrodialysis, photocatalysis and ultrafiltration were among the physicochemical removal techniques explored. In terms of application, their benefits and downsides were assessed. Microorganisms have a function in biological methods of treatment by settling sediments in the solution. Industrial wastewater is treated with trickling filters, activated sludge, and stabilization ponds. Bioadsorption is a novel biological technology that uses a variety of low-cost bioadsorbents (forest waste, agricultural waste, algae, industrial waste, and so on) to remove heavy metals from wastewater to the greatest extent possible. Bioadsorption methods, rather than chemical and physical approaches, are the most environmentally acceptable techniques for removing heavy metals from wastewater. Chemical techniques, on the other hand, are the best treatments for harmful inorganic chemicals produced by several industries that cannot be eliminated through biological or physical means (Gunatilake 2015).

Environmental contamination from pesticides and heavy metals has raised worries about toxic potential to a variety of species, therefore their removal from water is critical. The goal of this study was to use physical and biological approaches to remove heavy metals, pesticides, arsenic, Diazinon and Malathion from water. Particle trapping techniques, which had straws to trap tiny and big particles, were used to physically remove the contaminants (Arash et al. 2018).

Biosorption, reduction/oxidation, bioaccumulation, precipitation, leaching, degradation, phytoremediation and volatilization are among the biological methods currently available or potentially available for removing and detoxifying toxic metalloids and heavy metals from contaminated sediments and water (bioaccumulation, biosorption, reduction/oxidation, precipitation, leaching, degradation, volatilization, and phytoremediation). They also go over the alternatives for recovering metals accumulated through biosorbents (with the right desorbing agents) as well as plant biomass and microbial (leaching through biological processes or chemical reagents, and thermal treatment in controlled systems) (Kikuchi and Tanaka 2012).

3 Transport Mechanisms of Heavy Metals

Due to their environmental permanence, mobility and toxicity in soils, heavy metals have sparked a lot of attention. Owing to the increase of mining companies, pesticide use, as well as other sociocultural activities, certain Chinese soils have been poisoned by heavy metals, causing the agro-ecosystem to have become damaged. Their study focus to provide light on the current state of contamination in China, as well as the sources of heavy metals, their transport modes, and the factors that influence their mobility. Additional studies on source identification and heavy metal movement features in soil will be presented in the future (Jing et al. 2018).

Although a set of genes encoding putative transporters have since been discovered, the methods used in the absorption of necessary heavy metal micronutrients are still unknown. The heavy metal (CPx-type) ATPases, the natural resistance associated macrophage protein family, and members of the cation diffusion facilitator family are the three categories of membrane transporters that have been accused in the transport of heavy metals in a variety of microorganisms and therefore could utilize such an important roles in plant. They hope to provide an overview of the main characteristics of these transporters in plants in terms of function, structure, and regulation, based on research in a variety of microorganisms (Lorraine et al. 2000).

There are three types of mercury fragments: semi-mobile mercury (Hg(0)-metal, Hg(0)), mobile mercury (EtHgCl, MeHgCl, and other mercuric compounds) and nonmobile mercury (EtHgCl, MeHgCl), as well as other mercuric compounds) (HgS, HgSe, and Hg_2Cl_2) (Yao et al. 2019).

Membrane transport of non-essential hazardous heavy metals (type 0 heavy metals) clearly defines their absorption, excretion from the body, and distribution, as well as restricts their access to Intracellular target sites. Membranes have a crucial role Several researchers have focused on the toxicity of class 0 metals, and significant data has been gathered on the mechanism(s) of metal transfer through membranes. Metal transport features are not exactly equivalent in cell populations, or even on separate sides with same cell, or under various physiological circumstances, and no single hypothesis to explain this process in all cells has been proposed. However, it's plausible that the various cell mechanisms hypothesized are variants on a few basic motifs (Foulkes 2000).

Adsorption, desorption and ion exchange, mobilization, aqueous complexation, and biological immobilization, mineral dissolution, plant uptake, and precipitation all influence lead dispersion in soil. Simultaneously, chemical mechanisms such as oxidation–reduction reactions, cation sorption on exchange complexes, and chelation with organic matter are responsible for lead dispersion as well as migration in soils (Kushwaha et al. 2018; Palansooriya et al. 2020).

Under fed conditions, two critical processes occur: iron oxide is decreased, and absorbed arsenic is discharged into solution phase; arsenate [As(V)] absorbed on solid phase is lowered to As(III), which is less effectively absorbed than As(V) and thus has a higher potential to separation into solution phase (LeMonte et al. 2017).

Various physical and chemical processes regulate cadmium's behavior in the soil. Rainfall and adsorption reactions help to keep it in the soil. The fundamental mechanism is rainfall, with anions in the forms of $PO_4{}^3$, OH, CO_2, and S_2, with cadmium adsorption on the surface of soil minerals occurring through both nonspecific and specific mechanisms (Zhang et al. 2018; Wang et al. 2022).

The majority of chromium in soils comes from weathered minerals found in ultramafc rocks. The chromite weathering process has two phases: a manganese oxide oxidation reaction to chromium (VI), and a chromium (III) hydrolysis reaction to $Cr(OH)_3$. Furthermore, chromium is found in significant amounts in spinels, clay minerals, and iron oxides (Christopher et al. 2011; Hausladen et al. 2019).

They leach into subsurface fluids, travelling down water routes and finally settling in the aquifer, or they are swept away by run-off into surface waters, causing water and soil contamination. Toxicity and poisoning are common in ecosystems due to coordination and exchange processes. They mutilate their structures and obstruct bioreactions of their functions when consumed, forming stable biotoxic chemicals (Ruangcharus et al. 2020).

4 Impact of Heavy Metals on Human Health and the Environment

Heavy metals can enter the body through the use of the intestinal system, the skin, or breathing. Toxic metals have shown to be a significant health risk, owing to their propensity to harm membranes and DNA, as well as disrupt enzyme activity and protein function. By attaching to free thiols or other functional groups, perturbing protein folding, accelerating the oxidation of amino acid side chains, or/and displacing critical metal ions in enzymes, these metals disrupt native proteins' activities. The biochemical and physiological implications of hazardous metal interactions with proteins and enzymes were accounted for in their study. Because heavy metal poisoning of the ecosystem is one of the most serious worldwide issues, certain detoxifying procedures are also discussed (Witkowska et al. 2021).

Toxic heavy metal pollution of terrestrial and aquatic ecosystems is an environmental issue that is a public health risk. Heavy metals accumulate in the ecosystem as persistent contaminants and harm food systems as a result. The buildup of potentially hazardous heavy metals in biota poses a health risk to their consumers, who include humans. This article examines the various elements of heavy metals as hazardous compounds in depth, with a particular focus on their environmental durability, toxicity for living beings, and bioaccumulative potential. These elements' trophic transmission in aquatic and terrestrial food chains/webs has significant ramifications for animal and human health. The amounts of potentially harmful metalloids and heavy metals in various environmental components and in the resident biota must be assessed and monitored (Ali et al. 2019; Madiha et al. 2022).

Cadmium poisoning has been linked to bone and kidney damage. Cadmium also has been discovered as a human carcinogen that can cause lung cancer. Lead poisoning affects fetuses, babies, and children's growth and neurobehavioral development, as well as raising blood pressure in adults. Mercury is harmful in both its inorganic and elemental forms, but the organic molecules, particularly methylmercury, that accumulate in the food chain, i.e. in predatory fish in lakes and seas, are the primary routes of human exposure. Long-range transboundary air pollution is really only one source of exposure to toxic metals, but due to their potential and persistence for global atmospheric transmission, atmospheric emissions have an impact on even the most remote places (Rafati et al. 2017).

Also, heavy metals can cause toxicity in some organs of the human body including as neurotoxicity, nephrotoxicity, skin toxicity, cardiovascular toxicity and hepatotoxicity, among other things (Saikat et al. 2022).

5 Recent Reports on Removal of Heavy Metals Through Bio-remedial

In the last few decades, rapid industrialization, increased population expansion, hazardous industrial and urbanization practices have all contributed to the growth of ecological pollution. Heavy metals are one of those contaminants that, due to its toxicity, are linked to public health and environmental consequences. Successful bioremediation can be achieved using both "in situ" and "ex situ" techniques, depending on the kind and quantity of contaminants, site conditions, and cost. Recent advancements in artificial neural networks and microbial gene modification aid in improving "in situ" heavy metal bioremediation at contaminated areas. For the efficient elimination of toxic metals using diverse indigenous microorganisms, multi-omics techniques are used (Oindrila et al. 2021).

Because of the damaging effects of long-term environmental contamination, heavy metal pollution poses a major threat to all forms of life in the environment. At low quantities, these metals are very sensitive and can be preserved in food webs, posing a severe public health danger. Several organic contaminants and metals are still not biodegradable and can persist for a long period in the ecosystem. Traditional chemical and physical techniques of remediation are inefficient and result in enormous amounts of chemical waste. Over the years, there has been a growing and strong interest in the equilibrium of dangerous metals. Biosensor bacteria are both environmentally friendly and cost-effective. As a result, microbes have a range of metal sequestration processes that allow them to have higher metal biosorption capabilities (Tarekegn et al. 2020).

Due to increased industrialization as well as certain other anthropological practices, substantial quantities of heavy metals are already being added to the soil and untreated sewage on a daily basis. Several more heavy metals are already non-biodegradable, so they continue to stay in circulation after being discharged into the ecosystem. Certain heavy metals could cause chronic and deadly disorders in people, as well as impact plants and animals metabolism, if their concentrations above the threshold level. Many of the current physical and chemical techniques for removal of heavy metals from industrial effluents, including ion exchange, electrochemical treatment, reverse osmosis, and precipitation, have not been proven to be cost effective, so a biological perspective might also demonstrate to be a substitute remediation innovation for accumulation of heavy metals. Microbes have developed techniques to resist, metabolize or detoxify heavy metals, including sequestration or active efflux with insoluble or proteins substances. In t heir, it was observed that, the relationship of microorganisms and heavy metals, as well as their uses in heavy metal remediation (Sanjay 2020).

6 Conclusion and Future Trends

Bacteria are one of the most important microbiological options for bioremediation; nevertheless, only a few studies have been conducted in this field, and more comprehensive and comprehensive investigations are needed to get the most out of bacterial systems as "heavy-metal pollution alleviators." Multidisciplinary methods are also required for the effective treatment of various heavy metals employing bioremediation. Further research into heavy metal bioremediation is needed, with an emphasis on strategies that have been proven to change various environmental characteristics and conditions, as well as refining the processes by which the co metabolic pathway to bioremediation works. There's also a requirement to assess appropriate conditions, deterioration rates, and lag times for diverse heavy metal genres. In addition, further research should be done on optimizing diverse environmental circumstances and improving vital growth conditions within site-specific variances. Furthermore, there is a need for current and credible research on bioaugmentation, as well as the specific microbe accountable for heavy metal breakdown and the exact factors involved.

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Municipal Wastewater as Potential Bio-refinery

Shipra Jha and Nahid Siddiqui

Abstract Globally and locally, there is need of acceptable water quality and waste water treatments are precedence. The various conventional waste water treatment methods are used for the removal of particulate matter, organic matter and nutrient load before releasing into river. And these treatment methods include higher cost, higher energy consumption and impact on environment. With increasing research evidence for the impact of contaminated water on environment and human health, wastewater biorefinery is gaining interest. Certain technologies include biorefinery can convert wastewater into valuable product and reduce economic and environmental burden. Due to the potential, to fill the gap between wastewater treatment and biorefinery. This chapter will provide wealth of information on new research on technological interventions on the implementation, design and municipal waste water for biorefinery and promoting a green and cleaner environment.

Keywords Municipal water · Environment · Technologies · Biorefinery · Green technology

1 Introduction

In developing countries the waste disposal becomes big problem due to poor infrastructure, budget limitations and lack of facilities to maintain practical standards. The waste pollutants become the reason behind air, water, and soil pollution, emission of green house gases and source of infection. Hence with the development of biorefineries, waste can be utilized and wide range of valuable products can be produced. With the increase in population, the demand for development of industrial sector and infrastructure also increases which in return dispose different waste effluent in

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environment. The wastewater management is difficult task due to the presence of excessive nutrients discharge in the environment leads to acidification and become risky for human health (Jerbi et al. 2020; Al-Zboon and Al-Ananzeh 2008; Arora et al. 2021; Bailey and Ollis 1986).

Globally, municipal biorefinery are gaining attention due to the waste management and produce products includes heat, fuel, valuable products and energy. Many experimental data provided by scientist using wastewater containing heavy metal, biomass, chemicals, plastics, leather, oil, detergent used in biorefinery (Belinsky et al. 2005; Bhatia et al. 2017). The scientific studies show that there are many operational and technical challenges to achieve economical benefits of biorefinery. The wastewater biorefinery represents complex network and it's important to identify the economical and sustainable development of biorefinery. The research study reported that agricultural waste, used cooking oil and poultry waste can also be used in biorefinery (Bhatia et al. 2020).

Conventional wastewater treatment plant were designed for the treatment of suspended solids, particles and biological oxygen demand even along with trace elements to release treated waste water to land water. For the purpose of reuse of wastewater and complete breakdown of pathogenic microbes, tertiary treatment may be used along with the combination of conventional method. It is important to develop economically feasible methodology for complete removal of hazardous organic compounds and trace elements. Because presently municipal sludge constituents can be controlled by maintaining waste water quality before treatment (Fisher-Jeffes et al. 2014; Burton et al. 2009).

Considering many scientific reports contributed by global research team, it is concluded that wastewater biorefinery has potential to develop various valuable products and provide solution to manage large municipal waste generate daily in urban areas. This chapter gives overview of the types of treatment, characteristic feature of Municipal wastewater biorefinery, potential of biorefinery and design of reactor (Narayanan and Narayan 2019; Carey et al. 2016; Coetzee 2012).

2 Potential of Municipal Waste Water Pollutant

The research studies shows that the municipal waste water contains different pollutant includes pathogens, toxic contaminants, phosphorous, nitrogen, organic matter and dissolved minerals. Most of the cities started collecting waste water from commercial places, household etc. and treating municipal wastewater through centralized treatment plant. The waste pollutant differs from source to source. The composition of waste belongs similar to same sources. In urban areas varieties of toxic pollutant are discharged into water through human activities (Bruin et al. 2004; Kreuk et al. 2007a). In household wastewater pollutant contain number of microbes along with different toxic chemicals due to which chemical and physical methods are used to remove contaminant. Based on physical and chemical approach contaminant are categorised into dissolved impurities, settle able pollutant, colloidal pollutant and suspended pollutant. The main objective behind municipal wastewater treatment to clear off the waste and reuse the water (Kreuk et al. 2007b, 2010).

With the vigorous research, scientist could develop municipal wastewater method to remove almost all the pollutant from municipal wastewater. The treatment plant consists of continuous series of system for the removal of complete pollutants (Details 2011).

3 Concept of Wastewater Biorefinery

Biorefinery are effective way to utilize bioenergy in a durable manner to reduce the usage of chemicals, to secure, to minimize environmental changes. Biorefinery an essential built-in which can convert various substrates into different usable produce includes energy, compounds and substances after extraction or further treatments like chemical or biological (Donofrio et al. 2009).

The operational condition to setup traditional Biorefinery plant considered to be expensive process in terms of social, environmental and economical concern. Due to the high cost of feedstock includes biopolymers, vegetables oil, glucose and diesels, pressurizing the agriculture sector for providing substrate and creating load on ecosystem by releasing heat, it become essential to explore wastewater biorefinery. The basic objective behind the construction of Waste water Biorefinery plant is to generate not only clean water but also the commercial valued products (Donofrio et al. 2009; Rabelo et al. 2011; Drosg et al. 2015; Sas et al. 2021).

Depending upon the types of raw material and technology for product development, Biorefinery are categorized into different seven types which includes Lignocelluloses Biorefinery, Thermo chemical Biorefinery, whole crop Biorefinery, conventional Biorefinery, Two-platform Biorefinery, Green Biorefinery and Marine Biorefinery (European Union 1986, 2009) (see Table 1).

Municipal wastewater plants passes through primary, secondary and tertiary treatment or even sludge treatment either to further utilize for water reuse purpose or to dispose. After the treatment of municipal wastewater reuse for crop production, agriculture irrigation, and sludge used for landfill purpose, store as ground water and for construction sites (EUROSTAT 2014; Fux and Siegrist 2004; Shizas and Bagley 2014). The wastewater treatment plant consist of preparatory treatment, primary, secondary treatment and if needed then advanced treatment to safeguard the quality of environment and public health known as tertiary treatment. A different phase of development used includes small particles or stone removal, waste screening, sedimentation and biological treatment (Fava 2012; Finger and Parrick 1980).

Table 1 Characteristic feature of Biorefineries

Technology used	Sources of raw material	Types of biorefineries	Stage of development
Cell disruption, extraction and product isolation	Seaweed, marine algae	Marine	Pilot plant, Research and Development stage
Biochemical and thermo chemical conversion	All types of raw material	Two platform	Pilot plant
Preliminary treatment, squeezing, partition, extract, isolate and digestion	Green crops and grasses	Green	Pilot plant, Research and Development stage
Initial treatment, enzymatic hydrolysis, fermentation and isolation	Material containing lignocellose like Wood, reed, straw and reed	Lignocelluloses	Pilot plant, Demo stage, Research and Development
Conversion through roasting, thermal decomposing, gasification, isolation, enzymatic synthesis	All types of raw material	Thermo chemical	Pilot plant, Demo stage, Research and Development
Wet or dry grinding, conversion through digestion, fermentation and harvesting or composting	Maize, rye, wheat and straw	Whole crop	Pilot plant and Demo stage
Biochemical conversion: enzymatic catalytic hydrolysis, fermentation, fractionation and product isolation	Plant oil, carbohydrate, terpenes, lignocelluloses rich material	Conventional	Phase-III (Advanced)

3.1 Initial or Preparatory Treatment

The purpose of initial treatment includes removal of stone, inorganic solid or particles and screening of wastewater to enhance water quality to ovoid interference during further processes (Fisher-Jeffes et al. 2014; Fytili and Zabaniotou 2008).

3.2 Primary Treatment

In primary treatment screened sediments, suspended particles, Biochemical oxygen demands and settled particles are easily removed for economical purpose before secondary treatment. The research study shows that primary treatment may reduce pathogens, reduce nutrients concentration in wastewater, harmful organic compounds and trace elements (García-Martíneza et al. 2019).

3.3 Secondary Municipal Wastewater Treatment

The research study shows that chemical and physical method is not effective in case of secondary treatment. In secondary wastewater treatment, microorganisms used in activated sludge, pond or trickling filters known as biological method. In biological method oxidation of some portion of organic material takes place to release products and carbon dioxide, and rest part utilized by microbial growth and development (Ginni et al. 2021; Graedel et al. 2009; Harding 2009). The microorganisms form biological aggregate and separated from sediment tanks known as secondary sludge. Due to microbial aggregates, wastewater can linked to secondary sludge. The ammonia can be reduced by secondary treatment method (Guimarães et al. 2016).

3.4 Advanced or Tertiary Wastewater Treatment

Tertiary treatment utilized for municipal wastewater when there is requirement for high quality water treatment after secondary wastewater treatment. For the removal of pathogenic microorganisms, trace elements, viruses and organic compounds advanced treatment method is used (Harding et al. 2007; Jung et al. 2009; Harrison et al. 2016; Heidrich et al. 2011). By adding coagulant Biochemical oxygen demand and suspended solid can be reduced. Trace elements and organic compounds can be removed by using activated carbon. Through chemical precipitation or microorganisms, phosphorous is easily removed and using nitrification nitrogen content can be removed from waste water (Henze et al. 2008) (see Figs. 1 and 2).

The characteristic feature of Municipal wastewater biorefinery includes:

- To handle complicated and poorly managed wastewater effluent containing diversity in nature and concentration.
- Municipal wastewater treatment require less amount of energy for its operation.
- Easily adaptable system which can adjust internal and external environment.
- Cheaper Cost of waste treatment in robust environment.
- Comparison to conventional biorefinery, wastewater biorefinery deliver more effective ecological service.
- Ability to give double benefit includes more important by-products along with preserving natural legacy.
- Potential to allow easy construction of business model for Eco-Industrial systems.

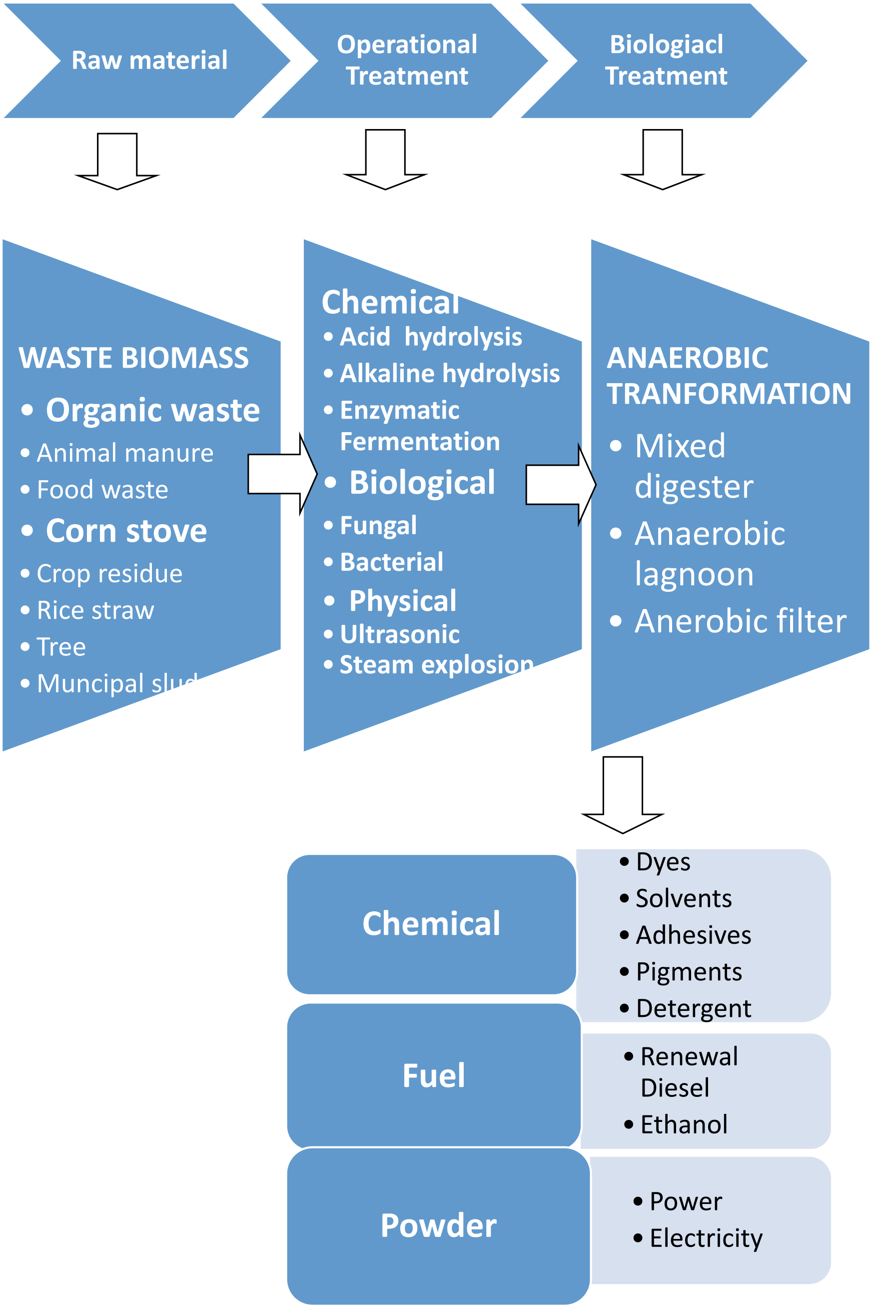


Fig. 1 Flowchart shows potential wastewater biorefinery

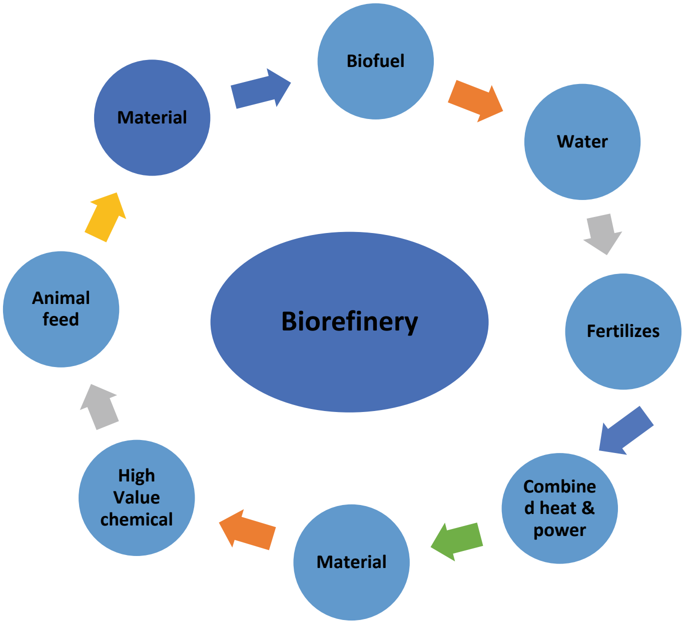


Fig. 2 Modern Biorefinery concept

4 Application of Wastewater Biorefinery

Municipal wastewater nutrients used for producing biomass of Nannocholoropsis species. Municipal water nutrients open up new ways of enhancing economy for the production of valuable product from microalgae. A municipal water nutrient contains growth promoting nutrients and growth inhibiting pollutant (Huang et al. 2011).

4.1 Different Categories of Raw Material for Wastewater Biorefineries

To design the reactor for biorefiney, it becomes very essential to classify the wastewater as feed. Based on the reactor, wastewater classify into three factors –quantity of constituents present, variable constituents and their number present in the wastewater and flow rate. Depending upon three factors, biorefinery scale up to produce desired products (Jackson et al. 2009; Iranpour et al. 2002). Large volume of waste water enters into streams per day and may get diluted with minor to major components. The heavily diluted waste water includes dyes, chemicals, detergent, acids, paints, cooking oil with changing concentrations enters from various sources becomes mega challenge for their treatment. Waste water Biorefineries can be designed based on the volume flow (Coetzee 2012).

4.2 Waste Water Source for Production of Valuable Products

The main objective behind biorefinery, to enhance water quality and number of products which yields after the complete treatment. Due to heavily dilute wastewater, desired product can be recovered by proving different growth condition through microbial activity (Jeong et al. 2010). The wastewater biorefinery products can be classified into three types: [a] to generate power or electricity, biogas produced under anaerobic tank [b] break down of large molecule into smaller one includes ethanol which can further utilized for complex industrial products and [c] third classified group includes super molecules with simple purification functional based products include soil conditioners, bioflocculants (Kleerebezem and Loosdrecht 2007; Kosaric et al. 1984). During the course of wastewater treatment, there are various valuable products includes alginate, pigments, organic acids, volatile substances, enzymes and polymers produced at the end of the treatment cycle. The behaviour of products always depends upon the bioactive agent and type of wastewater treatment method (Koskan et al. 1998; Lalloo et al. 2010).

4.3 Wastewater Biorefineries Source for Irrigation

Globally, waste free irrigation water is main concern due to limited clean water. The scientific studies reported that with the advancement in drainage system, crop development process, waste water which is of low quality can be used for crop irrigation (Lettinga 1995; Li 2009; Liu and Tay 2004). Low quality water not only affects crop development but also interfere in soil properties (Libutti et al. 2018). Many research studies shows advantage and disadvantages of wastewater irrigation which affects the plant growth includes chlorophyll content, length of leaves, root length, seed size, soil quality in terms of nutrients and may increase salt concentration. Limited water resources are forcing to invite more studies to explore waste water treatment method. And the concept of Municipal waste water biorefinery used for treating contaminated water to improve water quality. The key aim of wastewater biorefinery to use distinct unit to release clean water and to produce variable products after complete treatment of wastewater (Baghel et al. 2018).The research studies indicated that multiple waste includes poultry waste, used edible oil, agro-industrial waste can also be used in biorefinery for water treatment and treated water can be utilize for fast-growing plant irrigation which may help soil to hold nutrients (Mitra and Sandhya Mishra 2019; McCarty et al. 2011).

4.4 Prevent Waterborne Pollution

Water clarification is essential part of biorefinery treatment system which involves removal of floating matter and suspended particles. Through the process of adsorption, ion-exchange, reverse osmosis, precipitation and oxidation heavy metals are removed. Adsorption is considered as most efficient in removing heavy metal as compared to other methods due to high cost and operational limitations. Slit and sand can also be removed by using equipment and washed water may be reused for irrigating plants (McCarty et al. 2011; Mohan and Ramesh 2006; Wang et al. 2018).

5 Integration of Waste Water Treatment

Increasing population and urbanization around the world has resulted in scarcity of water even in areas which were initially rich in water resources and water supply. This brought up the need to reclaim the wastewater and then reuse it for non drinkable purposes. Therefore the purpose of management of wastewater from different sources, gained importance which would decrease the burden of water pollution as well as use the treated waste water for purposes other than drinking and washing (Narala et al. 2016; Narayanan and Biswas 2015; Pandey et al. 2010). New technologies and methods are in place to help treat the waste water coming from different sources. Combination of the conventional and modern treatment processes are employed to reclaim the water to its original quality. The use and reclamation of waste water for irrigation, agriculture and landscaping is the most cost effective solution for protection of environment and also to mitigate the lack of water resources around the world. Integration of water and reclaimed water is done effectively. Planning with respect to the facilities like site of wastewater treatment plant, reliability of the treatment process, financial support and also future use of the reclaimed water, quality of water, regulatory mechanisms etc. is necessary for this to have a sustainable method in place (Rabelo et al. 2011).

Treating wastewater is done by the combination of chemical, Physical and biological processes. Also, these treatment plants require high-cost infra structure, highly skilled workers, and lots of energy. Constant efforts are being made to find an alternative and cost effective approach for the wastewater treatment. Wastewater rich in nutrients is produced from different industrial sources such as municipality, textile, pharmaceutical, dairy, food and many others (Ramaswamy et al. 2013; Richardson 2011; Ren et al. 2019). Being rich in inorganic and organic substances, these cause eutrophication in the environment, which is harmful for the environment. Eutrophication mainly affects the irrigation, agriculture, fisheries and causes the growth of different microbes and pathogens in the environment (Li 2009; Richmond and Cheng-Wu 2001). Above all, if left untreated, it will contaminate the ground water, soil and air as well, adding to the pollution woes of the environment and causing a potential damage to the ecosystem. Traditional methods use the physical and chemical methods of treatment but these are expensive and generate sludge/ slurry, which is again needed to be, treated (Guimarães et al. 2016).

Using the traditional method of treatment, pose a lot of challenge to the wastewater industry. Methods have to be adopted to valourize the wastewater, turn it into a useful resource and at the same time reduce the environmental and economic load. Researchers are exploring different methods for treatment and valourization of the components of the wastewater (Ramaswamy et al. 2013). Strategically optimization to enhance the effectiveness of the waste treatment, and better utilization of the bioresources with regard to the impact on environment and economy. Therefore, integration of environmental engineering and Bioprocess technology seemed to be the need of the hour which would help to produce useful and sustainable products from the waste and at the same time help improve the environment and help in the remediation of contaminants. Wastewater used as nutritive substrate followed by further treatment yields useful bioproducts which can be put to use (Saratale et al. 2020; Sheik et al. 2014). This can be termed as Wastewater biorefinery. When put into action, the wastewater biorefineries can extract the valuable components from the wastewater, valorize them and reinsert them for economic use and at the same time bring up the remediation of the pollutants. These products must be easily recoverable and should fulfil a role in ecology, for which sturdy treatment system should be maintained. The biorefineries should be able to produce fewer footprints, should be strong and resilient, should have the capability to generate more than one co-product and should need least energy for operation. In addition to these, the competitiveness of the biorefineries can be improved by considering systems based of renewable resources such as sunlight, gravity based flow systems for the economic, social and environmental issues. Waste water biorefinery can aim to enhance the wastewater industry by improving through industrial ecology. So that environmental and ecological sustainability can be achieved. Taking care to achieve the better final water stream is of top priority of the biorefineries (Shizas and Bagledy 2004).

One of the effective and sustainable approaches appears to be the integration of Microalgae in wastewater treatment (Ramaswamy et al. 2013). Microalgae grow in waste water and convert sunlight and carbon dioxide into biomass. The biomass produced contains different biomolecules like lipids, carbohydrates and other important organic compounds which are further utilized for the production of bio fuels. And these can have other applications (Bhatia et al. 2020; García-Martíneza et al. 2019) as these cells do not use the energy for the growth and development but store it within them. This stored energy with the biomass can be used for the production of biofuels, so, technologies based on such biorefineries have taken up a lot of interest of researchers. The Biological waste water treatment using microorganisms to degrade the pollutants is an integral step of the treatment system. Protozoa, Algae bacteria, fungi, nematodes are used for the breakdown of unstable organic wastes to convert them into stable inorganic forms, using aerobic or anaerobic methods (Shizas and Bagledy 2004). Microalgae based technologies are the most viable methods of waste water treatment which allows almost 100% of the recovery of the nutrients from the waste water. The wastewater environment is non-sterile in nature, thus the microorganisms for the product formation should be selected accordingly such that the microbial ecology is maintained. Also beneficial culture conditions and products are selected which can have a selective contribution to the microbial community of interest (Show et al. 2017).

Two-step degradation process is used for the production of biofuels.

1. Microalgae are grown in the wastewater aerobically.
2. Biofuel is produced from the biomass anaerobically.

   Biodiesel can be produced from lipid while as fermentation of other components of the biomass can be yield other biofuels (both liquid and gas) like as bioalcohol. Dark fermentation can be used for the production of biohydrogen and anaerobic co-digestion to yield biomethane (Stafford et al. 2013).

Microalgae can be cultivated in different types of cultivation systems on a small scale as well as large scale (Stefanakis and Tsihrintzis 2012a, b). Choice of the system depends upon the type of the microalgae selected, availability of type of nutrients and the utilization of the biomass thus produced. Open and closed system of cultivation is most preferred but advanced method so cultivation is also available, but at times these might overlap. For large scale production, Open System cultivation uses open spaces such as ponds, tanks for cultivation. These are low cost, and more economical as compared to the closed systems, but have few disadvantages. Since these are open, evaporation of water, Co_2 diffusing in the atmosphere causing pollution, poor utilization of light by the cells and requirement available land for the cultivation are a few disadvantages with this system (Verster et al. 2013). Open systems there are not preferred for pilot scale production. Closed systems, most appropriately calls Photobioreactors (PBR) are preferred as there is no direct exchange of gases and no contaminants in the surrounding environment can affect the system (Berg 2009).

Another study based on the integration of the Willow as biorefinery was evaluated with primary effluent wastewater irrigation was done. Wastewater irrigation led to increase as lignin, phytochemical and glucose yield in the biomass. Also this could treat waste water in a more sustainable manner (Shizas and Bagledy 2004).

6 Bioreactor Design Requirement of Wastewater Biorefinery Includes

Large volume reactor-

- Semi continuous or Continuous flow.
- Large commodity.
- Decouple hydroluric and sludge retention time.

Complex variable

- Targeted non-sterile or microbial community.
- Create environmental niche and target to product benefits.

Environment

- Allow to flow water into environment.

Downstream processing

- Product can formed in different phase.
- Recovery of product.
- Design reactor for load balancing and elimination.

Reactor designing to increase residence time

- Recovering before cell settling.
- Recycle after settling.
- Rectors in parallel.

7 Designs

Bioreactor refers to any device or system which may be manufactured or engineered which a biological system with active environment, in which growth of microorganisms can take place. Recovery of the useable resources from the wastewater can be done using a Biorefinery reactor, the design of which is optimized as per the requirement. Downstream processes (DSP) are more developed, easily adaptable in a given reactor in which the separation mode is depended upon a few properties such a size, charge, solubility, separation properties and volatility (Sterr and Ott 2004; Stuart and El-Halwagi 2012). Primary objective of an environment efficient and a cost effective DSP is to obtain easy recovery from the bulk material and to reduce the amounts of different unwanted components. Design of the reactor towards product recovery is done which will reduce the loading on the DSP operation units and at the same time efficiency can be increased. The flow rates of gas (i.e., air, oxygen, nitrogen, carbon dioxide), pH, temperature, and agitation speed/circulation rate and dissolved oxygen levels, need to be monitored and controlled (Takkellapati et al. 2018).

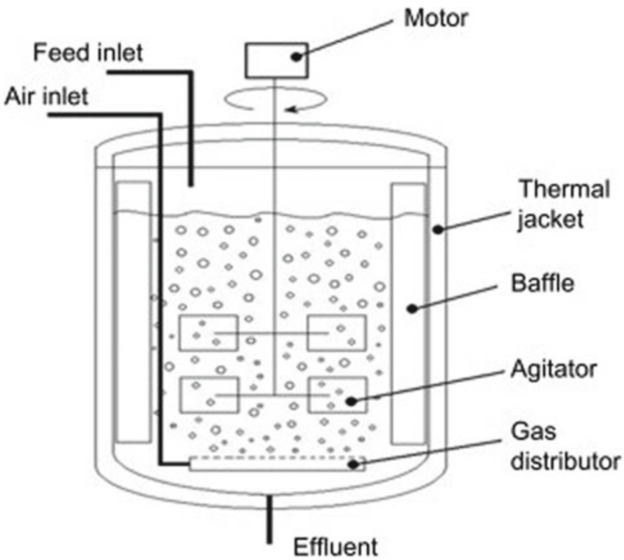
A few challenges are encountered with respect to the current design of the reactors. Firstly, Optimization of the system as a single unit will pose a challenge as it will not ensure desired results, therefore, a systems approach is needed where other productions are also considered along with the reactor design to have the maximum utility of the reactor functionally as well as deliver high productivity. Aerobic reactors working at low substrate concentration are the primary source of employment of energy, with increasing cost of energy supply this design needs to be reconsidered (Sung et al. 2007; Taniguchi et al. 2005).

Various types of Biological waste water treatment Biorefinery reactors are in application as below.

7.1 Stirred Tank Reactor for Aerobic Treatment of Waste Water

This process which has undergone large number of diversifications and modifications is one of the oldest methods of biotechnology for wastewater treatment. An activated sludge process employs a tank which is an agitated vessel in which the inoculum of the microbial sludge is introduced. Air at high pressure is introduced from the bottom to provide sufficient amount of dissolved oxygen to the medium in the tank. Due to large volume of the tank and less solubility of atmospheric oxygen in the medium, large volumes of gas has to be introduced in the tank, requiring huge compressors so that aerobic conditions inside the tank are maintained. The constraint with this type of a system is the high cost of the compressors although the system can be easily designed and installed (Tsihrintzis et al. 2007).

Oxidation of the dissolved organic matter, denitrification and nitrification can be achieved in this process. Conventionally, the system requires two stirred tanks in series-in the first one has aerobic conditions in which carbon removal and nitrification occurs and in the second tank denitrification is done anoxically. Using a number of small sized tanks in series or dividing the tank in various compartments are the different types of modifications in this technique, where in the cascade remains the same as in a single tank, but improves performance, as increased BOD destruction occurs, also reducing the deadzones and bypass streams (Tsihrintzis and Gikas 2010).



Source C.M. Narayanan and Vikas Narayan, Biological wastewater treatment and bioreactor design: Sustainable Environmental Research (2019)9, Article number: 33 (Kreuk et al. 2010)

1. Stirred tank reactor for Anaerobic treatment of waste water:

This process involves the treatment of wastewater using culture of microbes which are acidophilic, methanogenic or acetogenic. The products thus produced are converted into useful products such as Biogas. The sludge digested anaerobically can be used as a fertilizer directly or can be used in the production of phosphate rich fertilizer. This process this slow and the microbes especially the methanogenic ones can be sensitive to pH and temperature changes (Berg 2009).

Although not that economically cost effect, this process can be used with thermophilic microbes as well, the cost of the installation of the heating pipes may prove a constraint (Belinsky et al. 2005) Mesophilic bacteria can also be used.

These can be horizontal, vertical or tube like are the close systems, considered easiest to scale up. Algae and the growth media are continuously circulated through the tubes using a mechanical pump. A whole range of algae such as Chlorella, Porphyridiumetc can be successfully grown on a pilot scale using tubular photobioreactors. Problems like unfavourable Co_2 and pH gradients and high levels of Dissolved oxygen are a few problems encountered with this system (Verster et al. 2013).

7.2 Flatplate Photobioreactors

These are ideal for large scale indoor and outdoor conditions. Less accumulation do dissolved oxygen, lots of solar light on the plate and easy to use modular designs make these a better choice. Temperature controls, algal film formation on the plates, hydrodynamic stress are some of the drawbacks with this method (Sung et al. 2007).

7.3 Plastic Bag Photobioreactor

Plastic bags with a diameter of 0.5 m with aerators attached to it are use as photobioreactors, vertically hung inside plastic or metal cages and kept exposed to sunlight. Air is pumped from the bottom and the microbes are continuously mixed with the air. The drawback with this is the poor mixing of the microbes and air leading to the destruction of the cultures (Verstraete and Vlaeminck 2011).

7.4 *Packed Bed Biofilm Reactors*

Support particles such as activated carbon particles, silica granules, polymer beds etc. Microbial cells surround each particle forming a biofilm. The aggregation of particle and biofilm complexe form a distinct phase in the Bioreactors. Microbial cells in the biofilm grow and multiply till the thickness of, $\delta = 0.3$–0.5 mm is reached after which they slough off from the particle surface and get replaced by fresh cells on the particle. High rate of bioconversion is achieved as the biofilm thickness is low and the concentrated cell mass on the biofilm is high. Since the unconverted substrate and the product accumulation in the biofilm does not occur, the substrate and product inhibition for the growth of microbe is low in such bioreactors (Vymazal 2002).

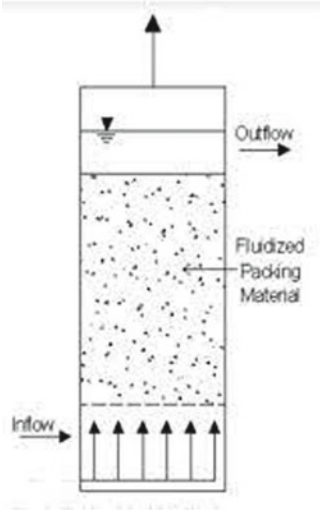
7.5 *Moving Bed Bioreactors*

As the name indicated in this system, the bed of the particles is not fixed in the column rather it is a fluidized type of a reactors in which the particle-biofilms aggregates move against the current of water. The aggregate is fed to the stirred tank bioreactor and remain suspended in the liquid substrate (wastewater) in the tank. Under aerobic conditions, Air sparged in at high pressure also keeps these aggregates suspended and moving in the liquid, thus the name moving bed. The microbes grow attached to the support particles so there is no need of the sludge to be recycled as the microbes do not leave the bioreactor. Batchwise method of operation of this system is preferred although it can be operated in the continuous mode as well. The biomass concentration is substantially high in the biofilms, BOD removal is high but a little resistance in the transfer of substrate in the biofilms is encountered (Yen et al. 2019).

The denitrification and aerobic tank bioreactors can be operated for the activated sludge process using the moving bed system.

7.6 *Fluidized Bed Biofilm Reactors*

These bioreactors can be operated at high velocity and flow rates. Used for large scale production, the industrial effluent enters from the bottom of the column at high velocity due to which all aggregates remain suspended due to the upstream flow of the fluid. The bioreactor performance is enhanced as all the biofilms are surrounded by the fluid on all sides making an intimate contact with the aggregates. The total volume of the reactor increases due to the expansion of the bed. Advantage with this reactor is that the pressure drop across the bed is close to constant (Show et al. 2017; Yen et al. 2019).



Source Neha Baghel*, Satyam Sopori, Bagwan Mohamadazrodin, Ashpak Rafik Saudagar, Saudagar Ajharoodinournal of Advances and Scholarly Researches in Allied Education, Vol. XV, Issue No.2 (Special Issue) April-2018, ISSN 2230-754 (Stafford et al. 2013)

8 Conclusions

Many wastewater treatment programme and waste segregation at community level result in the removal of contaminant from sewage water. Treated waste may increase the load of contaminant in soil which comes from sewage sludge, agricultural pollutant. The final product of municipal wastewater treatment is sewage solid or mud containing contaminant removed from wastewater effluent. In sewage mud containing nitrogen and phosphorous as nutrients similar to other organic compost which can be used as soil conditioner and improves soil properties. Currently, financial benefits for sewage mud are argumental issue.

With the increasing rise in biofinery technology, municipal wastewater treatment plant releases improved water quality for non-portable use. It has been found through many research studies that land irrigated continuously with treated polluted water which motivates municipal wastewater biorefinery to use for crop irrigation. Municipal wastewater biorefinery can be new key source to solve irrigation water deficit problem worldwide. In future with proper reactor designing, improved operational conditions, varieties in raw material may significantly enhance the irrigation water problem.

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Phytoremediation of Metals and Radionuclides

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Abstract Heavy metals and radionuclides are the hazardous pollutants that are need to be remediated from environment for safer health and well-being. Heavy metals are leached out into the environment via numerous activities. Industrialization and modernization of radioactive applications and metal-based compounds have ended up in environment. Wetlands paves greater advantage via removal of pollutants in a natural process. This chapter highlights the phytoremediation of metals and radionuclides compounds in a sustainable and economical way. Apart from plants, microbes were also used in phytoremediation process. Metal binding particles were also employed for enhanced microbial assisted phytoremediation process. Phytoremediation is a chemical free pollutant removal process, eco-friendly and environmentally safe, etc. However further research are needed in effective processing of Phyto remediated biomass source.

Keywords Phytoremediation · Heavy metals · Radionuclides · Microbial remediation · Wetlands

1 Introduction

The increased requirement for resources from industry, military, nuclear weapon industry and agricultural site leads to huge contamination on earth environment, i.e. major cause of accumulation of the toxic pollutants including heavy metals, radionuclides, and organic contaminants in terrestrial and aquatic (surface waters, and groundwater) fauna and flora, this is due to infiltration of human activities, lack of knowledge and carelessness (Thakare et al. 2021; Singh et al. 2022b) responsible for the highest damage in the ecosystems. Most of the heavy metals i.e. Mercury (Hg),

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Lead (Pb), Nickel (Ni), Cobalt (Co) or Chromium (Cr), Zinc (Zn), Arsenic (As), Copper (Cu) etc. present in ecosystem beyond permissible limit, are toxic in nature and get accumulated through the food chain; contaminate drinking water reservoirs and freshwater habitats leading to serious ecological and health effects. It alters the structure and physiological functioning of all form of living beings (Microorganisms, human, animal and plants). On account of their adverse effects, the presence of metals in water and soil poses serious challenge to environmental managers as the remediation options are not only limited but also expensive. As the heavy metal takes long time to degrade and there are lot of physical (carbon adsorption, air stripping, precipitation, extraction and membrane separation technologies), chemical (chemical extraction and leaching, hydrolysis, etc.) (Jhilta et al. 2021) and biological (biosorption and biofiltration) methods are available for example these are the most economical method and take more time to implement or accomplish. So, to overcome this problem researchers attempted to evaluate the potential phytoremediation, it is an advantageous technique which is comparatively one of the safer and cheaper for heavy metal removal. There are different types of phytoremediation, they all are potential approach for the remediation of heavy metals, metalloids, and radionuclides and most frequently used phytoremediations are phytodegradation, phytoextraction, phytofiltration, phytostabilization, and phytovolatilizationare (Singh et al. 2022b).

Plants have the highest capacity of up taking nutrients and mineral so they are highly magnetic towards contaminants present in terrestrial and aquatic ecosystem (Barbosa et al. 2016). Phytoremediation is an *in-situ* method which involves the straight use living plants for the degradation of contamination form, land, oil sludges and aquatic environment, this is because of plants use the energy from of solar power and this pollution removal is totally solar energy mediated method with low cost (Jhilta et al. 2021).

As mention above most of the other technology are economically expensive and could be applied on mini scale, unlike phytoremediation is proved by researchers for application in large scale and economical (Jhilta et al. 2021). The use of a biological material is an emerging and environmentally friendly technology with great prospects to effectively clean up toxic metals at low concentrations and possible recovery for re-use in industry. The energy savings and environment benefits associated with phytoremediation activities are also quite significant and novel approach. This is an emerging technology which will use for the treatment of polluted sites and produce guidelines on the use of plants for the remediation of contaminated land, enabling transfer of the approach to other sites.

2 Hazardous Wastes

Hazardous waste is defined as any waste that has the possibility of causing irreversible harm to human health or environment due to its physical, chemical, biological or infectious qualities. It is a waste material whose chemical composition renders it potentially hazardous to humans. These wastes are flammable, corrosive, poisonous and reactive compounds that pose a threat to safety, human health and the environment (LaGrega et al. 2010).

Heavy metals are categorized as harmful substances in the environment. Even at extremely low quantities, non-essential heavy metals are hazardous to plants, animals, and people. At high doses, even essential heavy metals have negative health impacts (Huat et al. 2019). Arsenic (Ar), chromium (Cr), zinc (Zn), lead (Pb), mercury (Hg), cadmium (Cd) and copper (Cu) are examples of common heavy metals (Kim et al. 2019; Shah 2020). Heavy metals are described as metallic elements with a higher density than water. Heavy metals are also termed trace elements due to their appearance in trace amounts (less than 10 ppm) in different environmental matrices (Kabata-Pendias 2000). Physical parameters such as temperature, adsorption, phase association and sequestration all have an impact on their bioavailability. It is also influenced by chemical parameters such as speciation at complexation kinetics, thermodynamic equilibrium and lipid solubility. Biological variables such as species features, trophic interactions and physiological or biochemical adaptability are also significant (Tchounwou et al. 2012).

Another significant aspect that endangers human health is radioactive pollution. The nuclear power sector has reached a critical stage in its evolution after decades of tremendous expansion. Nuclear technology's use in medical, industry and farming has also accelerated. With the fast advancement of nuclear power and its technical uses, the disposal of many forms of radioactive waste has become a concerning issue. However, with the uncertainty of long-term temporary storage, the potential safety concerns of radioactive wastes rise due to inadequate waste management (Xu et al. 2021). The most significant sources of radioactivity in the environment include radionuclides from the natural radioactive series of uranium (^{238}U), thorium (^{232}Th), and actinium (^{235}U) and non-series potassium (^{40} K) found in the Earth's crust. Based on the geological and geochemical structure of the region, the activity concentrations of these radionuclides differ from one location to another (Kayakökü and Doğru 2020).

2.1 Heavy Metals: Sources and Toxicity

Since the Earth's origin, these heavy metals have been found naturally in the Earth's crust. The massive growth in the usage of heavy metals has resulted in an impending influx of metallic compounds in both the aquatic and terrestrial environments (Gautam et al. 2016). Heavy metal pollution has evolved as a result of anthropogenic activity, which is the primary source of pollution, mining, smelting, metal leaching, waste dumps, excretion, animal and chicken dung and runoffs. Natural sources of heavy metal contamination include metal corrosion, metal evaporation from soil and water, volcanic activity, sediment re-suspension, geological weathering and soil erosion (Briffa et al. 2020; Masindi and Muedi 2018).

Heavy metal pollution in water and soils has increased dramatically in recent decades as a result of fossil fuel combustion, electronic waste, municipal waste disposal, pesticides, mining and smelting, fertilizer and sewage (Kim et al. 2019). Heavy metals are non-biodegradable contaminants, and even trace amounts of non-essential heavy metal ions (As, Hg, Pb, and Cd) may be harmful to living creatures. If essential metals including Zn, Cu, and Fe are present in amounts beyond their hazardous thresholds, they can become poisonous (Jaishankar et al. 2014).

Heavy metal increase in the human body causes serious damage to different organs, including the neurological, reproductive systems, respiratory as well as the digestive system (Huat et al. 2019). Heavy metals have been attributed to carcinogenesis, mutagenesis, and teratogenic effects. They produce reactive oxygenic species (ROS) and consequently cause oxidative stress. Numerous disorders and pathological conditions are caused by oxidative stress in organisms. Heavy metals can also be metabolic toxins. Heavy metal toxicity is caused by their interaction with sulfhydryl (SH) enzyme systems and consequent inhibition (Csuros and Csuros 2016). Heavy metals, such as Hg, Pb and Cd, are nephrotoxic, particularly in the renal cortex. The chemical form of heavy metals plays a crucial role in toxicity. The toxicity of mercury is primarily determined by Hg speciation. Patients with cancer and diabetes have increased levels of harmful heavy metals, such as Cd, Cr and Pb, and lower levels of the antioxidant element Se (Ali et al. 2019; Shafique et al. 2011). Human health concerns associated with arsenic poisoning include respiratory, dermal, immunological, reproductive, liver cancer, genotoxic, neurological, and mutagenic consequences (Järup 2003; Shah 2021). Excess Pb consumption can impair children's cognitive development and intellectual aptitude, elevate blood pressure, and increase the risk of cardiovascular disease in adults (Akoto et al. 2019).

2.2 Radionucleotides: Sources and Toxicity

The continual growth of the nuclear industry and other hazardous technologies required for the broad and ever-increasing usage of radiation and radioactive isotopes necessitates an evaluation of the baseline of natural radiation in order to identify man made pollution to secure the population and the ecology (Abd El-mageed et al. 2011). Radionuclides as they are exposed directly to through the environment, aquatic habitats also get a major portion of natural and artificial radionuclides that build on the soil by connecting the rivers (Ligero et al. 2006). The sources of radionuclides are nuclear power plants, Radioactive waste, nuclear explosions and radioisotopes. Many radioactive elements such as radium 224, uranium 235, uranium 238, thorium 232, radon 222, potassium 40 and carbon 14 occur in rocks, soil and water.

The consequences of radioactive nuclide contamination in surface and groundwater sources are serious health concerns that must be addressed. This is highly hazardous when they exceed the drinking water's recommended permissible limit. According to studies, multiple types of fatal tumors caused by radon, a radium descendent, consumed via drinking water could be equivalent to total lungs cancer caused by radon inhalation (Ahmad et al. 2019). Uranium isotopes, radon isotopes, and radium isotopes are the most significant isotopes in ground water. The most significant radium isotopes are ^{226}Ra and ^{228}Ra, both of which have potential health implications (Abbasisiar et al. 2004).

Leaching process carries ^{226}Ra (^{238}U), ^{228}Ra (^{232}Th), and their decay daughters, as well as the single non-series ^{40} K, to water from many types of rock formations, minerals, and ores with large quantities of terrestrial radionuclides. The consumption of radionuclide-contaminated water exposes human internal organs to alpha, beta, and gamma radiations (Ugbede et al. 2020).

3 Pollutant Remediation Strategies

There are various phytoremediation strategies involved for treating the contaminants using plants in order to reduce the heavy metal in soil. Such remediation technologies include phytostabilization, phytovolatilization, phytofiltration and phytoextraction. Figure 1 provides the different strategic approaches on pollutant removal via phytoremediation process. Each of the technologies are responsible to remediate pollutants using plants to extract and remove the heavy metals from soil; by using plants to absorb and release the heavy metal pollutants into the atmosphere as a volatile compound; by using hydroponically cultured plants to absorb and adsorb the heavy metal pollutant from the groundwater; breakdown of organic pollutants by performing the degradation process (Yan et al. 2020).

The performance of phytoremediation techniques can also be improvised by choosing a plant species having potential phytoremediation capabilities like slow growing, plant species should be capable to adapt at any environmental condition and should also be limited to the large scale applications (Kaur 2020). Genetic engineering has also been recently found to be a promising technique for improvising the abilities of plants with respect to the phytoremediation of heavy metals. While comparing genetic engineering with the traditional breeding, genetic engineering has great advantages in order to manipulate plant species with the desirable traits

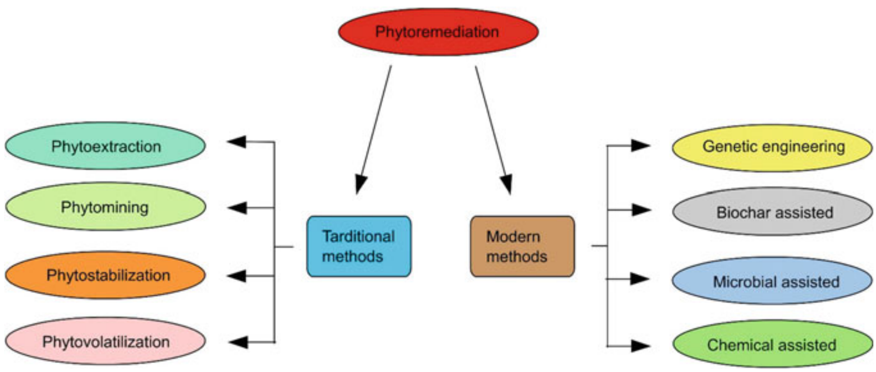

Fig. 1 Phytoremediation strategies on removal of toxic pollutants from wastewater environment

for phytoremediation in a stipulated time (Singh et al. 2022a). Genetic engineering can also help to achieve the transfer the specified genes from hyperaccumulation to sexually incompatible plant species which was found to be difficult with respect to the traditional breeding.

4 Phytoremediation

Accumulation of heavy metals in soil is increasing rapidly due to various natural processes and anthropogenic (industrial) activities. Because heavy metals are not biodegradable, they can remain in the environment, enter the food chain through crops, and eventually accumulate in the human body through bio expansion. Heavy metal pollution is toxic and poses a serious threat to human health and ecosystems. Therefore, remediation of soil pollution is of utmost importance. Phytoremediation is an environmentally friendly approach and can be an effective mitigation measure for cost-effectively revitalizing heavy metal-contaminated soil. To improve the efficiency of phytoremediation, it is essential to have a better understanding of the underlying mechanisms of heavy metal accumulation and tolerance in plants. This review describes the mechanism by which heavy metals are taken up by plants, transported and detoxified. We focus on strategies applied to improve the efficiency of plant stabilization and plant extraction, including the application of genetic engineering, microbial support, and chelation support approaches. Foreword With the progress of industrialization and urbanization, the abundance of heavy metals in the environment has increased significantly in recent decades, raising serious concerns around the world (Suman et al. 2018; Ashraf et al. 2019).

Heavy metals are a group of metallic chemical elements with relatively high densities, atomic masses, and atomic numbers. Common heavy metals/metalloids include cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), zinc (Zn), copper (Cu), nickel (Ni) and chromium (Cr). included. These heavy metals/metalloids, sewage sludge (Farahat and Linderholm 2015), metal mining and smelting (Chen et al. 2016), pesticide use (Iqbal et al. 2016), Electroplating and fossil fuel combustion (Muradoglu et al. 2015). Heavy metals are not decomposed by biological or physical processes, remain in the soil for long periods of time, and pose a long-term threat to the environment (Suman et al. 2018). Heavy metals can be divided into essential and nonessential, depending on their role in the biological system. Essential heavy metals such as Cu, Fe, Mn, Ni and Zn are required for physiological and biochemical processes during the life cycle of plants (Cempel and Nikel 2006). However, it can be toxic if present in excess. Non-essential heavy metals such as Pb, Cd, As and Hg are highly toxic, have no known function in plants (Fasani et al. 2018), cause environmental pollution and are involved in various physiological and biochemical processes of crops. Agricultural productivity (Clemens 2006), which has and can have serious implications. They can enter the food chain through crops, accumulate in the human body through bioexpansion, and pose a major threat to human health.

4.1 Pollutant Removal via Phytoremediation

Water is an essential resource for human life and is polluted by human influence. Industrialization by a more invasive population than other means (Diez-del-Molino et al. 2018). The collapse of such pollutants is the main reason for ecological pollution of all. A single key circle, especially the hydrosphere, lithosphere, and biosphere (Bilal et al. 2018). Waste from the dye industry, power plants, refineries, mines and pharmaceuticals Industrial sector. These contain both toxic and non-toxic metal ions and other components Littering directly into waterways, causing water pollution and causing a lot of health Impact on humans and plants. Cancer, human infectious diseases like humans Carcinogens, neuronal depletion, cholera, typhoid fever, gastroenteritis, diarrhoea, vomiting, skin and kidney problems (Haseena et al. 2017). In plants, it acts on seeds Germination, increased production of ROS (reactive oxygen species), cellular components It also alters various metabolic pathways, affecting plant growth, police yields, and biomass. Production (Stambulska et al. 2018). There are many methods used for treatment Contaminated water for adsorption, ion exchange processes, nanofiltration, agricultural applications, etc. Use of waste, reverse osmosis, distillation, plant engineering, and biological compounds.

Phytoremediation is an effective way to remove harmful heavy metals from your body Pollution environment. The genetic term phytoremediation consists of the Greek prefix phyto. Plants attached to Latin root remedies mean to correct or eliminate evil (Tangahu et al. 2011). Because contaminated water contains many components that are toxic metals Causes of many diseases and effects in humans and plants. Toxic metal concentration is determined using a variety of methods, including atomic absorption spectroscopy (AAS). Calorie measurement and ratio measurement fluorescent probe (Rasheed et al. 2018), different chromatography Methods include high performance liquid chromatography (HPLC) and gas chromatography (GC), Perkin Elmer Sciex Elan 6100 ICPMS, Flame Ionization Gas Chromatography Detection (GCFID) and Gas Chromatography-Mass Spectrometry (GCMS).

Table 1 explains the detailed note and collection of literature note on different plants and their heavy metal tolerance level. action of plants on these compounds is manifold: They can be immobilized, stored and evaporated, transformed various intentions (mineralized) or combinations from these, specific compounds, environmental conditions, vegetation mental types differ depending on the type of spirit. As a result, various plant technologies are happening, it will help to relieve organic contamination. Plant extraction is removal of contaminants from the ground and accumulation in the subsequent plant tissue (Sashin filtration is a preferred term that water source occurs it will be treated. Plant contrast agents contain chemicals correction of pollutants, usually they render harmful, followed by storage or elimination. Plants Ovolatitration takes contaminants made of soil or water and, they do not contain plants covering them in the atmosphere or convert them into volatile compounds. Plant stability reduces the bioavailability of contaminants by immobilization or binding to the substrate matrix. The availability of pollutants is obviously important Fiter Mediation Success Factors.

Table 1 Plants and their tolerance limit tested in literature towards heavy metals

Heavy metal	Plant name	Concentration (mg/kg)	References
Arsenic	*Pteris vittata*	8331	Kalve et al. (2011)
	Corrigiola telephiifolia	2110	Garcia-Salgado et al. (2012)
	Eleocharis acicularis	1470	Sakakibara et al. (2011)
Chromium	*Pteris vittata*	20,675	Kalve et al. (2011)
Cadmium	*Tumip landraces*	52.94–149.95	Li et al. (2016)
	Phytolacca americana	10,700	Peng et al. (2008)
	Prosopis laevigata	8176	Buendia-González et al. (2010)
Mercury	*Achiliea millefolium*	18.275	Wang et al. (2012)
	Silene vulgaris	4.25	Pérez-Sanz et al. (2012)
	Cicer arietinum	0.2	Wang et al. (2012)
Copper	*Pteris vittate*	91.975	
	Eleocharis acicularis	20,200	Sakakibara et al. (2011)
Manganese	*Alyxia rubricaulis*	14,000	Chaney et al. (2010)

The movement of organic compounds in ecosystems depends on it mainly its physicochemical properties (solubility) underwater, molecular size, charge, vapor pressure, etc.) interaction with surrounding molecules. Soil properties such as pH, texture, structure, organic matter Content is related to this context. Rhizosphere It can also be very influential (Dzantor 2007). From experience, before applying comprehensive phytoremediation techniques to contaminated areas, many issues need to be considered. Of particular importance are: (i) A detailed description of the location. Including the degree of pollution; (ii) Choosing the right plant Seeds of this site; (iii) Assessment of total cost Cultivation (planting, irrigation, management) and soil Change as needed. And (iv) determine the fate of Pollutants on plants. Estimated time Repairs that are heavily dependent on pollutants Uptake or removal rate is also an important parameter. Pilot tests are usually run to support this Estimate (McCutcheon 2003). Contamination level of each remaining facility Materials-especially underground materials, that is Expensive and difficult to remove.

The mechanisms concerned withinside the uptake, translocation and cleansing of those compounds. Plants absorb xenobiotics mainly thru roots and leaves (Wang and Liu 2007). Leaf absorption is usually a consequence of agricultural spraying with organo chemicals, although direct uptake of risky compounds may be additionally significant (Burken et al. 2005). Penetration into roots takes place specifically through easy diffusion thru unsuberized mobileular walls, from which xenobiotics attain the xylem stream. There are manifestly no unique transporters in plant life for those man-made compounds, so the motion charge of xenobiotics into and thru the plant depends in large part on their physicochemical properties. Because this motion is basically a passive bodily process, it's far as a substitute predictable and amenable to particularly easy modelling (Fujisawa 2002). Certain agronomic practices can also additionally beautify the effectiveness of the uptake process. For example, plant roots may be guided artificially closer to polluted sectors, and supplemental irrigation, fertilization or root oxygenation also can be applied. Likewise, plant life may be selected or engineered for suitable root architecture (Wang et al. 2006). Trees like poplar (Populus spp.) or willow (Salix spp.), with large root structures and high transpiration rates, keep unique promise for phytoremediation (Jansson and Douglas 2007).

The United Nations Environment Program has defined phytoremediation as “the efficient use of plants to remove, detoxify, or immobilize environmental pollutants”. Phytoremediation An environmentally friendly and beneficial technology for cleaning Contaminated media. Its mechanism includes absorption of harmful substances by roots, accumulation in body tissues, and decomposition. Converts pollutants into less harmful forms. Different phytoremediation techniques are used in different media Widely discussed by several workers around the world (de Campos et al. 2019; Favas et al. 2018). This technology is effectively used to clean up water pollution. It is receiving serious attention from scholars, government agencies and non-governmental agencies. But the use of plants in treatment the amount of wastewater started about 300 years ago (Carolin et al. 2017).

Various types of aquatic plants have been validated and recognized Efficiency in collecting inorganic and organic pollutants from water through hydroponics or field applications. Many species of aquatic plants like *Ranunculaceae, Remna, Kayatsurigusa, Haroraga, Hydrocharitaceae, Pondweed, Pondweed, Najadaceae, Pontederiaceae, Juncaceae, Zosterophyllaceae* are the most important representative of aquatic plant phytoremediation Environment (Prasad 2007). The technique of phytoremediation is an effective tool for tackling unprecedented pollution aquatic environment. With this technique, the first step is Identification and screening of highly effective plants Collect dissolved nutrients, metals and other pollutants (Lu 2009).

Plant selection for phytoremediation technology it should grow fast and be easy to handle and harvest. Other biological processes of plants such as growth and development, photosynthesis are important elements of sustainability. Successful phytosanitary system It also depends on the factors related to the seriousness of the pollution (Jamuna and Noorjahan 2009). In addition, various plant technologies such as plant degradation, plant stabilization, rhizosphere filtration, rhizosphere degradation, and plant volatilization have been applied to contaminated ecosystems (Cunningham and Berti 1993). The decrease in contaminants present in the soil is performed by rash Plant roots. Stabilize these roots, dismantling, non-moving, and binding Impurities are called vegetable stability processes. In addition, the root, adsorption, adsorption, and soil and water precipitation of specific plant species Immobilization Process. This is an important way to eliminate Organic and inorganic pollutants present in soil and sediments and the mud unit (Cunningham and Berti 1993). Plant extraction is a useful process for different floors impurity pollutants are also absorbed in the process As over-accumulated in different parts of the plant (Rulkens et al. 1998).

Plants have an excellent ability to consume and evaporate It releases pollutants directly into the atmosphere through the plant volatilization process. This process is

Table 2 Plants and their phytoremediation responses towards pollutants

Plant name	Phytoremediation response	References
Pistia stratiotes L.	Cell membrane damage, reduced root volume, less growth rate and photosynthesis, Increase in enzyme activity of peroxidase, catalase, superoxide dismutase	de Campos et al. (2019)
Echinodorus amazonicus	Reduce plant height, root length, plant growth	Sricoth et al. (2018)
Eichhornia crassipes	Lesser plant growth, Less plant height and root length and resulted in death of plant	
Ipomonea aquatica	Increased root size but reduced root length	Chen et al. (2010)
Lemna minor L	Less enzymatic activity and photosynthesis, reduced chlorophyll, root and shoot growth	Radić et al. (2010)

cost effective in removing contaminants from soil, groundwater, tailings and sludge (Girdhar et al. 2014). Plants partially metabolize pollutants Compounds produced in plant tissue Plant conversion/plant decomposition process (Chaudhry 1998). Table 2 provides the note on different type of plants and their response to phytoremediation process. For resofiltration, Metal pollutants on surrounding growth substrates Root zone. In this process, groundwater contamination and surface water are performed by plant roots (Dushenkov et al. 1995). Plants release tightened different organic compounds Microbial communities present on the ground help the root of the impurities. This Phytoroma Mediation Technology provides an alternative to replace rehabilitation purposes. Bio-solvitation is a physicochemical process used as a method for. Metal removal (Verma et al. 2008). I used this method As an alternative technique for removing HMS from wastewater (Ali et al. 2020).

5 Challenges and Conclusion

Unawareness or negligence in knowing that water is essential for all forms of life to survive in the universe. This water resource is continuously contaminated due to anthropogenic activities. Government should make stringent rules and ensure that authorities are in constant surveillance on water localities. Beyond this modern and hybrid mode of water treatment techniques need to be identified and experimented for effective wastewater treatment with focusing on reduce and reuse strategy. This book chapter was dedicated to focus especially on phytoremediation of wastewater contaminated with heavy metals and radionuclides. Phytoremediation process helps in improving the COD and BOD level of water bodies. Physiochemical parameters like concentration, variety of contaminant, plant type used, experimental design.

In future integrated approaches of mechanical, physical, chemical and biological approaches need to be engaged in phytoremediation process.

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Anaerobic Biotechnology: Implementations and New Advances

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Abstract Anaerobic technology has gained widespread acceptance in environmental sustainability as a low-cost alternative for pollution control. The anaerobic technologies for contaminants treatment have three essential returns, i.e., bioenergy recovery, energy-saving and low sludge production. Therefore, the anaerobic process will be the favored green treatment technology for a sustainable environment in years to come. Currently, anaerobic treatment remains to flourish in several features, such as reactors development, bio-hythane production, molecular techniques for microbial studies and kinetic modeling and extending applications to a wide range of waste and wastewater effluents. Therefore, this chapter brings together the most up-to-date information on the new developments in anaerobic technology. Also, it sheds light on the current conversion methods and technologies for energy recovery with a focus on the use of natural materials as sustainable and environmentally friendly sources for creating new materials used in this regard.

Keywords Anaerobic technology · Bioenergy recovery · Pollution control · Annamox process · Emerging pollution

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1 Introduction

Anaerobic biotechnology is important for two important challenges in our society: environmental protection and resource recovery. Today, 7.9 billion people (2022) live in this global community, sharing an increasingly polluted environment and quickly dwindling resources. The existence of our global community is further affected by the ongoing growth of the human population, which may exceed 12 billion by the end of the twenty-first century. Anaerobic technology has been proved as a viable treatment procedure for numerous types of waste and wastewater after decades of development. Despite its inherent benefits and demonstrated effectiveness, this biotechnology is still frequently overlooked by many today. In comparison to the more traditional aerobic approach, the anaerobic process for waste and wastewater treatment has three inherent advantages: energy savings, lower sludge yield, and biofuel generation. Because of these benefits, as well as recent technological advancements, anaerobic treatment technology has become a cost-effective way of pollution management, leading in the installation of thousands of full-scale treatment plants across the world during the last two decades. These benefits also make the anaerobic process a long-term environmental technology with considerable promise. Today, anaerobic technology is thriving in many areas, including the creation of new types of reactors, the application of molecular tools for microbial investigations, and kinetic modeling, bio-hydrogen generation by fermentation and a microbial electrolysis cell, with expanded applicability to municipal wastewater, chemical industrial effluents, and agricultural wastes with high lignocellulose content. Therefore, this chapter shits light on identifying influencing factors on anaerobic sustainable management for energy and resource recovery. Moreover, encourage further research work that would help expand and improve the best technologies to ensure affordability, suitability, and environmental sustainability.

2 Anaerobic Technology as a Tool to Promote a Sustainable Development

As a result of a paradigm shift in the treated waste/wastewater as renewable resources, a reduction of dependency on fossil fuels and dropping in the amount of pollution can be addressed simultaneously. The clean water and energy supplies available are depleting due to population growth, competition among users and lack of studies concerning the risk assessment of emerging pollutants (EPs) (Hernando et al. 2011; Patel et al. 2020). Additionally, the wastewater effluent became more complex and dangerous over time, due to innovation and marketing in the various sectors (Zăbavă et al. 2019). This leads to higher levels of the EPs (i.e., toxic organics and metal) in the ecosystem (see Fig. 1). The polluted water is graded according to its sources and is defined accordingly (Hsien et al. 2019). Generally, wastewater includes three major groups. The first is domestic wastewater, which refers to flows discharged mainly from residential sources created by food preparation, washing, cleaning, and personal hygiene. Secondly, it relates to industrial wastewater produced through production and commercial activities such as everyday products industries, pharmaceutical industries, and agro-industries. The third is agricultural wastewater which refers to flows discharged principally from agricultural activities such as poultry wastewater, dairy farming wastewater, and nutrient runoff. Nowadays, water resources are becoming increasingly scarce in Middle East Countries (MEC) and Egypt is expected to be absolute water scarcity by the year 2030 due to the rapidly increasing population that reached now near 100 million inhabitants and economic growth (Miralles-Wilhelm et al. 2018). In addition to recalcitrant organic pollutants (ROPs), the wastewater effluents include potentially toxic substances, along with various pathogenies. Thus, this will require a lot of effort to be made for more efficient management of wastewater treatment at low cost and sustainable solutions, to secure sustainability and development for the coming generations. To date, there is no economically and rapidly attractive solution to treat wastewater effluents due to socio-economic factors (Foteinis et al. 2018). However, among various technologies used for wastewater treatment, biological technology is a promising technology because of its simplicity and a promising net positive energy, even when heating of the liquid is required, could be produced from wastewater containing recalcitrant and toxic organics by different operation conditions and bio-systems. Therefore, this review sheds light on the current conversion methods and technologies for energy recovery from wastewater sludge treatment with a focus on the use of natural materials as sustainable and environmentally friendly sources for creating new materials used in this regard.

Clean and renewable water supplies are essential for establishing and maintaining a wide range of human activities. Accordingly, the emerging pollutants caused by human activities such as industrial and agricultural production are a permanent concern throughout the world. Which requires a lot of effort to find and develop effective treatment technologies for wastewater treatment with energy-saving in mind.

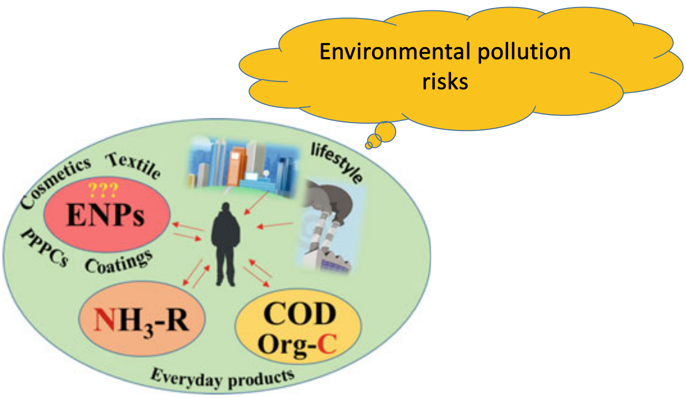

Fig. 1 Economic growth and sustainable society

Data in the literature show that there are three main technologies for wastewater management which are physical, chemical and biological treatments. Each of these technologies is characterized by advantages and disadvantages, benefits and technological limitations (Cheremisinoff 2001; Bui et al. 2019). The physical/chemical technologies such as membrane technologies, coagulation & flocculation and chemical transformations, have been studied extensively for pollutants and pathogens removal from wastewater effluents (Abdullah et al. 2019; Ho et al. 2020; Ahamed et al. 2019). However, there are still various limitations in the implementation of the above technologies such as cost and operational difficulty (Bello et al. 2019). The bio-treatment of aerobic and anaerobic technologies, however, presents a prosperous technology. In this technology, microbes adapt to toxic pollutants and naturally produce new resistant strains that convert various toxic substances into less harmful substances. Several reports established that anaerobic technology is one of the promising and suitable technology to treat a wide range of wastewater effluents and bioenergy production (Stazi and Tomei 2018). The biodegradation of complex and toxic pollutants is achieved efficiently by enzymatic mechanisms with energy-saving options and low-cost management, such as those associated with agricultural wastes (Jiang et al. 2017, 2018), cellulosic wastewater (Gadow and Li 2019; Gadow et al. 2019a), and recalcitrant textile dyes (Gadow and Li 2020a, b; Gadow et al. 2019b). Consequently, a new classification of wastewater treatment technologies has been proposed, which takes into account using of natural materials as sustainable and environmentally friendly sources for creating new materials used in this regard and the energy-saving option.

Although the energy-consuming technologies for wastewater treatment have been recommended to eliminate pollutants and pathogenic microorganisms, they still face some challenges including issues of cost and operational difficulties (Farooq and Ahmad 2017). As for several chemical technologies such as chemical transformations, the main concern for consuming large amounts of energy and chemicals and often cause secondary contaminations under ineffective management (Zhang et al. 2019). Consequently, these methods are economically unfavorable or technically complicated and are only used in special cases of wastewater treatment. Energy recovery during wastewater treatment is particularly important because of its effectiveness in comparison with other techniques, on minimizing treatment costs and reduction of sludge. Figure 2 shows the potential wastewater-energy recovery options by using different technologies. A significant amount of renewable energy such as biogas, chemical, liquid fuel, heat, and electricity generation can be recovered from sludge management (Jiang et al. 2018; Gadow and Li 2019; Thanos et al. 2020). There are four different technologies to extract energy during wastewater treatment including combustion, pyrolysis, gasification and anaerobic digestion (Shukla et al. 2019; Shi et al. 2019; Shareef 2020). The benefits and drawbacks of these technologies and their technical weaknesses will be discussed in the following sections.

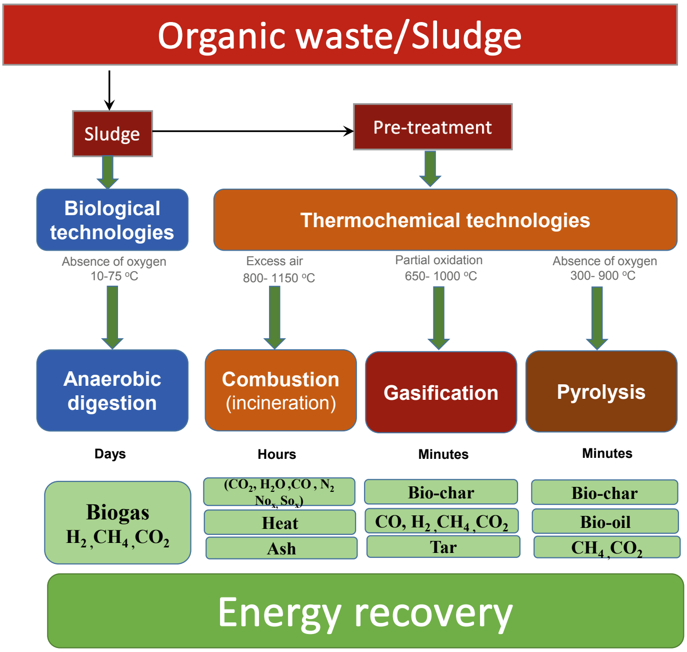

Fig. 2 Methods of energy recovery from organic waste and/or sludge

3 Anaerobic Granulation and Granular Sludge Reactor Systems

Anaerobic wastewater treatment is a biological conversion of organic pollutants with high water content to produce biogas mixtures of hydrogen, methane and carbon dioxide (Jiang et al. 2018; Gadow and Li 2020a). Recently, anaerobic digestion is an important and promising industrial technology to treat a wide range of wastewater including industrial, municipal and farming effluents (see Fig. 2). The general advantage of the anaerobic digestion process is that it can be applied for low-strength wastewaters and high-strength organic solid waste to the proper reactor configuration (Xiong et al. 2020). Moreover, it can accommodate high COD loads, which adapt to remove various toxicant components provided that adaptation time is allowed for the anaerobic waste/wastewater (Luo et al. 2019). Generally, up to 95% of organic material will become biogas as a renewable resource by the properly run digester (Stazi and Tomei 2018; Li et al. 2015). On the other hand, the improper operation of the anaerobic plant will cause the following drawbacks to become more evident: (1) low growth by high temperature (Gadow et al. 2012). (2) The process is sensitive to COD overloads and toxicant shock loads (Xiong et al. 2020).

Especially treating for the high C/N ratio waste/wastewater (Gadow et al. 2019a). (3) The optimum pH for the process lies in a narrow range near neutrality and with an inapposite operation such as the temperature and substrate concentration, the process will be inhibited due to intermediates accumulation (Jiang et al. 2018; Lim et al. 2020). Therefore, the biodegradation of recalcitrant pollutants is always a permanent concern to achieve environmental suitability with energy-saving in mind.

3.1 Hydrogen Production

Hydrogen is considered to be the promising energy carrier due to its highest heating value (142 MJ/kg Higher Heating Value (HHV) and leaving pure water as the only end product. Dark hydrogen fermentation is considered a sustainable approach to waste/wastewater treatment issues and has the added advantage of yielding bioenergy (Gadow et al. 2013a; Meky et al. 2020). The organic pollutants are converted by anaerobic hydrogen-producing bacteria (HPB) grown. The HPB is able to use energy-rich hydrogen molecules by hydrogenases to generate H_2. During dark hydrogen fermentation, acetic acid, butyric acid, and ethanol are the main soluble by-products as shown by Eqs. 1, 2 and 3, respectively (Balachandar et al. 2020; Gadow et al. 2016). Accordingly, it can be noted that the hydrogen yield is greater when coupled with acetic acid rather than butyric acid and ethanol.

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 4H_2 + 2C_2H_4O_2 + 2CO_2 \quad (1)$$

$$C_6H_{12}O_6 \longrightarrow 2H_2 + C_4H_8O_2 + 2CO_2 \quad (2)$$

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2H_2 + C_2H_4O_2 + C_2H_5OH + 2CO_2 \quad (3)$$

The ratio of butyrate/acetate is very important and a suitable indicator to evaluate the hydrogen production efficiency.

Table 1 shows the stoichiometry of different reactions to produce hydrogen in the dark fermentation and the maximum theoretical reached 4 mol H_2/mol hexose with acetate as an only soluble by-product (Gadow et al. 2013a). However, the formation of other by-products, such as propionic and butyric acids as well as methanol, butanol, or acetone, lowers the amount of hydrogen produced in fermentation. Several studies reported that there is a diversity of microorganisms including facultative and strictly anaerobic such as bacteria and archaea able to produce hydrogen by dark fermentation. Two bacterial genera that have been studied extensively are enteric and clostridia (Collet et al. 2004). The importance of using a mixed culture of obligate and facultative bacteria is valuable since facultative bacteria make an anaerobic condition for the oxygen-sensitive obligate bacteria. For continuous bio-hydrogen production, the hydraulic resistance time is an important operational parameter in the anaerobic bioreactors. The hydrogen-producing bacteria have a long specific growth rate which reached 0.172 h^{-1}. Therefore, short HRT is suitable for hydrogen production and eliminating other hydrogen-consuming bacteria. Zhang et al. (2006) confirmed that

Table 1 The theoretical maximum H_2 yield under different reactions

Reaction	Stoichiometry	References
Acetate production	$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 4H_2 + 2HCO_3^- + 4H^+$	Thauer et al. (1977)
Butyrate production	$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2H_2 + 2HCO_3\ 3H^+$	Thauer et al. (1977)
Acetate and ethanol production	$C_6H_{12}O_6 + 3H_2O \rightarrow CH_3CH_2OH + CH_3COO^- + 2H_2 + 2HCO_3^- + 3H^+$	Hwang et al. (2004)
Lactate production	$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOO^- + 2H^+$	Kim et al. (2006)
Butanol production	$C_6H_{12}O_6 + H_2O \rightarrow CH_3CH_2CH_2OH + 2HCO_3^- + 2H^+$	Chin et al. (2003)
Propionate production	$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H_2O + 2H^+$	Hussy et al. (2003)
Valerate production	$C_6H_{12}O_6 + H_2 \rightarrow CH_3CH_2CH_2CH_2COO^- + HCO_3^- + H_2O + 2H^+$	Ren and Gong (2006)
Acetate fermentation to H_2	$CH_3COO^- + 4H_2O \rightarrow 4H_2 + 2HCO_3^- + H^+$	Stams (1994)
Butyrate fermentation to H_2	$CH_3CH_2CH_2COO^- + 10\ H_2O \rightarrow 10H_2 + 4HCO_3^- + 3H^+$	Sawers (2005)

decreasing the hydraulic resistance time from 8 to 6 h led to significantly increasing hydrogen production efficiency (Zhang et al. 2006).

3.2 Methane Production

The anaerobic digestion of methane production from waste/wastewater is promising and growing worldwide due to its economic and environmental benefits. Furthermore, the other crucial benefits offered by the use of biogas over natural gas are as follows: (i) it is produced from renewable resources, (ii) it does not add any greenhouse gases to the atmosphere, (iii) it is produced locally without any dependency on other energy supplies, (iv) it helps in reducing the pollution produced by the organic wastes, which account for most freshwater pollution, and, (v) it helps in retarding the waste management problems.

The anaerobic digestion will begin by hydrolysis of complex organic substances such as carbohydrates and proteins into sugars, amino acids and respectively (see Fig. 3). The Acetogenic bacteria and other fermentative bacteria convert the reduced compounds to short-chain volatile fatty acids (VFAs) and hydrogen. The final step in the anaerobic digestion process is methanogenesis, where the methane was produced by converting the acetic and hydrogen by methanogenic bacteria such as a consortium of microbes (Ali et al. 2019). Methane fermentation technology is the most efficient way of handling and energy generation from waste/wastewater, in terms of energy output/input ratio (28.8 MJ/MJ) in comparison to all other technology of energy production through thermochemical routes of energy conversion processes (Deublein and Steinhauser 2011). Since anaerobic digestion of methane production is a biological reaction, environmental conditions such as pH, temperature and growth medium characterization have a significant effect. Additionally, operational factors such as the hydraulic retention time and organic loading rate are the factors affecting the amount of methane produced. This approach is guaranteed to reduce environmental risks and create renewable sources. The produced methane can replace natural gas and the residues resulting from anaerobic digestion can use as fertilizer and bio-conditioner for agricultural purposes. The hydrolysis steps of anaerobic digestion can achieve at a wide range of pH, however, the optimum pH value of methanogenesis around is neutral. Several studies state that the pH below 6.5 and high than 7.5 decreases the rate of biogas production (Khan et al. 2016). Therefore, in order to preserve the optimal pH level required for methanogenesis, an adequate amount of bicarbonate alkalinity in the solution is necessary. As for operational parameters, the loading rate is not limited by substance supplement, but by the processing capacity of the microflora. Consequently, it is essential to retain a sufficient bacterial mass in the anaerobic bioreactor. The specific growth rate of methanogenic bacteria is very low (0.0167–0.02 h^{-1}), when compared with hydrogen-producing bacteria (0.172 h^{-1}) (Kamyab et al. 2019). Therefore, in many studies, where a mixed consortium, such as sewage sludge, is used, long HRT is preferable to increase methanogenic bacteria. An interesting study reported that bio-methane production was increased from 0.11 to 0.24 L/L leachate significantly by increasing HRT from 12 to 48 h (Kaparaju et al. 2010).

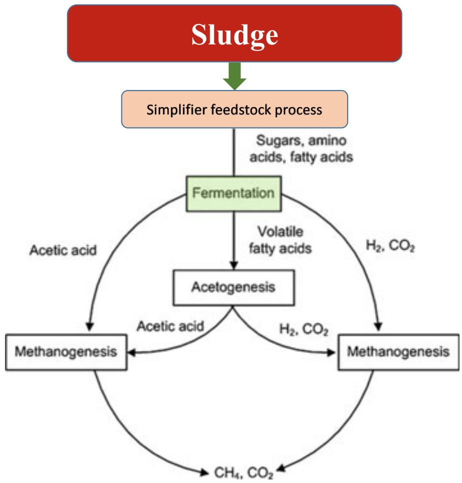

Fig. 3 Hythane production pathway

3.3 Bio-hythane Production

Bio-hythane is defined as a mixture of hydrogen and methane gases, which are produced by a biological process in two and/or single-stage (see Fig. 4) and trade-marked in 2010 by Eden. It is also has been utilized commercially in India and the United States of America and several individual companies like Volvo and Fiat have taken Hythane into account as well. Bio-hythane production has been proposed as a promising approach for waste/wastewater treatment. Several studies reported that bio-hythane production is required a short resistance time compared with conventional anaerobic digestion (Krishnan et al. 2019). It also found that high biodegradation over 95% was achieved with high energy recovery efficiency and better process control and resilience under two-stage treatment. The concentration of hydrogen in the gas mixer ranged from 5 to 25% in the dark fermentation (Gadow and Li 2019, 2020a). Therefore, the H_2/CH_4 ratio can be easily regulated under the biological process by microbial community structure design and improvement (Ramos et al. 2019). Recently, there are many efforts to produce bio-hythane in a single-stage process, thus making the treatment and bioconversion an attractive and suitable solution for wastewater management (Gadow and Li 2019).

The pH value is a crucial factor for bio-hythane production. The pH value affects Fe–Fe hydrogenase activity therefore, metabolic products significantly change. The optimum pH for hydrogen production (first stage) ranges between 5.0 and 6.5, however, the optimum pH for methane production (second stage) is around the neutral condition. Therefore, the recirculation process in the two-stage bio-hythane production provides efficient performance in the overall COD degradation efficiency and bioenergy recovery, however, it is still remaining unclear its effects on microbial community structure in the anaerobic bioreactors.

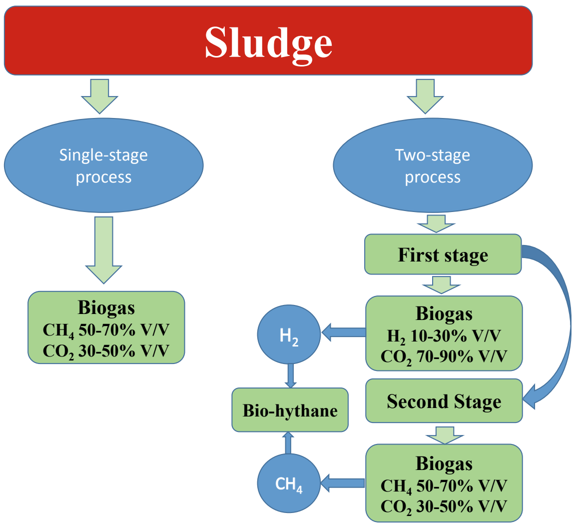

Fig. 4 Bioenergy optimization by anaerobic dark fermentation

4 Challenge and Recent Advances in Anaerobic Technology

In fact, the pollutants in industrial wastewater become more complex over time, due to the innovation and marketing in the various industry sectors (Yi et al. 2020). This leads to higher levels of the emerging pollutants (Eps) (i.e., toxic organics and engineering nanoparticles (ENPs)) in the ecosystem (Hao et al. 2020). The untreated agricultural-based industries cause environmental pollution and harmful effect on human and animal health and create different problems with climate change by increasing the number of greenhouse gases. Egypt is an agricultural country and agro-industrial waste products are abundantly available. Therefore, the agro-industrial waste uses for wastewater treatment is attractive technology because of its good adsorption potential due to the presence of carboxyl, hydroxyl, and amino groups over their surfaces as well as their easy availability, low cost (Amor et al. 2019). The conventional treatment systems are designed and constructed to reduce BOD, COD, and nitrogen compounds but not for the EPs. They could be washed and released in the wastewater (domestic or industrial) and able to kill the microorganisms and/or change the microbial community structure in wastewater treatment plants (WWTP), which could lead to a threat to drinking water safety (Li et al. 2016). Therefore, the scientific communities need to explore innovative, cost-effective, energy-saving and sustainable solutions to reduce the risks to acceptable levels in the treated discharge.

4.1 Rapid Start-Up Under Ambient Temperature

The recalcitrant, complex and toxic pollutants are the most important limiting factor for energy optimization and biodegradation efficiency in the anaerobic degradation process. Therefore, the hydrolysis step is a permanent concern in recent studies to enable fast start-up under ambient temperature (Gadow and Li 2020a; Kong et al. 2019). Aside from the hydrolysis step, there are many parameters such as temperature, pH, microbial community design and reactor configuration, which would affect the bio-reaction speed and the end product characterization. Under two-stage technology, acidogenesis and methanogenesis occur in two separate reactors and each stage required different environmental conditions. Under the acidogenic phase, the first stage, the recalcitrant, complex and toxic pollutants are hydrolyzed and converted to simple compounds, which is slow-rate bioconversion. On the other hand, the HPB is rapid growth compared with methanogenic bacteria which means the bio-system configuration is very important to improve the energy recovery efficiency by hydraulic retention time (HRT) optimization (Gadow and Li 2020b). In recent times, different reactor configurations and operational conditions were applied to validate the wastewater treatment level and bioenergy recovery improvements. Gadow et al. (2013a, b) reported that the temperature affects not only the rate of biogas production but also the microbial community structure (Gadow et al. 2013b).

4.2 Inhibitors

It is essential to obtain high performance and deal with inhibitory factors for sustainable energy production (Hou and Ji 2018) under dark fermentation technology. It has been proposed that resilience plays an important role in maintaining the process (Gadow and Li 2020b). Microbial acclimation plays an important role in the sustainability of the process. Strategies to achieve and maintain optimal microbial communities by acclimation in hydrogen and methane fermentation can enhance its economic viability and implementation (Zhao et al. 2020; Soares et al. 2020). A variety of different inhibitors have been reported in the literature for a single or two-stage process such as toxic pollutants, temperature, pH and metal ions (Hou and Ji 2018; Habets and Boerstraat 1999). Consequently, only when the composition of wastewater and operational parameters is well-known can the stable activity of the anaerobic reaction be assured. For example, under acidogenesis, the production of hydrogen coupled with butyrate and ethanol means the degradation process was not complete and we still need to remove such unfavorable by-products economically. As for methane production, two mechanisms for methane formation from acetate have been reported. Aceticlastic is the first one, which is produced by Methanosarcinaceae. The second reaction involves a two-stage reaction in which acetate is initially oxidized into H_2 and CO_2 and then converted into methane with those products (Shah 2020).

4.3 Internal Circulation Reactor

UASB reactor is being used to handle the extremely high loads of COD which needs high retention time using Aerobic reactor, by mean of keeping a high level of TSS (100,000 ppm or higher), Keeping this blanket in the bottom of the reactor is the key of the successful treatment, this can't be achieved with Up-flow high velocity (maximum velocity 1 m/s), as a result of this UASB reactor needs high area footprint and large retention time 24 h. UASB also suffers from bad mixing due to low velocity inside the reactor, also the poor separation of gas in the bottom part causing the extra needed volume to avoid solid drift up. The disadvantages of UASB are reclaimed and minimized in the smart idea of IC UASB reactor, IC tends to Internal circulation (Shah 2021).

Reactor Process Description

Feed Water flows from the bottom of the reactor in static fins causes cyclone pattern flow the high velocity in the centre of cyclone eye of cyclone leads to slight vacuum this make the suction needed for internal circulation, and achieve very good missing also the rotary motion enhance the granulation of the Sludge blanket components (Fig. 5). Water flows up to the top of the reactor with relatively high velocity, and to avoid drifting the biomass up to the reactor top losing its effect, the smart design takes into consideration 3 phase separator in the 1st bottom 1/3rd part of the reactor

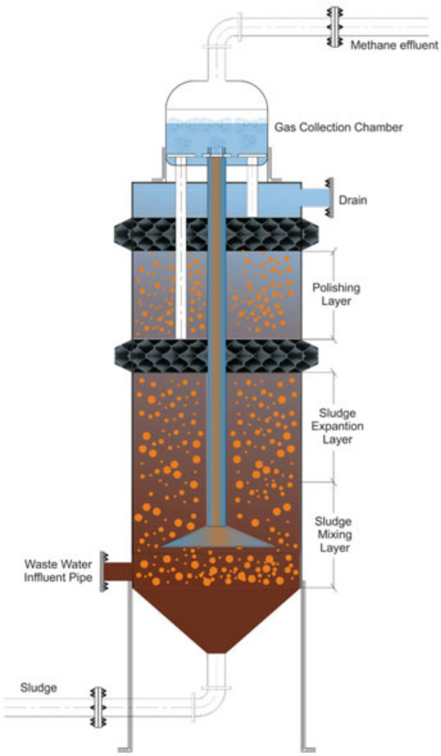

Fig. 5 Internal Circulation Up-Flow Anaerobic Sludge Blanket (IC-UASB)

and the 2/3rd up part of the reactor, both 3 phase separators helps the achieving of high up-flow velocity, better mixing, better gas separation and lower retention time/Footprint. Cycles of gas removal are being increased and this improves the reaction forward speed by getting red frequently of all biogas generated inside the reactor. This very efficient flow pattern doesn't need any type of mixers or moving parts, energy-saving design, near to Plug flow reactor.

5 Conclusion

Anaerobic technologies have grown in relevance in the field of environmental technology because they are the major mechanism controlling the result of anaerobic microbial metabolism. Energy recovery during waste/wastewater treatment is particularly important because of its effectiveness in comparison with other techniques, on minimizing treatment costs and reduction of sludge. A significant amount of renewable energy such as biogas, chemical, liquid fuel, heat, and electricity generation can be recovered from sludge management. The general advantage of the anaerobic digestion process is that it can be applied for low-strength wastewaters and high-strength organic solid waste to the proper reactor configuration. Moreover, it can accommodate high COD loads, which adapt to remove various toxicant components provided that adaptation time is allowed for the anaerobic biomass. Generally, up to 95% of organic material will become biogas as a renewable resource by the properly run digester. However, the complex composition of organic pollutants remains its main limitation due to the hydrolysis step which affects the safety of treated water, increases energy input and increases the cost of treatment. All the technologies discussed in this review demonstrate the need for further research and development in the operation condition and energy recovery optimization while reducing cost and emissions. An interesting observation is the lack of data to integrate biological and thermochemical technologies to improve energy recovery. This is easily conceived as an integrated approach to bio-refinery that can be appropriately designed to maximize energy recovery to reduce harmful environmental impacts. Regardless, all of these processes must be tailored to suit specific cases and require an in-depth technical assessment to determine their sustainability in a low-carbon future.

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Remediation of Soil Contaminated with Heavy Metals by Immobilization with Organic and Inorganic Amendments

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Abstract Heavy metals and metalloids are hazardous chemicals that very difficult undergo microbial or chemical degradation. Their presence in natural environment results mainly from anthropogenic sources, such as agriculture, oil and gas production, mining industry, and military activities. Soil pollution with heavy metals is recognized as "hot spots" posing a risk to the environment, agricultural production, food safety, and human health. Various technologies have been developed to reduce the potential for the release of metal ions into the environment and to scale down changes in the land use pattern. In situ remediation of contaminated soils by supplementing amendments is considered as a sound alternative both environmentally and economically. This method provides a long-term, relatively cheap remediation solution by reducing metal mobility and availability to plants. As steams from literature, amendments' application can improve soil biological, chemical and physical properties and consequently enhance the plant growth. The present chapter presents the current trends (from the last decade) in the remediation of soil contaminated with heavy metal ions by their immobilization with various by-products and low-cost materials. The focus was put on the factors which determine the metal binding and transformation into more stable forms. An assessment of the effectiveness of these amendments on the soil properties and the phytoavailability to plants has been made as well.

Keywords Heavy metals · Contaminated soil · (Bio)remediation techniques · Immobilization · Organic and inorganic amendments

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1 Introduction

Heavy metals are well known toxic chemicals. Their presence in soil, resulting mainly from the anthropogenic activities, poses a threat to the natural environment as a whole, as well as to the production of healthy food and consequently to human health. Agriculture, oil and gas production, burning of fossil fuels, and mining industry are the main sources of soil pollution with heavy metals (Kamari et al. 2015; Rodríguez-Eugenio et al. 2018; Hannan et al. 2021a; Irfan et al. 2021; Li et al. 2021; Quoc et al. 2021; Shao et al. 2021). Heavy metal contaminated soil can be a serious barrier to the cultivation of many plant species. On the other hand, vegetation grown in polluted areas may pose a risk to humans/animals due to bioaccumulation/biomagnification of toxic metals in the plant biomass that may become a component of the human diet or animal feed (Radziemska et al. 2022).

Soil washing (extraction), phytoremediation (accumulation by plants) and chemical immobilization (solidification/stabilization, vitrification, chemical treatment) are available technologies used for cleaning up of heavy metal contaminated sites. These conventional remediation technologies have encountered numerous obstacles, such as long operation time, high chemicals cost, large energy consumption, secondary pollution, soil degradation by the excessive soil nutrient loss, etc. (Hamid et al. 2018; Shah 2020; Zhou et al. 2020; Liu et al. 2021; Pei et al. 2021; Quoc et al. 2021). Therefore, low-cost and ecologically sustainable in situ remedial options are desired to reduce the risk associated with heavy metals, to make the land resource available for agricultural production, and to enhance food security (Mahmoud and Abd El-Kader 2015; Shah 2021; Lwin et al. 2018; Liu et al. 2021; Shao et al. 2021).

One approach used in the remediation of contaminated soil is the application of organic and/or inorganic soil amendments for immobilization of heavy metal ions. These additives decrease the mobility and bioavailability of heavy metals ions to plants (Hu et al. 2014; Lwin et al. 2018; Shan et al. 2020; Liu et al. 2021). Their use is not aimed at eliminating heavy metals from the soil, but at lowering their activity and blocking their entry into the food chain (Hu et al. 2014; Mahmoud and Abd El-Kader 2015; Hamid et al. 2018; Hannan et al. 2021a). Low input, high efficiency and less land disturbance are the main advantages of this cheap and environment friendly method (Hu et al. 2014).

Certain waste biomass from forest industry (e.g., bark and wood chips, bark saw dust), agricultural operations (e.g., straw, animal manure), kitchen waste, sewage sludge from wastewater treatment, humic acid as well as produced from this waste biomass compost/vermicompost or biochar can be used as inexpensive sorbents for immobilization of heavy metal ions in the soil (Lwin et al. 2018; Xu et al. 2020; Zhou et al. 2020). These materials are available in large quantities and their large-scale applications would fit the worldwide need for circular economy. Besides organic amendments, inorganic binders, such as natural minerals (e.g., sepiolite, zeolite, kaolinite, bentonite, etc.), liming materials (e.g., calcium oxide, calcium carbonate, etc.), gypsum, cement, nano-scale materials (e.g., nano-Fe, ZnO, MgO,

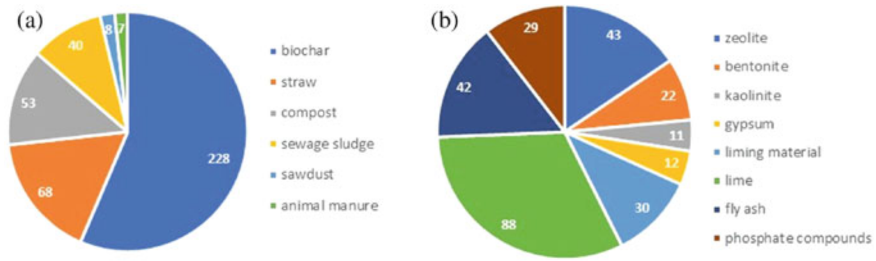

Fig. 1 Soil amendments: (**a**) organic and (**b**) inorganic used in the immobilization of heavy metals in polluted soil (on the basis of Web of Science database, access on 28 February 2022)

etc.), phosphorus-containing materials (e.g., apatite, calcium phosphate, diammonium phosphate, etc.), industrial by-products (fly ash, red mud, slags etc.) can also serve as amendments in the remediation of polluted soil (Lwin et al. 2018; Zhou et al. 2020; Pei et al. 2021). Figure 1 presents the most often used organic and inorganic additives in the immobilization of heavy metals in polluted soil. The data comes from the Web of Science database—in the abstracts of the publications, the following phrases were found: "immobilization of heavy metals in soil" and "a given soil amendment", for example "biochar". Nonetheless, as pointed out in some publications, amendments can negatively affect soil properties and microorganisms (Quoc et al. 2021). Therefore, the choice of amendment should be made taking into account the particular type of element(s), soil composition and its properties (Palansooriya et al. 2020). Properly selected soil additive not only stabilizes heavy metals in soil, but also reduces their uptake by plants (roots, shoots, leaves, grain) and promotes plant growth in the contaminated soil (Wang et al. 2020).

The aim of the present chapter was to present the current trends in the remediation of soil contaminated with heavy metal ions by immobilization with organic and inorganic sorption materials. This chapter was prepared based on the recent publications (2012–2022) from the Web of Science, Scopus, PubMed, ScienceDirect, and Google Scholar databases.

2 Sources of Heavy Metal Ions in Soil

Soils are the major sink for heavy metals released into the environment from a wide variety of anthropogenic sources such as: metal mine tailings, disposal of high metal wastes, leaded gasoline and lead based paints, application of fertilizer, animal manures, biosolids (sewage sludge), compost, pesticides, coal combustion residues, petrochemicals, and atmospheric deposition (Dhaliwal et al. 2020). So far, there has been no sufficient data to provide an overview of the global distribution of heavy metals in soil. In the European Union the most comprehensive source of information is FOREGS data (produced by the EuroGeoSurvey), which provides continuous map sheet based on sampling density 1 site/5000 km^2. More reliable view was provided by Tóth et al. (2016), who analyzed the metal content in a soil sample with a density 1 site/200 km^2. The analysis of heavy metals distribution by using the LUCAS Topsoil Survey, reveals that 137,000 km^2 of agricultural land in the European Union is affected to a certain degree. About 2.6% of the samples from agricultural land contain heavy metals in concentration qualifying for soil remediation.

Soil itself has the ability to immobilize heavy metals, which depends primarily on the soil composition, grain size distribution, and pH. In general, acidic environment enhances heavy metals solubility and hence their mobility. Fine grained soils, with large quantities of organic matter, silt, and clay, have higher capacity to retain heavy metals than coarse soils, which favor the infiltration of water and spread of dissolved metal ions. The inorganic colloidal fraction of soil is comprised of clay minerals, oxides, sesquioxides and hydrous oxides. All mentioned factors influence both the presence of certain forms of heavy metals in the water-soil environment and immobilization properties of soil.

3 Immobilization of Heavy Metal Ions in Polluted Soil

The immobilization process of heavy metals with the use of waste biomass (metabolically inactive) or other inorganic materials remains unaffected by toxicity, does not require any growth/nutritional medium and is flexible to environmental conditions (Lwin et al. 2018). In turn, employment of living biomass for bioremediation may not be a viable option owing to highly toxic metals which can accumulate in cells and interrupt metabolic activities resulting in cell death (Dzionek et al. 2016). Conversion of contaminants from their original form into a more physically and chemically stable form due to soil amendments action, reduction in heavy metal mobility, solubility, bioavailability, and toxicity in soil is regarded as an environmentally friendly and economically viable remediation strategy (Kamari et al. 2015; Hannan et al. 2021a; Malik et al. 2021; Pei et al. 2021; Quoc et al. 2021).

The effectiveness of the immobilization process depends on many parameters such as soil pH, the content of organic matter, bulk density, cation exchange capacity (CEC), heavy metal form, and parent material of the amendment (Hannan et al. 2021a; Malik et al. 2021; Shao et al. 2021). Because there is a direct correlation between soil physicochemical properties and toxic metals mobility and availability to plants, soil characteristics provides guidance for selection of the most efficient amendment (Palansooriya et al. 2020).

The immobilization process results in changing metal ions speciation from initially highly bioavailable forms (i.e. free metals) to the much less bioavailable fractions associated with organic matter, metal oxides, or carbonates (Mahmoud and Abd El-Kader 2015; Pei et al. 2021). More specifically, toxic metal ions are taken up by the dead waste biomass/inorganic sorbents through sorption—biosorption/adsorption or complexation or precipitation, which results in their lower availability for plants and lower content in cultivated crops (Lwin et al. 2018; Wang et al. 2020; Pei et al. 2021).

Organic amendments in the form of waste biomass with a high content of organic matter can decrease the mobility of some heavy metals due to the formation of stable chelates (Kamari et al. 2015). This method effectively reduces the ecological risk associated with the movement of toxic metals in soil. Simultaneously, the application of biomass residue or products derived from it (e.g., biochar, compost) as an organic soil amendment can improve the biological, chemical and physical properties of the soil. An increase in the content of soil organic matter and nutrients such as N, P and K, increases the cation exchange capacity, improves soil structure and soil microbial and enzyme activities and finally enhances plant growth (Hu et al. 2014; Lwin et al. 2018; Hannan et al. 2021a; Irfan et al. 2021; Liu et al. 2021; Malik et al. 2021; Pei et al. 2021). Due to improvement in soil nutrient, water retention capacity, and porosity, soil additives establish a suitable environment for soil microbiota. Consequently, immobilization of heavy metals in soil reduces their stress on microbiota and increases enzymatic activities, which promote plant growth (Liu et al. 2021).

Chemical fixation of heavy metal ions on inorganic sorption materials causes stabilization rather than decontamination, which relates to transformation of metal into an inactive form. It is claimed that there is no single mechanism responsible for the immobilization of metal ions. Most likely, several processes are taking place simultaneously, among which precipitation, electrostatic interaction, surface adsorption, structural sequestration and complexation are the most often mentioned. Their share relates directly to sorption material properties as well as to the process' conditions, and can change over time influencing the degree of reduction in the bioavailability of metal ions. Common features of inorganic sorption materials are porosity and rough morphological surface with honeycomb-like anatomical or other irregular structures. The primarily properties, which characterize the material sorption abilities are cation exchange capacity (CEC) related to the quality and quantity of surface functional groups, and the size of sorption surface area (SSA) directly related to material's porosity (Palansooriya et al. 2020).

Despite many advantages, (bio)remediation of contaminated soil with organic/inorganic amendments has some disadvantages. In case of in situ immobilization, heavy metals are not removed from soil. They remain in the environment as a potential future pollutant (Hannan et al. 2021a). It is also hypothesized that due to degradation of organic amendments, under changing environmental conditions, previously bound metals may be released and become bioavailable again over time (Kabata-Pendias 2011; Hu et al. 2014; Kamari et al. 2015; Palansooriya et al. 2020). A special attention should also be paid to the chemical composition of amendments themselves. Some of them, especially produced from waste biomass (e.g., sewage sludge and animal manure) may contain toxic elements such as Zn, Cu, Cr, Ni, Pb, Cd, and Hg, which may cause secondary soil contamination (Ayaz et al. 2021; Li et al. 2021).

In the vast majority of cases, the efficacy of soil amendments to immobilize heavy metal ions is evaluated in pot experiments (Table 1). Therefore, there is a need to perform detailed in situ experiments to check the degree of degradation of organic amendments in soil over time, accumulation of heavy metals in cultivated plants, as well as in the (bio)remediated soil. Long-term field experiments are needed to assess the safety, biological toxicity and stability of these amendments used in immobilization of heavy metals in soil.

3.1 Materials Used in Heavy Metal Ions Immobilization in Soil

Table 1 presents the examples of (a) inorganic, (b) organic, as well as (c) comparison of inorganic versus organic and (d) mixed (inorganic and organic) amendments used in the immobilization of heavy metal ions in polluted soil and their effect on accumulation of heavy metal ions in plants and crop growth. Most of the remediation experiments, performed in the last decade, were carried out in pots with the use of maize, wheat, grass, rapeseed, rice or spinach as model plants. For the immobilization of heavy metal ions, naturally contaminated soil (multi-metal-contaminated soil collected from polluted areas) or soil artificially polluted in the laboratory (usually contaminated with a single heavy metal) were used. Most of the research concerned the verification of soil amendments effectiveness in the immobilization of cadmium and lead. Soil amendments used in the heavy metals immobilization can be applied at several rates (low, medium, and high) ranging usually from 0.5 to 5% (Zhou et al. 2020).

There are two main parameters used to evaluate an individual plant's ability to accumulate and translocate heavy metal ions. The bioconcentration factor (BCF) refers to the ratio of metal content in plant (stem and leaf tissues) to metal content in soil. The transfer/translocation factor (TF) is the ratio of the heavy metal content in plant (stem and leaf tissues) to that in the root (Cakmak and Marschner 1992; Kabata-Pendias 2011; Kamari et al. 2015; Wang et al. 2020).

Inorganic Sorbents

Clay minerals are ubiquitous phyllosilicate minerals found principally in soils, marine sediments and argillaceous shale rocks. Their formation is a result of a hydrothermal action, sedimentation or weathering of aluminosilicate rocks. The basic structural unit of clays comprises tetrahedral silicate sheet(s) connected to an octahedral aluminum hydroxide sheet by weak electrostatic interactions. Arrangement of the tetrahedrons in layers results in a hexagonal network, while the octahedral layer in an octahedral configuration. Both networks comprise strong covalent bonds inside their sheet. The basis for clay minerals classification is the ratio of tetrahedral to octahedral sheets which can be 1:1 or 2:1. The main representatives of clay minerals are: (1) kaolinite, represented by $Al_2Si_2O_5(OH)_4$, is a main component of kaoline and other clays. Its 1:1 structure consists of a silicon-centered tetrahedral sheet and an aluminum-centered octahedral sheet; (2) bentonite

Table 1 The examples of (a) inorganic, (b) organic, (c) comparison of inorganic versus organic and (d) mixed (inorganic and organic) amendments used in the immobilization of heavy metal ions in polluted soil and their effect on soil/plants

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
(a) Inorganic amendments				
Pb, Cd, Zn	Clay mineral—bentonite, Na form	Field experiment	Reduction in metal ions plant uptake (30% of Pb, 46% of Cd, and 30% of Zn), increase in soil pH	Vrînceanu et al. (2019)
Pb—120 mg/kg, Cu—230 mg/kg, Ni—270 mg/kg, Zn—340 mg/kg	Clay mineral—montmorillonite, 25 kg per cubic meter of soil	Percolation test/7 days	Increase in Zn^{2+} and Ni^{2+} adsorption but no effect on the retention of Pb^{2+} and Cu^{2+} when compared with the adsorption by soil alone	Correia et al. (2020)
Pb—700 mg/kg, Cu—430 mg/kg, Zn—1800 mg/kg	Clay mineral—vermiculite, 0.2 g per 1.8 kg of soil	Maize (*Zea mays*)/greenhouse pot experiment/4 months	Decrease in Pb accumulation in leaves, stalks, and roots by 27.6, 22.6, and 69.7%; Cu accumulation by 29.2, 15.3, and 19.9%; Zn accumulation by 15, 14.6, and 13.2%, respectively	Parmar et al. (2022)
Cd—7.4 mg/kg, Cu—229 mg/kg, Ni—140 mg/kg, Zn—228 mg/kg	Zeolite—clinoptilolite from 1.25 to 10% w/w in a contaminated soil	Ryegrass (*Lolium multiflorum*)/greenhouse pot experiment/12 weeks	Addition of 2.5% w/w of natural zeolites caused a significant decrease in Cu and Zn content in soil and in plant tissue; Cd, Cu, Ni, Zn content in roots decreased with 10% zeolite addition rate by 17, 21, 7, and 42% while in shoots by 37, 45, 93, and 56%, respectively	Contin et al. (2019)
Cd—5 mg/kg	Zeolite obtained by geopolymerization of metakaoline	Batch study, soil mixed with geopolymer-supported zeolite/20 days	Cd immobilization efficiency up to 58.7%	Wu et al. (2021)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
Pb—9876 mg/kg, Cd—224 mg/kg, Zn—654 mg/kg	Phosphate compounds—$(NH_4)H_2PO_4$	Indian mustard (*Brassica juncea*)/pot experiment/6 weeks	Variation in the solubility of P compounds and the nature of metal phosphate compounds formed influenced the differential effect of P-induced immobilization; the addition of amendment increased supply of P and decreased bioavailability of heavy metals by 56.7% for Cd, 78.9% for Pb, and 39.2% for Zn	Seshadri et al. (2017)
Cd—1.5 mg/kg, Pb—100 mg/kg	Fly ash /30 g per 3 kg of soil	Rapeseed/greenhouse pot experiment/65 days	Reduction of Pb (14.7%) and Cd (3,6%) in contaminated soil primarily through increased soil pH; significant reduction of Pb and Cd in rapeseed plant tissue (66.1% and 48%, respectively)	Shaheen and Rinklebe (2014)
Cd—0.89 mg/kg, Pb—47.35 mg/kg	$Ca(OH)_2$	Field experiments, $Ca(OH)_2$ was applied into alluvial soil 15 days before nursery of rice seeds were transplanted	Application of lime up to 3600 kg/acre, increased soil pH which induced changes in hydroxide and carbonate precipitation, and caused deprotonation of adsorption sites at soil surface; 21% improvement of the rice grains yield and significant reduction in metal content in roots, shoots, and grains	Hamid et al. (2019)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
(b) Organic amendments				
Pb—800 mg/kg	Biochar from wheat straw/20, 40 g/kg; chicken manure/20, 40 g/kg; a combination—each at 20 g/kg	Maize (*Zea mays*)/pot experiment/45 days for measurement of antioxidant enzyme activity of leaves, 80 days for remaining measurements	For all amendments: (1) increase in maize plant height, biomass weight, activity of superoxide dismutase, peroxidase and catalase, decrease in the malondialdehyde content; (2) significant decrease in Pb content in maize (roots > stems > leaves), bioconcentration factor, translocation factor, available Pb in soil; biochar alone: more effective at soil alkalinization and Pb immobilization; chicken manure alone: more effective at maize growth increase and antioxidant enzymatic activity; combination: most significant decrease in Pb in maize tissues (roots—53.9%, stems—75.5%, leaves—67.5%) and soil available Pb	Liu et al. (2021)
Cd—0.38 mg/kg	Biochar from corn stalk; polyethyleneimine (PEI)-modified biochar/2600, 5200, 13,000 kg/ha	Wheat/field experiments	Significant difference in the Cd content in different parts of wheat for the same biochar treatment: root > stem and leaf > glumes > grains—lower than in the control group; with the increase in the biochar dose, decrease in Cd content in wheat—the best results for modified biochar at 13,000 kg/ha; enhancement of the catalase, urease, alkaline phosphatase and sucrase activity in soil	Tang et al. (2022)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
Pb—20 mg/kg, Cd—10 mg/kg, Cr—20 mg/kg	Biochar from maize straw; compost/0.5, 1, 2, 4%	Maize (*Zea mays*)/pot experiment/60 days	Decrease in heavy metals availability in soil treated with biochar and compost; biochar was more effective in immobilization of heavy metals in soil as compared to compost; for 4% biochar, the highest decrease in shoot Pb content—by 71%, Cd—by 63% and Cr—by 78%, and then 50, 50 and 71%, respectively for 4% compost when compared to the control	Irfan et al. (2021)
Cd—1 mg/kg	Peanut shell biochar (BC); crop straw (CS)/5% d.w	Peanut/pot experiment/4 months	Better results (statistically significant) for BC than for CS—enhancement of the peanut biomass and physiological quality; greater impact on Cd immobilization in soil; remarkable decrease in Cd bioavailability	Chen et al. (2020)
Cd—6.54 mg/kg and Pb—1344 mg/kg	Kitchen waste biochar (KWB); corn straw biochar (CSB), peanut hulls biochar (PHB)/20, 40, 60 g/kg	Swamp cabbage (*Ipomoea aquatica*)/pot experiment/30 days	Enhancement of soil pH, reduction in the extractable Pb and Cd in soil by all amendments; decrease in Cd and Pb accumulation in roots, stems, and leaves by 45.4–97.7% for KWB, 59.3–96.6% for CSB, and 63.9%–99.3% for PHB; immobilization performance order: KWB > CSB > PHB	Xu et al. (2020)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
Brown soil polluted with Cd	Peanut shell biochar (HBC); Mg-modified peanut shell biochar (MHBC)/1, 2%	Spinach/pot experiment/five weeks	Better amendment effect for MHBC than HBC; decrease in bioavailable Cd in soil by 26.2% to 50.1% for MHBC; higher reduction in Cd content in roots and shoots for MHBC than for HBC; decrease in Cd accumulation in roots and shoots with increase in biochar dose	Shan et al. (2020)
Lead smelting slag-contaminated soil Pb—18 300 mg/kg	Compost (C) from wild sunflower and poultry liter (3:1) for 12 weeks; rice husk biochar (RHB); cashew nut shell (CNSB) biochar; compost-modified biochar (RHBC and CNSBC)/0.05, 0.1, 0.2, 0.4 g/g of soil	Maize/pot experiments/6 weeks	Reduction in maize Pb uptake by all amendments, but to different extent; better performance of compost-modified biochars than the amendments used singly; the least Pb uptake by maize in soil for compost-modified biochar RHBC followed by CNSB, RHB, CNSB and then C; for 0.1 g/g dose of RHBC—decrease in root content by 91% and in shoot by 86%	Ogundiran et al. (2015)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
(c) Comparison of inorganic vs. organic amendments				
Ni—50 and 100 mg/kg	Biochar from bamboo; mussel shell; zeolite; limestone/3%	Rapeseed (*Brassic napus*)/pot experiment/90 days	Reduction in Ni bioavailability after soil addition of amendments; the best results for biochar and shells—decrease in Ni content by 30.4% and 25.1% in roots and by 54.5% and 50.6% in shoots, respectively for 50 mg/kg of Ni in soil; for 100 mg/kg of Ni in soil, the highest decrease in Ni content for shells and biochar—by 41.2% and 38.0% in roots, and 48.4% and 44.2% in shoots as compared to the control	Hannan et al. (2021a)
Multiple-metal-contaminated soil: Cd—2.6 mg/kg, Pb—1796 mg/kg, Zn—1603 mg/kg	Pine shoots biochar (BC); cow dung-based manure (DM), hydroxyapatite (HAP)/0.1, 1%	Maize (*Zea mays*)/pot experiment/60 days	HAP (optimal one for stabilizing heavy metals in soil): significant decrease in Cd, Pb, and Zn content in shoots and roots (better effects for the higher dose)—for 1% reduction of the shoot Cd, Pb, and Zn content by 67%, 85% and 84%; DM: decrease in the shoot Cd and Pb content and root Zn content (for 0.1 and 1%), but only for 1% DM decrease in the shoot Zn and root Pb content; BC: decrease in the shoot Cd and Pb content (for 0.1 and 1%), but decrease in the shoot Zn and root Pb content only for 1%	Wang et al. (2020)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
Multiple-metal-contaminated soil: Zn—2273 mg/kg, Cu—372 mg/kg, Pb—816 mg/kg	Coconut tree sawdust (CTS); sugarcane bagasse (SB); eggshell (ES)/1, 3%	Water spinach (*Ipomoea aquatica*)/pot experiment/8 weeks	Decrease in shoot metal content with the increase in amendment dose; significant reduction in metal uptake for 3% ES; reduced bioavailability of metals—for example Zn was reduced by 74.5% for 3% ES, 56.2% for 3% SB and 31.0% for 3% CTS	Kamari et al. (2015)
Cs—100 mg/kg	Coconut shell biochar (BC); incinerated sewage sludge ash (ISSA); zeolite/10%	Napier grass (*Pennisetum purpureum*)/pot experiment/1–7 months	For all amendments—enhancement of the grass growth (the best for ISSA treatment); decrease in Cs content in the leaf blade, leaf sheath and root of grass (the best for BC treatment—decrease up to 95% as compared to the control group—without soil amendment)	Shao et al. (2021)
Cd—0.86 mg/kg	Maize straw biochar (BC); zeolite (ZE); humic acid (HA)/1, 2.5, 5%; lime (L); sodium sulfide (SS); superphosphate (SP)/0.5, 1, 2%	Wheat (*Triticum aestivum*)/pot experiment	5% BC, 1% ZE and 0.5% L are recommended for Cd immobilization in acidic or neutral soils; better immobilization effect for high doses than for the low doses; significant decrease in Cd content in the roots within the range of 23.7–27.2% for BC, 29.5–33.0% for ZE, 16.3–37.4% for HA, and 48.2–65.5% for L and significant decrease in Cd content in the straw for 2.5 and 5% ZE and 5% HA as compared to the control	Zhou et al. (2020)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
(d) Mixed (inorganic and organic) amendments				
Multiple-metal-contaminated soil: Pb, Cd	Corn stover biochar (BC)/coal ash incorporated biochar (CA/BC)/1, 3, 5%	Paddy rice (*Oryza sativa* cv. Nipponbare)/pot experiment/35 days	Significant enhancement of immobilization efficiency of CA/BC for Pb –77% and 43% for Cd as compared to BC; for 5% CA/BC reduction in the content of Pb (by 81%) and Cd (by 62.5%) in rice as compared to control and significant promotion of rice growth—by 3.1, 2.2 and 2.0 times in terms of root, stem length and dry mass as compared to the control	Xia et al. (2021)
Multiple-metal-contaminated soil: As—39.5 mg/kg, Cu—33.6 mg/kg, Zn—108 mg/kg	Bentonite (B); talc (T); activated carbon (AC); corn starch (CS); composites (BAC, BCS, TAC, TCS)/2%	Lettuce/pot experiment/45 days	Small content of As in shoots after amending soil with B, AC, BAC, T and TAC as compared to the control; no effect of the treatments on As accumulation in roots; reduction in Cu uptake by roots and shoots for tested amendments (significant for AC); decrease in the Zn content in plant tissues after amending soil (in particular for BCS)	Quoc et al. (2021)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
Multiple-metal-contaminated soil: Cu—783 mg/kg, Cd—14.5 mg/kg, Zn—477 mg/kg	Coal fly ash/5%; straw/2%; their combination (homogeneous mixture of the part of two treated soils)	Rape (*Brassica napus*)/pot experiment/6 weeks	The highest stabilization efficiency for the combined application of amendments—reduction in extractable Cu, Cd and Zn by 94.6, 67.1 and 73.3%, respectively; significant reduction in Cu, Cd and Zn content in plant shoot for coal fly ash and the mixture of coal fly ash and straw	Hu et al. (2014)
Multiple-metal-contaminated soil: Zn—667 mg/kg, Ni—860 mg/kg, Pb—586 mg/kg, Cd –122 mg/kg	Phosphogypsum (PG) used alone and in combination with rice straw compost (CP) (1:1)/10, 20 g d.w./kg of soil	Canola (*Brassica napus*) pot experiment/10 weeks	PG alone or in combination with CP immobilized heavy metal ions (better effect for PG used alone); optimal dose was 10 g/kg; mixture of PG and CP gave the highest canola growth at 10 g/kg (increase by 66.8% as compared with the control group)	Mahmoud and Abd El-Kader (2015)
Cd—2.5 mg/kg, Pb—300 mg/kg, Zn—500 mg/kg	Willow biochar; zeolite and their combination/0.5% (15 t/ha)	Grass species: tall fescue (*Festuca arundinacea*) and cocksfoot (*Dactylis glomerata*)	Zeolite was a more effective soil amendment than biochar in soil remediation; higher biomass production for zeolite alone or mixed with the biochar than in control treatment; zeolite applied alone significantly affected root biomass and root morphology; no effect of biochar on most root morphometric parameters	Głąb et al. (2021)

(continued)

Table 1 (continued)

Heavy metal/content in soil	Soil amendment/dose	Plant species/type of experiment/duration	Effect of sorbent on soil/plant	References
Contaminated paddy field: Cd—0.51 mg/kg, Pb 107 mg/kg	Lime, biochar, Fe-biochar, Ca-Mg-P fertilizer, GSA-2 (a combination of biochar, lime, sepiolite and zeolite), GSA-3 (a combination of organic and inorganic additives), and GSA-4 (a combination of manure, lime and sepiolite)/1%	Rice (*Oryza sativa*)/field experiment—mixing of amendments thoroughly on the upper surface of soil, mechanical ploughing into 0–20 cm plow layer 15 days before transplanting	General decrease in Cd and Pb content in roots, shoots, grains and husk after amendments application as compared to the control; more effectivity of a combined amendment (GSA-4) for immobilizing Cd and Pb in field as compared to other tested amendment; the best effect on reducing Cd and Pb phytoavailability in soil and uptake by early rice for GSA-4 treatment—reduction in Cd and Pb uptake in shoot (42% and 44%) and in grains (77 and 88%, respectively); improved rice growth (56%) and grains yield (42%) also for GSA-4 as compared to the control	Hamid et al. (2018)
Aged Ni contaminated soil—100 mg/kg	Mussel shell (MS); bamboo biochar (BC); and their combination (BC + MS, 3:1; 1:1; 1:3)/3%	Rapeseed (*Brassica napus*)/pot experiment/90 days	The combination of MS and BC was more effective in the Ni immobilization, reduction of metal toxicity to plants and the improvement of soil biology; the lowest content of Ni in shoots and roots for BC + MS (3:1) as compared to other experimental groups	Hannan et al. (2021b)

is represented by a chemical formula $(Na)_{0.7}(Al_{3.3}Mg_{0.7})Si_8O_{20}(OH)_4{\cdot}nH_2O$ and is composed of montmorillonite, hectorite, saponite, beidellite and nontronite. Its 2:1 structure comprises of an aluminum-centered octahedral layer sandwiched between two silicon-centered tetrahedral layers; (3) montmorillonite is represented as $(Na,Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2{\cdot}nH_2O$. Its 2:1 structure comprises of an aluminum-centered octahedral layer sandwiched between two silicon-centered tetrahedral layers; (4) illite, represented as $(K,H_3O)Al_2(Si_3Al)O_{10}(H_2O,OH)_2$, has a basic 2:1 structure—two tetrahedral silica layers surrounding an octahedral aluminium layer in the center but with greater silica-to-alumina ratio compared to montmorillonite; (5) vermiculite is represented by $(Mg,Fe_3,Al)_3(Si,Al)_4O_{10}(OH)_2{\cdot}4H_2O$, which structure is often referred to as 2:1 phyllosilicate. Two tetrahedral silicate layers are bonded together by one octahedral magnesium hydroxide-like layer (Shaikh et al. 2017).

Layered plate-like structure provides clay minerals with plastic properties on wetting as well as hardness on drying. The only exception is illite, which due to the poor hydration properties of the interlayer K^+ exchangeable cations, is a non-swelling clay. The unique swelling properties provide significant capacity to adsorb water. Furthermore, the empty metal centers (no occupied by Al) in the octahedral layers act as adsorption centers for metal ions dissolved in water. Metal ions are attracted to siloxane faces (Si–O–Si siloxane bridges with the O atom at the surface) by small negative charge arise from isomorphous substitution of Si^{4+} by Al^{3+} in surface of tetrahedral sheets. Metal ions can be also adsorbed on the crystal edges on the places resulting from bearing primary bonds Al–O and Si–O. These are so-called permanent active sites with a pH independent charge. In the contrary, the variable active sites are influenced by pH. The change of pH causes protonation/deprotonation of the silanol (≡Si–OH) and aluminol (=Al–OH) hydroxyl groups. The aluminol sites are negatively charged at pH > 9, while silanol sites at pH < 9. Both sites interact with positively charged metal cations via H-bonding. The resulted outer-sphere complexes (X_2Me) contain water or hydroxyl ligands between the metal center and the mineral surface. Their formation is effected by ionic strength. Inner-sphere surface complexation is related to direct covalent bonding of metal ions to the variable active sites on the clay mineral surface. The effect of ionic strength on inner-sphere complexes formation is negligible. The amount of variable sites decreases along with the decreasing pH, and at about pH 5.5 adsorption occurs exclusively at permanently charged sites. Metals that form hydrolysis products more readily (e.g., Pb, Cu) start to adsorb onto variable active sites, while metals with lower tendency to hydrolysis (e.g., Zn, Cd) are more eager to be adsorbed on permanent active sites. The amount of variable and permanent sites available for a given metal ion adsorbed from multi metal solution can be estimated from an appropriate equilibrium model (Matłok et al. 2015).

Zeolites are crystalline aluminosilicates with three-dimensional frameworks of SiO_4 and AlO_4 tetrahedral units. The aluminum ion is small enough to occupy the position in the center of the tetrahedron of four oxygen atoms, while the isomorphous replacement of Si^{4+} by Al^{3+} produces a negative charge in the lattice. The negative charge is balanced by the interchangeable positive ions such as Na^+, K^+, Ca^{2+}, and Mg^{2+}. These cations are exchangeable with certain cations in solutions, such as heavy metals.

Clinoptilolite is the most common natural zeolite, which belongs to the heulandite family. Its chemical formula is $(Na,K,Ca)_4Al_6Si_{30}O_{72}\cdot 24H_2O$. The characteristic tabular morphology shows an open reticular structure of easy access, formed by open channels of 8- to 10-membered rings. There are two channels running parallel to each other: 10-member (tetrahedron) ring of the size of 4.4–7.2 Å and 8-member ring with the size of 4.1–4.7 Å, and 8-member ring channel run parallel with the size of 4.0–5.5 Å. Porous structure of the clinoptilolite consists of microporosity presented by nanotube system of aluminosilicate framework, mesoporosity formed by slot pores determined mainly by cleavability of the zeolite crystallite, and macropores consisted of various forms located between blocks of the zeolite crystallite and other minerals in the zeolitic tuff. Clinoptilolite occurs in andesite, rhyolite, and basalt rocks as veins and impregnations. But the large industrial deposits are connected with volcanic sedimentary high-silica rocks. Zeolite tuffs often contain more than 70% clinoptilolite. Associated minerals are usually quartz, cristobalite, calcite, aragonite, thenardite, feldspars, chlorite, montmorillonite, and other zeolites.

Fly ash-based geopolymers (GPs) are alkali-activated aluminosilicates with tri-dimensional network structure of –Si–O–Si(Al)– bonds. In opposite to zeolitic materials, tetrahedra of SiO_4 and AlO_4 are linked by sharing all the oxygen atoms. The GP low-CO_2 binders are synthesized via an alkali-activation method of a solid source of SiO_2 and Al_2O_3. The most commonly used aluminosilicate raw materials are metakaolin and, coal fly ash, bottom ash, red mud, slag, and biomass fly ash. They can be used as a substrate of the geopolymers synthesis. The GPs formation mechanism includes dissolution, rearrangement, condensation, and resolidification and is affected by the temperature, amount of water, mixing method, and physical properties of the source material. Depending on reaction conditions and an alkaline activator used for their manufacture, the resulting material is amorphous or semicrystalline zeolites. The geopolymers prepared with NaOH show more crystalline morphologies compared to the geopolymers prepared with Na_2SiO_3 which feature more amorphous phases. Anyway, fast kinetics process and reaction pace do not allow for the gel or paste to grow into a well-crystallized structure (Provis 2014). The favorable properties of geopolymers are high compressive strength and fire and acid resistance which make them an alternative material to cementitious binders. GPs application significantly reduce soils' swell-shrink behavior, responsible for damage of foundations, roads, or embankments. The study of Vitale et al. (2017) reveals that when alkali activated binders contact with contaminated soil, they promote the formation of new mineralogical phases responsible for the mechanical improvement of treated soil. Furthermore, large total porosity, well-defined pore size distribution, and low permeability of geopolymer structures enable immobilization of heavy metal in both cationic and an anionic forms. It opens up the possibility of GPs usage as filtration media in sand filters, permeable reactive barriers, or point-of-use water treatment filters (Adewuyi 2021).

Phosphate compounds such as phosphate rocks, synthetic hydroxyapatite, KH_2PO_4, CaH_2PO_4, H_3PO_4, commercial phosphate fertilizers, bone meal (from animal bones), and slaughter-house waste products are able to immobilize heavy metals in contaminated soils by the formation of stable minerals. The variation in the solubility of the P compounds influences the mechanism of metal ions immobilization. In case of soluble phosphates, the process occurs with interaction of metal ions with P-induced adsorption sites, and their precipitation in a form of insoluble phosphates, e.g., with Pb: chloropyromorphite ($Pb_5(PO_4)_3Cl$), hydroxypyromorphite ($Pb_5(PO_4)_3OH$), and fluoropyromorphite ($Pb_5(PO_4)_3F$); with Cu and Zn: hopeite (Huang et al. 2016). The reaction of P with Pb proceeds through the formation of the highly insoluble pyromorphites [$Pb_5(PO_4)_3(OH, Cl, F)$]. Importantly, the formed precipitates are not digestible for plants. The parameters that affect the process efficiency are pH and soluble salts content. Especially the presence of $H_2PO_4^-$ and SO_4^{2-} ions in soil enhances metal ions adsorption. pH decrease causes increase in the negative charge on iron and aluminum oxides on soil surface. It favors formation of metal-carbonate and metal-Fe–Mn-oxide bounds (in e.g., ferromanganese oxyhydroxides) (Andrunik et al. 2020). Nevertheless, the immobilization of metals was relatively ineffective when phosphate rock was applied to calcareous soils, which can be attributed to the poor reactivity of apatite. The possible mechanism of this process is an ion exchange between metal cations and Ca^{2+} from the apatite particle. Dissolution of apatite is rather limited in alkaline soils what diminishes lead immobilization as pyromorphite. Phosphorus fertilizers, added to the contaminated soil, release phosphoric acid, which promptly dissociates into phosphate ions and acidic hydrogen ions. This process supports the solubilization of metal ions and their subsequent precipitation. Natural phosphate rocks applied as fertilizers not only release P and Ca but can also reduce soil acidity by releasing $CaCO_3$ (Seshadri et al. 2017).

Lime materials have been recognized as carbonates, oxides, and hydroxides of Ca and Mg as well as a set of wastes e.g., eggshells, mussel shells, and oyster shells, limestone, sugar beet factory lime, and cement kiln dust (Hamid et al. 2019). Liming materials enhance soil quality by ameliorating soil acidity, thereby aiding crop productivity and immobilization of heavy metals by precipitation as carbonates. On the other hand, liming materials increase the content of Cd bound to mobilizable soil fractions at the expense of the most-environmentally-inert fractions. Hence, the combined use of liming and vegetation may increase the long-term environmental risk of Cd solubilization and leaching. Too higher dosages of CaO (above 10% w/w) could negatively affect plant growth, increase metal ions phytoavailability, and immobilization of beneficial micronutrients such as Fe, Mn, Zn Cu, and B. Therefore, determining soil qualities and its pH are important considerations prior to lime application. Combined application of liming materials with appropriate organic amendments (sewage sludge, manure compost) can minimize heavy metals mobility and phytoavailability (Holland et al. 2018).

Organic Sorbents

Waste biomass from agricultural operations (e.g., straw, leaves, bagasse from sugar cane, rice hulls from rice processing, animal manure—cattle, poultry), forest industry (e.g., bark and wood chips, bark saw dust, wood ash), mushroom cultivation (mushroom residue), wastewater treatment (sewage sludge), produced compost/vermicompost or biochar may become inexpensive sorbents of heavy metals for soil bioremediation. These materials are characterized by large porous structure and surface area with abundant functional groups responsible for contaminants binding. The examples of organic amendments used in the immobilization of heavy metal ions in polluted soil and their effect on soil/plants are presented in Table 1b.

The most popular soil amendment is biochar, a carbonaceous material, being the major product of pyrolysis or gasification (pyrolysis combined with partial oxidation) of biomass (EBC 2012; Lwin et al. 2018; Guo et al. 2020; Li et al. 2021). Biochar can be obtained from a variety of feedstocks, for example wheat (Liu et al. 2021) or maize straw (Xu et al. 2020; Zhou et al. 2020; Irfan et al. 2021), corn stalk (Tang et al. 2022) and stover (Xia et al. 2021), rice husk (Ogundiran et al. 2015), as well as kitchen waste (Xu et al. 2020), bamboo (Hannan et al. 2021a, b), pine shoots (Wang et al. 2020), willow (Głąb et al. 2021), pig manure digestate (Ayaz et al. 2021), coconut shell (Shao et al. 2021), peanut hulls (Xu et al. 2020) and shells (Chen et al. 2020; Shan et al. 2020), etc. The feedstock source material determines the biochar properties and sorption abilities as well as the effect on morphometric parameters of cultivated plants (Guo et al. 2020; Xu et al. 2020; Głąb et al. 2021). Guo et al. (2020) in their review provided a detailed characteristics of biochars obtained from different organic feedstocks and under different carbonization conditions, together with examples of the biochar-facilitated remediation of heavy-metal-contaminated soils.

Li et al. (2021) indicated that biochar produced by co-pyrolysis of different feedstocks is more effective in removing heavy metals from contaminated soil than the pristine biochar. Depending on the feedstock, biochar can vary in the content of organic carbon and ash, plant nutrients (N, P, K), base cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), as well as pH. The carbonization conditions, for example temperature, solid residence time and the heating rate, also influence the biochar quality (e.g., Irfan et al. 2021; Li et al. 2021; Liu et al. 2021; Tang et al. 2022). With the increase in pyrolysis temperature, decrease in the cation exchange capacity (CEC) is observed, which is a measure of negatively charged functional groups (e.g., $-C=O$, –COOH, and –OH) on the sorbent surface binding positively charged ions (Guo et al. 2020). On the other hand, the temperature increase favors micropore development and causes increase in the specific surface area of sorbent (Guo et al. 2020).

The biochar properties, closely associated with heavy metal ions stabilization in soil, are alkaline pH (increases soil pH) and high CEC (responsible for uptake and release of N, P, K, Ca, Mg, S to soil) (Lwin et al. 2018; Guo et al. 2020; Shao et al. 2021). In an alkaline environment, the majority of metals appear to be less soluble (Hu et al. 2014). Additionally, biodegradable organic carbon can be released from biochar to soil (Shao et al. 2021; Irfan et al. 2021). Organic matter increases soil metal adsorption capacity by promoting exterior complexation reactions that result in metal–organic ligand complexes (Hu et al. 2014). High content of organic matter (in opposite to inorganic amendments) significantly impacts the ability of metal ions retention over the long period of time (Hannan et al. 2021a). Furthermore, environmental recalcitrance of biochar, estimated at 90–1600 years, is a significant advantage over compost and other raw bio-materials (Singh et al. 2012). In this study, long-term (5 years) laboratory experiment revealed that between 0.5 and 8.9% of the biochar carbon was mineralized depending on biochar feedstock (papermill sludge, *Eucalyptus saligna* wood and leaves, cow manure and poultry litter) and pyrolysis temperature. The carbon in manure-based biochars was mineralized faster than that in plant-based biochars, and carbon in biochars produced at 400 °C mineralized faster than that produced at 550 °C.

Other popular organic amendments used in polluted soil bioremediation are crop straw and compost. The first one is known to have a positive effect on soil structure, stimulation of crops growth and development. Returning straw to the field can result in the production of dissolved organic carbon (DOC), which acts as an organic ligand of heavy metals complexation (Xu et al. 2016; Chen et al. 2020). The same concerns compost, which can also be used to stabilize heavy metals in polluted soil. Ogundiran et al. (2015) showed that compost obtained from wild sunflower (*Tithonia diversifolia*) and poultry liter released dissolved organic matter faster than biochar produced from rice husk and cashew nut shell due to the action of microorganisms. As a result, more functional groups were available for binding heavy metal ions in soil and compost was more effective in stabilization of Pb than biochar. Different results were obtained by Irfan et al. (2021) and Chen et al. (2020) who showed that biochar had better immobilization properties of heavy metals than crop straw. This indicates the complexity of the immobilization process of heavy metals in soil by additives, which is influenced by many factors from the preparation of the additive itself, to soil properties and others.

It is also possible to use a mixture of organic amendments for contaminated soil treatment. Liu et al. (2021) showed that the combined use of biochar from wheat straw and chicken manure resulted in the most significant decrease in Pb level in maize tissues (reduced uptake) and soil Pb bioavailability, and increase in soil enzyme activity and maize growth when compared with single amendments treatments.

Some soil amendments (e.g., traditional biochar) have certain limitations in their adsorption capacity, therefore more research is focusing on sorbents modification to improve their surface properties (Li et al. 2021; Tang et al. 2022). This can be done through the acid or alkali, redox or magnetic modification of biochar (Tang et al. 2022). Several modified biochars have already been tested in the bioremediation of contaminated soil, for example biochar modified with polymer—polyethyleneimine was used to immobilize Cd (Tang et al. 2022), coal ash incorporated biochar for the treatment of multimetal (Pb, Cd) contaminated soil (Xia et al. 2021), Mg-modified peanut shell biochar to immobilize Cd (Shan et al. 2020), etc. Shan et al. (2020) showed that an increase in Mg content in biochar after modification promoted the exchange of Mg^{2+} loaded on the biochar surface with the Cd^{2+} in soil, thereby fixing Cd^{2+} on the biochar surface.

Inorganic Versus Organic Amendments

In order to select the most effective additive for the immobilization of metals in contaminated soil, comparative studies—organic versus inorganic amendments—are carried out. Usually biochar is compared with inorganic amendments, like zeolite, limestone, hydroxyapatite etc. (Table 1c). There is no clear indication which type of amendment—organic or inorganic—exhibits higher ability to immobilize heavy metals. Usually all tested additives reduce heavy metals mobility in soil and their bioavailability to plants. The differences in the immobilization degree of heavy metals resulted mainly from the physicochemical characteristics of the used additives. For example, Shao et al. (2021) showed that biochar from coconut shell and zeolite exhibited higher CEC and surface negative charges than the incinerated sewage sludge ash, indicating that these two amendments had higher electrostatic adsorption abilities for Cs^+. Interesting results were presented by Zhou et al. (2020)—to achieve a similar degree of immobilization of Cd in the acidic and neutral soil, biochar from maize straw is recommended at a dose of 5% while zeolite at a much lower 1% dose. Comparison of the raw biomass (unprocessed e.g., by pyrolysis) such as coconut sawdust or sugarcane bagasse with eggshells indicated better affinity for Zn, Cu and Pb of inorganic amendment (Kamari et al. 2015).

Mixed (Organic and Inorganic) Amendments

More and more frequently the immobilization of heavy metals in contaminated soil is done by the application of combinations of organic and inorganic amendments (Hu et al. 2014; Li et al. 2021; Pei et al. 2021; Quoc et al. 2021). Table 1d presents the examples of mixtures containing organic and inorganic amendments used in soil remediation. Integrated application of adequate amendments is recommended to maximize their efficiency (Palansooriya et al. 2020). It is hypothesized that blending amendments could provide more binding sites for heavy metal(loid) stabilization in soil (Li et al. 2021; Quoc et al. 2021). Analysis of amendments mixtures by several morphological and physical techniques revealed that they have a higher adsorption capacity towards metal ions than amendments applied individually (Hannan et al. 2021b). The treatment of contaminated soil with organic and inorganic additives not only effectively immobilizes heavy metal ions, but also has a greater impact on soil fertility parameters (e.g., higher availability of N, P, K, soil organic matter, and microbial biomass C and N) and on microbial community composition (Głąb et al. 2021; Pei et al. 2021). Several researchers have confirmed better heavy metal immobilization efficiency and better plant growth stimulation for combined fixes than for single treatments (e.g., Hu et al. 2014; Mahmoud and Abd El-Kader 2015; Hamid et al. 2018; Hannan et al. 2021b; Xia et al. 2021). Nevertheless, as steams from the work of Hu et al. (2014), the increase in the rape (*Brassica napus*) biomass was observed only in the group treated with 2% straw, but not in the group with 5% coal fly ash and their combination case. It probably resulted from provision of plentiful organic matter produced during straw decomposition.

3.2 Characteristics and Mechanism of Action of Sorbents Used in Immobilization of Heavy Metal Ions in Soil

There are many instrumental techniques, which can be used to characterize physicochemical properties of amendments in order to identify their immobilization performance towards heavy metals. As examples can serve: Brunauer–Emmett–Teller (BET) to determine specific surface area of immobilizing agents using N_2 sorption analysis (Hannan et al. 2021b; Quoc et al. 2021; Shao et al. 2021); Scanning Electron Microscopy (SEM–EDS/EDX) to visualize the morphology of amendments as well as their elemental composition (e.g., Kamari et al. 2015; Wang et al. 2020; Głąb et al. 2021; Hannan et al. 2021b; Quoc et al. 2021; Shao et al. 2021); Fourier Transform Infrared Spectroscopy (FT-IR) to determine functional groups on the surface of amendments, which participate in ion exchange (e.g., Kamari et al. 2015; Zhou et al. 2020; Hannan et al. 2021a, b; Quoc et al. 2021); X-ray Fluorescence (XRF) (Quoc et al. 2021) or flame atomic absorption spectrometry (Quoc et al. 2021) to analyze the chemical composition of amendments; X-ray Diffraction (XRD) to confirm the formation of metal related ligands (e.g., Wang et al. 2020; Hannan et al. 2021a; Hannan et al. 2021b). Identified characteristics of the soil amendments enables prediction of the (bio)remediation mechanism.

Mechanism of Action of Inorganic Sorbents

An in situ field experiment carried out on severely polluted paddy soil revealed that alkaline clay minerals such as bentonite, sepiolite, and palygorskite immobilized heavy metals by increasing soil pH, and consequently forming $CdCO_3$ and $Cd(OH)_2$ precipitates (Liang et al. 2014). Generally, where clay minerals were used as an amendment to soil, there was an increase in the biomass of plants, whereas accumulation of metals decreased. Addition of Na-bentonite and Ca-bentonite reduced the labile fraction of heavy metal, improved wheat shoot dry matter production by decreasing shoot metal concentration below phytotoxicity levels and decrease in water-soluble metals in contaminated floodplain soil. It is attributed to bentonite's larger surface area and sorption capacity, and ability to increase soil pH which allow forming metal ions precipitates (Vrinceanu et al. 2019). Nevertheless, raw clay minerals have poor stabilization property and the adsorbed heavy metals can be released in the long term through ion exchange. One of the methods to enhance their stability is their thermal and chemical activation with e.g., magnesium carbonate. The treated mineral particles are covered by $MgO/Mg(OH)_2$ which results in decrease in pore volume and surface area. An increase in the pore diameter is linked with the formation of $Mg(OH)_2$ clusters in the mineral interlayer space, which induces a new phase of $MgSiO_3$. The magnesium-montmorillonite amendment increases alkalinity of the treated soil. This in turn causes a reduction of heavy metal solubility in basic surrounding due to the hydrolysis reaction and electrostatic adsorption on mineral surface as well as to precipitation with $Mg(OH)_2$. It is also supposed that the reduction of heavy metal bioavailability results from the fact that montmorillonite promotes the activity of soil bacteria to some extent and activates the expression of functional genes (Qin et al. 2020). Clay minerals amendments can enhance soil micronutrient availability as well. For example, vermiculite application allowed to obtain an optimum foliar Mn concentration with a simultaneous reduction of translocation factors for root-stalk and stalk-leaves (Parmar et al. 2022). This opens up the possibility of using clay minerals as complementary fertilizers, aiming at partially decreasing the high fertilization rates.

The mechanism associated with the successful use of natural zeolites was identified to be preliminary an ion-exchange. Beside, adsorption processes and surface precipitation/co-precipitation controlled by insoluble/soluble products interacting with other minerals can occur as well. The use of zeolites in acidic soils causes an increase in pH, reduction of electrical and hydraulic conductivity and lowering pore size of soils. As a consequence heavy metals solubility and bioavailability for plants are reduced. However, the increase in soil pH cannot be considered as a major factor influencing plant growth. 10% w/w zeolite addition resulted in decrease of heavy metals uptake by plants by 20.1% for Cd, 23.4% for Cu, 29.2% for Ni, and 26% for Zn. Additionally significant decrease in metal ions concentration was observed in plant tissue and shoots with efficiency following the order: Ni > Cu > Cd > Zn. The decreased leachability of metals followed almost a reverse order: Cu > Cd > Ni > Zn (Contin et al. 2019). Zeolites are unstable under acid conditions. Their presence in soils may influence the precipitation of some metals, which in turn changes charge density, pore distribution, and particle aggregation, subsequently affecting stabilization and accumulation of organic C. Thus, the use of zeolite for immobilization of soil heavy metals in urban or residential areas needs to be deeply evaluated in the long-term perspective. The beneficial effects of zeolite application as soil amendment is a significant increase of soil's K and Ca exchangeability. Zeolites in homoionic K form may serve as an effective slow-release potassium fertilizer and also as a potential soil conditioner that can improve soil infiltration and hydraulic conductivity. The controlled release of potassium, ammonium or phosphates improves nutrient use of plants and decrease runoff and sediments amount by increasing the soil water holding capacity. Moreover, zeolites can act as carriers, stabilizers and regulators of mineral fertilizers and macro- and micronutrients supplier. Application of zeolite significantly increased Zn uptake by spinach plants and favorably influenced the root growth of *Castanea sativa* plants (Chatzistathis et al. 2021). The increase in plant growth due to the addition of zeolites was observed in crop yield of potato, maize, rice, tomato, eggplant, carrot as well as cultivation of different cereals, forage crops, vine and fruit crops (Jakkula and Wani 2018). It could result from both alleviation of metals toxicity stress and an improvement of plant nutrition.

The mechanism of heavy metals immobilization by fly ash-based geopolymers is complex and can combine the ion-exchange with charge-balancing cations (Na^+, K^+), covalent bonding to the aluminosilicate network, the precipitation of hydroxides and carbonates and silicates in the matrix, and the physical encapsulation in a low-permeable geopolymeric matrix (Eleswed 2020). Siliceous composition enhances the formation of high-surface-area three-dimensional geopolymer gel. It straight translates into specific surface area, which is lower than 100 m^2/g. Addition of various boron compounds to the PGs synthesis reaction increases the value beyond 200 m^2/g (Siyal et al. 2018). Synthesis of GPs from fly ash precursor treated with 1 M Na_2SiO_3 (at 90 °C, 24 h) as alkali activator allowed to obtain the material with specific surface area up to 2464 m^2/g (Mondal et al. 2020). The increase in Ca/(Si + Al) and Al/Si ratios in GPs material decreases efficiency of Pb^{2+} removal. While, the increase of Na/Si ratio as well as the mass percent of the amorphous phase facilitate the Pb^{2+} uptake from soil. Moreover, higher Na content enhances ion exchange of Na^+ by Pb^{2+} in the charge balancing sites of the geopolymer framework. Nevertheless, the microstructure composition influences the kinetics of metal ions uptake more significantly than the equilibrium uptake. Decrease in the Si/Al molar ratio enhances the number of negatively charged Al tetrahedra and the pores volume but decreases the strength of GP. The presence of borax, anhydrous borax, boric acid, amorphous and crystalline lithium tetraborate, and colemanite limits the decrease of compressive geopolymer strength but causes heavy metal leaching (Rozek et al. 2021). It was also observed that the metal ions washing is lower in case of the ash-based GP synthesized under higher alkaline conditions.

The literature review suggests that fly-ash-based geopolymeric adsorbents are generally more effective compared with raw fly ash and zeolite adsorbents for the sequestration of aqueous and air contaminants. Nevertheless, practical application of GPs to soil stabilization is rather limited considering civil engineering requirements. The incorporation of metal ions into geopolymer matrix reduces its compressive strength and stability which leads to crushing and pulverization. It questions safe disposal of the material into landfill. GPs application for soil amendments has not been reported so far. Recently, Wu et al. (2021) presents in situ synthesis of zeolites by metakaolin geopolymerization in alkaline solution. The obtained materials were able to immobilize up to 58.7% of Cd(II) in paddy soil (Wu et al. 2021).

Mechanism of Action of Organic Sorbents

Organic sorbents possess a variety of functional groups (e.g., carboxyl, carbonyl, hydroxyl, phenolic hydroxyl groups, etc.), which provide active sites for ion exchange and adsorption of heavy metal ions as well as for nutrient retention (Lwin et al. 2018). Other factors influencing immobilization process are large surface area, high porosity and stability (Liu et al. 2021). Some of the amendments, like biochar exhibit alkaline pH (Liu et al. 2021). Biochar's ability to immobilize heavy metals in soil depends on its acid neutralization ability and high cation exchange capacity (Irfan et al. 2021). Increase soil pH transfers heavy metals into their less mobile ionic forms, controls their bioavailability in soil and reduces their movement from soil to plant tissues (Liu et al. 2021). Zhou et al. (2020) predicted that the increase in soil pH caused by the addition of biochar from maize straw caused Cd immobilization by precipitation and adsorption. Additionally, higher pH values trigger precipitation of heavy metals in a form of hydroxide or carbonate, lowering metals content in soil (e.g., cadmium) (Bian et al. 2013; Xu et al. 2020). Heavy metals are immobilized by biochar amendment primarily due to its liming effect (Bian et al. 2013).

Surface adsorption, ion-exchange, formation of stable complexes with organic ligands, complexation and precipitation of heavy metal ions by organic soil amendments are recognized as main mechanisms decreasing their content in plant tissues (Mahmoud and Abd El-Kader 2015; Lwin et al. 2018; Liu et al. 2021; Xia et al. 2021). In more details, the immobilization process of heavy metal ions by organic amendments could be divided into following phases: (i) the bioavailable heavy metal ions in water solution are transferred to near-surface of soil by physical attraction forces (i.e. hydrogen bond, electrostatic attraction, and van der Waals' force); (ii) the metal ions are adsorbed by inner and outer active sites on the surface of organic amendment; (iii) the adsorbed metals are immobilized by precipitation/co-precipitation (CO_3^{2-}, PO_4^{3-}, $CaCO_3$ and $MgCO_3$), surface complexation (Si–O bond), cation exchange (K, Ca, Na and Mg ions), and cation-π interaction (π electrons in lignin) to form stable metal–organic amendment complexes (Guo et al. 2020; Li et al. 2021; Xia et al. 2021).

The dose of soil amendment is also an important factor in soil (bio)remediation (Wang et al. 2020; Zhou et al. 2020; Głąb et al. 2021). Wang et al. (2020) showed a significant dose-dependent effect for immobilization of Cd, Pb, and Zn in polluted soil. More active sites for metal adsorption, precipitation, and complexation, as well as more beneficial nutrients for plants, can be provided with a higher dose of amendments. In comparison to the 0.1% dose of hydroxyapatite and cow dung-based manure applied individually, 1% dose caused lower content of Cd, Pb, and Zn in maize but higher plant biomass. However, high-dose amendment application may result in high costs and environmental side effects.

4 Conclusions

As steams from the literature review, immobilization is a method where relatively cheap materials are added to (or introduced into?) contaminated soil to decrease mobility and bioavailability of heavy metals. Various organic and inorganic by-products or natural minerals could be used as soil amendments. The studies reveal that there are various and complex interactions among sorption materials, heavy metals, and soil which leads to different immobilization efficiencies. Thus, a practical application of a given sorption material requires the priori analysis of soil composition and properties as well as quantitative and qualitive analysis of metal ions mixture, in order to identify the best soil remediation conditions.

In comparison with other sorption materials, biochar has higher porosity, larger surface area and consequently bigger sorption capacity. This material can be obtained from any bio-based mass resulted from agriculture or farm or food production. On the other hand, biochars have a higher than natural sorbents energy footprint, resulting from its production via pyrolysis.

The review also revealed large gaps in the knowledge about soil remediation via sorption materials amendments. First of all, most of the reported researches have been conducted in short-term laboratory experiments, while the long-term trials regarding mobility and bioactivity of metal ions in the field soils are very rare. It should be pointed out that heavy metals can never be entirely removed from soil and the immobilization effect may diminish over time. Though, the sorbent-amended soils need to be regularly monitored for heavy metal toxicity. The most suitable methods for this process are percolation tests that take into consideration the influence of the soil's properties and hence reproducing the conditions of a real field situation. Secondly, there is a lack of research on the optimization procedures with a view to arrive at a set of conditions that yields optimal immobilization and uptake effects. The sorption materials discussed are more suitable for the removal of cationic forms of heavy metals due to the negative charge of materials' sorption surface. However, some metalloids (e.g. As, Cr, I, U) can be present in environment in anionic forms. Furthermore, multicomponent studies, despite being time consuming and more complex to handle, should be done in the future to have a better understanding of the competition between different types of pollutants on the immobilization efficiency. Last but not least, practical application of low-cost amendments for heavy metals immobilization should be preceded by cost − benefit evaluations and by comparison of available technologies, aiding progress toward large-scale applications and long-term exposure on environmental conditions.

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Poly-γ-Glutamic Acid and Its Application in Bioremediation: A Critical Review

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Abstract Poly-gamma glutamic acid (γ-PGA) is an anionic bacterial polymer constituted by glutamic acid residues only. It has the intrinsic ability to strongly interact with positively charged ions and flocculate them. For this reason, a large body of literature has accumulated on its application in bioremediation, particularly targeted to positively charged heavy metals. In this work, the most important characteristics of γ-PGA and of its production are summarized, highlighting the advantages, but also the limits, in its application in bioremediation.

Keywords PGA · Bacteria · Bioremediation · Polymer

1 γ-PGA Biosynthesis

Poly-gamma glutamic acid (γ-PGA) is a natural anionic, high molecular weight (Mw), homo-polyamide, made up of repeating units of L- and/or D-glutamic acid residues connected by amide linkages between α-amino and γ-carboxyl groups; these pseudopeptide bonds are resistant to classical proteases. The ribosome-independent synthesis, that can lead to molecules most often above 10^6 Da, occurs via a transmembrane ATP-dependent γ-PGA synthase complex, which also secrets the polymer outside the cell (Sung et al. 2005). The synthase is constituted by the products of four genes, which are known as *pgsB*, *pgsC* and *pgsA* in species releasing the polymer in the environment. A fourth small gene, *pgsE,* completes the *pgs* operon in *B. subtilis*, *B. amyloliquefacens*, *B. pumilus* and *B. licheniformis* (Fujita et al. 2021). In *B. anthracis* and other capsule forming bacteria, the *pgs* homologues are known as *cap* genes (*capBCA*). In this latter group, the three biosynthetic genes are followed by *capD*, which encodes a product that bears resemblance to γ-glutamyl transferases, responsible for anchoring the polymer to the cell wall (Candela and Fouet 2005).

Microbial fermentation is the only viable way to obtain γ-PGA in significant amount, as the chemical synthesis of γ-PGA molecules longer than few residues

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is troublesome, inefficient, expensive and non-sustainable, requiring the constant protection of the α-carboxylic group.

Significative amounts of γ-PGA are produced by Gram-positive bacteria, mainly *B. subtilis*, *B. licheniformis* and *B. amyloliquefaciens* species. Aerobic, spore-forming, ubiquitous in soil and plants, *Bacilli* are ideal workhorses for γ-PGA production for many different reasons: they are generally non-pathogenic, fast growing, amenable to genetic manipulation, have simple nutritional requirements, and their products possess a long track record of safe use in human food products (Tamang et al. 2016). *B. subtilis* is indeed the model organism for Gram-positive bacteria and a large number of scholars, all over the world, have focused their studies on this organism. In fact, scientific investigations on *B. subtilis* date back to 1835, although *B. subtilis* got its present name only in 1872 by Ferdinand Cohn (Harwood 1989). These has led to the accumulation of endless and thorough knowledge on its genetics and physiology in general, and on γ-PGA production, in particular.

The polymer characteristics greatly vary among different producer strains, different growth media and fermentation conditions. Neither the enantiomeric composition nor the Mw are fixed. Analyses of purified γ-PGA showed that it can be made of D-glutamic acid, L-glutamic acid, or both enantiomers in different ratios. The pathogen *B. anthracis* produces γ-PGA made of D-glutamate only (Jang et al. 2011), while γ-PGA produced by the laboratory strain of *B. subtilis* JH642 (Srivatsan et al. 2008; Shah 2020) contains roughly 20% L-glutamate (Scoffone et al. 2013; Morelli, personal communication). The ratio of the two stereoisomers has been reported to vary according to the amount of Mn ions present in the growth medium (Wu et al. 2006), although this has not been confirmed by our preliminary data (Calvio and Morelli, unpublished). γ-PGA is also highly polydisperse, but the Mw of the molecules is on average extremely high, above 10^6 Da (roughly corresponding to 7500 Glu residues), even though it is extremely difficult to accurately measure the Mw of a polydisperse polymer which forms inter- and intra-molecular bonds (Park et al. 2005). The average size appears to change during bacterial growth (Scoffone et al. 2013), according to the producing organism, in dependence to the growth conditions, and might be reduced during purification. For example, addition of NaCl or $(NH_4)_2SO_4$, which increase ionic strength, positively impacts on γ-PGA molecular weight (Birrer et al. 1994; Park et al. 2005).

Being a natural secondary metabolite, γ-PGA is produced according to strictly regulated intracellular mechanisms. In *B. subtilis*, the activation of the biosynthetic operon is regulated by two different signal transduction pairs, the ComP/ComA and the DegS/DegU two-component systems; besides these signalling systems, the SwrA protein must be present. The most common *B. subtilis* laboratory strain 168, although possessing the genes necessary for the synthesis of γ-PGA, does not transcribe the *pgs* operon unless mutations that increase the intracellular level of DegU-P and produce a functional SwrA protein are introduced (Stanley and Lazazzera 2005; Osera et al. 2009; Ohsawa et al. 2009). In a recent work, an activating mutation in *degS*, the histidine kinase of the DegS/DegU two-component system, was shown to induce elevated and prolonged γ-PGA production in domestic and wild *B. subtilis* strains (Ermoli et al. 2021).

2 The Physiological Role of γ-PGA in Bacteria

The evolutionary advantage of γ-PGA biosynthesis may depend on the environmental niche the producing organism inhabits. For soil organisms that release it into the environment, γ-PGA may represent a formidable defence system against extreme desiccation, high osmolarity or the presence of toxic metal ions thanks to its water-holding and metal chelating capacity (Luo et al. 2016). Besides this role, the wide diffusion of γ-PGA-hydrolases in phages infecting *Bacillales* indicates that the polymer plays a fundamental role as anti-phage shield (Mamberti et al. 2015). Conversely, the fact that the polymer is secreted at the end of the logarithmic phase of growth, when nutrients are in short supply (Hsueh et al. 2017), cast serious doubts on the hypothesis that γ-PGA might be produced as nutrient reserve, as reported by several authors (Candela and Fouet 2005, Luo et al. 2016). However, once released in the environment, the polymer represents a perfect source of carbon and nitrogen to be exploited by both producers and non-producer strains (Mamberti et al. 2015).

Conversely, peptidoglycan-bound γ-PGA acts as virulence factor in pathogens, by constituting a passive barrier and masking surface epitopes to the surveillance of host immune response, protecting the capsulated bacteria from phagocytosis and antimicrobial peptides (Kocianova et al. 2005; Scorpio et al. 2007).

3 Degradation of γ-PGA

The degradation of γ-PGA is achieved by the action of unspecific and specific γ-PGA hydrolases, as the polymer is intrinsically resistant to common proteases. The unspecific hydrolases are enzymes belonging to the class of γ-glutamyltransferases (GGT, E.C. 2.3.2.2), which are ubiquitously present from bacteria to humans. GGTs from *Bacilli* and other Gram-positive bacteria, but not from *E. coli*, can act as γ-PGA exo-hydrolases, slowly releasing free glutamate from the N-terminal end of the polymeric chain, independently from the stereochemical configuration of the cleaved glutamate moiety (Calvio et al. 2018; Shah 2021a, b). As for the γ-PGA-specific enzymes, most producer strains carry *pgdS*, a gene encoding a secreted γ-PGA-specific endo-hydrolase, possibly involved in the release of the nascent chain from the synthase complex (Scoffone et al. 2013). In *B. subtilis*, *pgdS* is located just downstream of the *pgs* operon, but it is independently transcribed by a promoter regulated by SigD, the sigma factor responsible for motility genes' expression.

Bacillus phages are endowed with other highly efficient enzymes that hydrolyse γ-PGA and are possibly released from infected cell to break down the γ-PGA shield of surrounding target cells and ensure the reaching of cognate surface receptors for the subsequent infection cycle (Mamberti et al. 2015; Ramaswamy et al. 2018). Genes encoding those phage-derived enzymes are encased in several *Bacilli* genomes, both in prophage regions and in their vestigial remnants. The bewildering extensive occurrence of these enzymes also in genomes of non-γ-PGA-producers, most typically soil bacteria, is thought to have occurred through horizontal gene transfer events. The selective advantage conferred by γ-PGA hydrolases in these non-*Bacillales* hosts is possibly represented by the ability conferred to the organism to degrade environmentally dispersed γ-PGA and feed on the released glutamate (Mamberti et al. 2015).

As producer strains often contain a minimum of one γ-PGA hydrolyse (GGT), it cannot be excluded that part of the polydispersity observed in γ-PGA might be related to post-secretion hydrolytic degradation.

4 Strategies to Improve Microbial Productivity

Although microbial production of γ-PGA is established at the industrial level, the cost of production and purification, which ultimately affects the market price, is currently extremely high ($261 per 100 mg high-purity γ-PGA, sodium salt; CAS number: 208106-41-6; Sigma Aldrich). Such prohibitive cost constitutes the major limitation to a more widespread application of γ-PGA and, at the same time, the driving force for research activities aimed at improving productivity.

Such strategies have followed several parallel routes, such as the identification of new and more efficient natural producer strains, or the engineering of the genes and pathways involved in the biosynthesis and degradation of the polymer, or the development of better fermentation conditions (Luo et al. 2016).

γ-PGA producer strains come in two flavours: those requiring an external supply of L-glutamic acid and those that can produce it even in the absence of an external source of the amino acid. Productivity is much higher for L-glutamic acid-dependent strains, but polymer yield is directly correlated to the amount of L-glutamic acid added, which impacts on the production costs. Despite major efforts have been devoted to the isolation of new glutamic acid-independent strains, their productivity is scant in the absence of exogenous glutamate or its metabolic precursors (Zhang et al. 2012).

To reconcile needs and costs, several efforts were dedicated to the development of genetically engineered strains characterized by high γ-PGA productivity or simply to improve the intracellular glutamate synthesis, which is the limiting factor for γ-PGA synthesis (Cai et al. 2018). The genetic and metabolic optimization of all processes responsible for γ-PGA production is relatively easy in *Bacilli's* genome thanks to the existence of well-established techniques and molecular biology tools (Appelbaum and Schweder 2021), supporting the improvement in production yields and reduction in fermentation costs. Over the past years, a number of genes that are involved in γ-PGA production have been characterized, and much has been published on how different parameters affect productivity and costs.

As one of the main costs for commercial-scale γ-PGA production is the requirement of large amount of L-glutamate in the fermentation media (Nair et al. 2021), huge efforts were posed on strategies designed to reduce or replace this expensive component. These approaches generally aimed at rewiring the central carbon metabolism to enhance the carbon flux toward the tricarboxylic acid (TCA) cycle, as α-ketoglutarate is a direct precursor of γ-PGA. Metabolic models now available can predict genes to be targeted to enhance γ-PGA synthesis (Massaiu et al. 2019). Another successful approach was taken by Feng and collaborators' group, who took inspiration from a well-known glutamate producer microorganism: a 9.1% improvement in γ-PGA production was obtained by introducing the NADPH-dependent GDH pathway from *Corynebacterium glutamicum* into a glutamate-independent *B. amyloliquefaciens* strain; the yield further improved by 66.2% by manipulating the enzymes of the TCA cycle (Feng et al. 2017). Also, the deletion of two γ-PGA hydrolases, GGT and PgdS, was effective in doubling productivity in the presence of glutamate (Scoffone et al. 2013). However, the simple over-expression of the *pgsBCA* operon was shown to lead to a reduction in polymer yield, possibly because the excess of the synthase complex imbalanced other membrane-associated metabolic processes (Feng et al. 2015). In more systemic approaches, several of the previously explored genome engineering strategies were joined in modular way in the same strains, leading to a substantial increase in productivity (Feng et al. 2015; Cai et al. 2018).

Bacilli are considered as "strictly aerobic" and grow poorly in oxygen-limiting conditions (Nakano and Zuber 1998). For this reason, γ-PGA is mainly produced by aerobic fermentation in liquid media. However, during growth, oxygen transfer from the gas phase to the liquid phase is progressively reduced due to the high viscosity of the medium caused by the polymer that accumulates. This phenomenon limits cell growth and leads to a decrease in γ-PGA yield at later stages of cultivation. To overcome this problem a successful strategy was the introduction of the gene encoding the *Vitreoscilla* hemoglobin in *B. subtilis* chromosome, enhancing cell growth and increasing γ-PGA yield (Su et al. 2010). Although, the efforts dedicated to genetic improvement, the polymer is still too expensive; therefore, alternative routes have been parallelly explored to decrease γ-PGA market price.

5 Strategies to Reduce Fermentation Costs

Besides improving strains productivity, reduction of γ-PGA production costs is sought through the replacement of expensive components of the fermentation media with low-cost substrates. The dominant carbon sources used in the γ-PGA biosynthesis are glucose and citric acid, that are mainly derived from sources that are in competition with human nutrition, inevitably raising social dilemmas and raising the production costs imposed to the process. Recently, the urge to valorise agro-industrial waste as feedstock for bacterial fermentations has encouraged investigations on solid-state fermentation also for γ-PGA production. Indeed, a wide range of abundant and renewable lignocellulosic biomasses or agro-industrial wastes, such as rice straw, cane molasses, rapeseed meal, soybean residue, corncob fibres, crude glycerol from biodiesel plants, macroalgae, goose feathers and paper waste have been proposed as cost-attractive and environmentally sustainable carbon sources (Fang et al. 2020). Such biomasses not only represent cheap substrates for microbial growth, but their valorisation through fermentation mitigates the environmental impact they would have on eutrophication; moreover, the solid-state fermented substrate can be directly used as agricultural fertilizer, exploiting the beneficial effects of both *Bacillus* species and γ-PGA on plant performance (Zhang et al. 2017). Among the industrial wastes, untreated cane molasses and monosodium glutamate waste liquor, by-products of refining sugarcane and from the glutamic acid fermentation process, respectively, or powdered fulvic acid, recovered from the wastewater of molasses fermentation by yeast, were successfully exploited in cost-effective biosynthesis of γ-PGA (Zhang et al. 2012; Li et al. 2020). However, most often, direct and efficient fermentation of lignocellulosic feedstock is unfeasible; pretreatments are generally required for the utilization of the sugars therein contained. For this reason, bacterial feedstocks based on biomass hydrolysates are preferentially used. Hydrolysates of rice straw or corncob fibers have been tested for the convenient fermentation of γ-PGA (Hassan et al. 2014; Tang et al. 2015; Zhu et al. 2013). Unfortunately, lignocellulosic biomass hydrolysis often implies the use of polluting agents to break the recalcitrant matrix. An environmentally sustainable alternative for hydrolysing biomasses is the application of microbial degradative enzymes for the pretreatment of feedstock biomasses. Altun (2019) employed goose feathers from poultry processing plants as source of protein hydrolysates; in this case, feathers were first used as the sole carbon and nitrogen source to produce keratinolytic enzymes by *Streptomyces pactum*. The lyophilized crude enzymatic extract was applied to obtain feather hydrolysates, which was finally used as a cheap and renewable medium for γ-PGA production (Altun 2019).

Also, short fibres produced from paper linerboard recycling could be enzymatically turned into fermentable sugars and later used as carbon source for the biosynthesis of γ-PGA with yields compared to the one obtained by using glucose (Scheel et al. 2019).

Many additional industrial wastes have been suggested for production of γ-PGA, as well as of other biocommodities. All these approaches combine the valorisation of industrial wastes for the creation of value-added products, thus introducing circularity in the economic process.

6 The Applications of γ-PGA

γ-PGA is basically a long chain of glutamic acid residues joined by pseudopeptide bonds; the composition in glutamic acid mainly in the D-form makes it biocompatible, non-immunogenic and even edible; in fact, it is one of the main components of *natto* and *chungkookjang*, traditional Japanese and Korean foods, respectively, made from soybeans fermented with *Bacillus subtilis* species. The slow γ-PGA degradation profile prevents inflammatory responses. Furthermore, microbial production is sustainable and safe also for the environment, as the polymer is not only bioproduced but also easily biodegradable.

The configuration of the γ-amide bond confers to the polymer particular chemical-physical characteristics that make it a valid anionic biomaterial in various application fields (Ogunleye et al. 2014). However, the extreme viscosity of the high Mw chains makes the polymer not easily manageable. The problem can be alleviated by random reduction of the Mw by alkaline hydrolysis, sonication, or enzymatic degradation; on the other hand, excessive size reduction limits some properties of the polymer, which are dependent on the length of the chains (Shih and Van 2001).

The presence of several pending and negatively charged carboxyl groups in position α profoundly affects the solubility of the polymer: in basic conditions the – COOH groups are deprotonated, making the polymer hydrophilic, hygroscopic and superabsorbent, and favouring the formation of γ-PGA salts in the presence of metal ions; conversely, in acidic environments, the carboxylic groups are protonated, making the molecule water-insoluble. Besides, these pending groups represent ideal reactive centres for conjugation and crosslinking of any type of molecules. Thanks to the above properties, high Mw γ-PGA represents a versatile candidate for various industrial applications, as attested by the massive literature accumulated over the last few years. Biocompatibility has prompted several potential applications in the biomedical and food sectors. The long hydrophilic moiety of γ-PGA, which is metabolized at a slow pace in mammals, represents a perfect drug carrier to improve the administration of poorly soluble drugs. It can also be transformed in various γ-PGA-based composites, including hydrogels, nanofibers, and nanoparticles for medical applications, such as biological adhesives or scaffolding materials for tissue engineering; in these composites, the conjugated materials provide adhesion or mechanical strength (Park et al. 2021). γ-PGA-nanoparticles have been explored as substitute of adenoviral vectors for the delivery of anticancer agents (Khalil et al. 2018). In addition, the ability to chelate metal ions (e.g., magnesium or calcium), and their slow release in the digestive tract, has been exploited to create highly effective controlled-release mineral supplements for human and farm animals health.

Another well-developed field in which γ-PGA has already found wide application is processed food production; it is used as an additive to improve the rheological properties of wheat gluten by increasing the water-holding capacity (Xie et al. 2020), as viscosity enhancer for beverages, as cryoprotectant for frozen food, aging inhibitor, texture enhancer or bitterness relieving agent. It also acts as preservative for probiotic bacteria during freeze drying (Bhat et al. 2015).

γ-PGA can absorb water at an amount that is several hundred times higher than its original weight without dissolving. The high-water absorbability can be further increased by combining the polymer with other materials or by intramolecular crosslinking, leading to the formation of hydrogels. The super-absorbent capacity is the key property in cosmetics and personal care products, such as γ-PGA-based moisturizer, exfoliating and anti-wrinkle creams. γ-PGA has been shown to enhance skin elasticity more than collagen and hyaluronic acid (Lee et al. 2014); moreover, it has been explored also in products as diapers and napkins, as substitute of more toxic acrylates (Castrillon et al. 2019).

Another class of γ-PGA-based products which are already on the market are fertilizers and other agricultural adjuvants. In these types of applications, γ-PGA is considered an environmental-friendly fertilizer synergist for improving plant uptake of nitrogen, phosphorous, and potassium; it is often provided together with the producing organisms, as raw fermentation broth, thanks to the numerous positive effects that *Bacillus* species have on vegetable growth, making them perfect Plant Growth Promoting bacteria (PGPB) (Zhang et al. 2017).

Moreover, γ-PGA can also positively impact on soil quality indirectly, by promoting water retention (Guo et al. 2021) and by sustaining the flourishing of the soil microflora (Xu et al. 2013).

7 γ-PGA in Bioremediation

Bioremediation of contaminated matrices exploiting γ-PGA has been extensively investigated by several authors. At the basis of its application in this field lays the ability to efficiently chelate and flocculate polluting metal ions such as nickel, copper, cadmium, cobalt, chromium, aluminium, uranium, arsenic or lead, as well as various organic compounds. The basis for these property is the presence of multiple negatively charged carboxyl groups in the polymer structure, that readily bind to several environmental cations, forming both intra- and inter-molecular hydrogen bonds favouring the formation of flocks (McLean et al. 1990; Inbaraj et al. 2009).

7.1 Wastewater Treatment

Detection and removal of heavy metal ions in water is of paramount threats for the planet and for human and animal health; however, the decontamination solutions should not impose an additional threat to already endangered sites. Aluminium- or polyacrylamide-based coagulants, often used in water treatment plants, have indeed been linked to the development of neurodegenerative diseases (Bondy 2010; Pennisi et al. 2013). Being safe and biodegradable, γ-PGA represents instead the ideal solution for the removal of pollutants from wastewater, in particular of heavy metals, as it promotes the formation of small flocs entrapping the metal ions, that readily agglomerate into larger and sedimentable particles, without leaving toxic traces behind. The dynamics of complexes formation with bivalent lead ions at various concentrations of both adsorber and ligands and the characteristics of the flocks at different pH values were deeply characterized (Bodnár et al. 2008). One of the requirements for the activity of γ-PGA as adsorber and flocculant is the maintenance of a pH that allows the ionization of the −COOH groups, which must be neither below the pK_a of γ-PGA (4.09 according to Inbaraj et al. 2009), nor to basic, so that cation exchange reactions can occur rapidly and efficiently. In fact, purified acidic γ-PGA, which is insoluble in water, was shown to adsorb cesium from radioactive wastewater more efficiently than the corresponding sodium salt, even if cesium is preferentially bound with respect to sodium and calcium. Moreover, the adsorption equilibrium is attained in very short time (Sakamoto and Kawase 2016). Indeed, commercial bioadsorbers containing γ-PGA, among other components, are already on the market (http://www.poly-glusb.jp/basic.html) and their efficacy was experimentally tested, for example on wastewater from ethanol distillation, characterized by high levels of organic and inorganic matter. Results showed that the use of γ-PGA combined with sodium hypochlorite and sand filters removed about 70% of the turbidity and reduced chemical oxygen demand by 79.5% (Carvajal-Zarrabal et al. 2012).

The flocculation properties of γ-PGA can also be improved by coupling with other materials. As an example, the sequential addition of chitosan and γ-PGA was shown to substantially reduce the chemical oxygen demand from the wastewater of a potato starch plant, subtracting nitrogen and phosphorus and improving turbidity. The synergistic effect is thought to be linked to the ability of chitosan to neutralize negative charges, thereby reducing electrostatic repulsion, while γ-PGA provides a bridging function that promotes flocculation. By using biodegradable components, the sediments, rich in organic matter, could be classified as potential soil fertilizers (Li et al. 2020). A super Cu^{2+} adsorber was developed by cross-linking γ-PGA onto *Pseudomonas putida* cells displaying two recombinant metal-binding proteins on its surface. The biocomposite biosorbent allowed the quantitative recovery of copper from liquid matrices over a sufficiently varied range of pH and temperatures (Hu et al. 2017).

However, there are completely different approaches in which the multiple properties of γ-PGA can be highly valuable in wastewater treatment, e.g., as biostimulant for other microorganisms or plants directly involved in the bioremediation processes (Wojtowicz et al. 2022).

For the bioremediation of trichloroethylene-contaminated groundwater sites, reductive dechlorination can be carried out by anaerobic bacteria which use H_2 as electron donor to replace chlorine atoms with hydrogens. A mixture containing γ-PGA and emulsifiers, vitamins and a degreaser could be successfully injected in contaminated sites to speed up bioremediation by acting at multiple levels: (i) as physical adsorption agent for trichloroethylene; (ii) as slow-release carbon supplement for the maintenance of anaerobic dechlorinators (i.e. as biostimulant); (iii) as pH-stabilizer, thanks to the neutralizing effect of the amine groups released by the degrading polymer (Luo et al. 2021).

The complexation with γ-PGA was also shown to improve the performances of a catalytic dechlorination method based on the used palladium-doped zero-valent iron nanoparticles. γ-PGA complexation stabilized and provided electrostatic and steric repulsion to prevent particle aggregation due to attractive magnetic forces. The positive role of γ-PGA was demonstrated by comparing the dechlorination activity of naked and γ-PGA-complexed nanoparticles on chlorophenol as model pollutant (Zhang et al. 2018).

In marine sediments treatments, a new role for γ-PGA was devised as biological glue and protective agent to wrap oil-degrading bacteria adsorbed on solid zeolite particles, thus preventing marine currents dispersion of the microbial species directly responsible for the bioremediation of sediments contaminated by crude oil (Zhao et al. 2018).

Moreover, due to its highly efficient cation-binding character, it can also be used as biosensor of metal contamination. γ-PGA-stabilized gold nanoparticles have been synthetized and used to sense trivalent chromium levels in aqueous solution, which could be conveniently assessed via a colorimetric change occurring upon chromium binding. The reported sensitivity is very high (up to 0.2 ppb) and the biosensor was successfully tested in different liquid matrices (Yuan et al. 2020).

7.2 Soil Bioremediation

The suitability of γ-PGA properties in soil washing simulations has been poorly explored. This is probably linked to the existence of several alternative soil remediation methods and to the technical problems in envisaging the scale up of soil-washing procedures based on an expensive bio-adsorber as γ-PGA. The only real advantage that the polymer offers with respect to similar conventional techniques relies on the fact that upon γ-PGA washing, soil characteristics do not change, and the soil microbiota appears unaffected (Peng et al. 2020), which is a fundamental asset for future restoration of degraded soil (Coban et al. 2022).

However, the metal-chelation properties of γ-PGA can be indirectly exploited in contaminated soils. γ-PGA, applied to soil spiked with Cd and Pb, reduced the growth inhibitory effects of those metal ions on cucumber seedlings by reducing their bioavailability, as demonstrated by the lower metal ions content in the plant tissue (Pang et al. 2018).

Moreover, in phytoremediation of hypersaline soil, the growth of two different halophytes was shown to increase upon addition of γ-PGA solutions to the soil (Mu et al. 2021). This effect is supposedly linked to the γ-PGA-mediated reduced bioavailability of Ca^{2+}, Mg^{2+} and NO^{3-}, the ions analysed in this study, which mitigates the salt stress thereby promoting plant growth. The increase in plant biomass, in turn, led to a larger amount of ions adsorbed by the plants and, ultimately, to a more efficient phytoremediation of the hypersaline site (Mu et al. 2021).

Analogously to what described for wastewater treatments, γ-PGA can also be used as biostimulant for the activity of microbial consortia able to degrade specific pollutants. This strategy has been applied for the bioremediation of soil samples contaminated by petroleum hydrocarbons by Wojtowicz and collaborators (2022). An autochthonous degrading microbial consortium was introduced in soil derived from a contaminated site either alone or together with γ-PGA, in a combined bioaugmentation and biostimulation approach. The rate of pollutants degradation over a 6-month period improved significantly in the presence of the polymer with respect to the non-biostimulated consortium (Wojtowicz et al. 2022). This approach has the advantage of being potentially applicable in situ even to urbanized polluted sites.

8 Open Challenges and Perspectives

A large body of literature explored the propensity of γ-PGA to flocculate several cationic compounds rapidly and efficiently, a feature that has prompted a wide array of research studies on its applicability in bioremediation. The efficiency of ion binding is pH dependent, and the selectivity mainly depends on compounds' relative concentration and charge. However, the field implementation of the lab-conceived applications is still far from being realized. γ-PGA, as a commercial product, is currently too expensive for massive use in large wastewater and soil treatment plants.

γ-PGA appears more valuable as a slow-release carbon and nitrogen source for the biostimulation of microbes degrading organic compounds, (Luo et al. 2021; Zhang et al. 2018; Zhao et al. 2018). It has also shown to be applicable as metals-immobilization agent to support phytoremediation techniques. However, its biodegradability in this respect is a double-edged weapon: it makes it a perfectly biologically safe immobilization agent, able to preserve and even improve soil quality, but it does not guarantee the long-term stability of the immobilized metals, because of the ubiquitous presence of viral, bacterial, and eukaryotic γ-PGA hydrolases. Experimental proof of its actual survival in real natural environments is still missing, as most of the data have been collected in simulated natural conditions (Pang et al. 2018). The unstable nature of γ-PGA in an uncontrolled environment is also a major shortcoming when considering the option of recycling it after desorption of the payload through mild acidic conditions.

A still unexplored strategy to decrease γ-PGA-based bioremediation costs and, concurrently, overcome its degradation might be represented by the use of γ-PGA-producing bacteria grown in situ, alone or in combination with other suitable microbial species to ensure a constant γ-PGA-based bioremediating action. Alternatively, in order to stabilize γ-PGA molecules in the natural environment, conjugated molecules can be explored, decreasing the degradation rate and prolonging its survival.

The high production costs may be overcome by exploring novel fermentation strategies, for instance, biofilm fermentation. This technique has been applied by Moni and colleagues (2022) for the convenient production of proteases by *Bacillus subtilis* (Moni et al. 2022). However, the suitability of this technique largely depends on the specific nature of the substrate for bacterial growth, the aeration conditions and the stability of the external conditions, therefore further investigations should be done for its application in γ-PGA production.

In conclusion, γ-PGA is an attractive biopolymer with high potential in several application fields. However, first and foremost, the hopes placed in new overproducer engineered strains and ideal fermentation conditions of low-cost recycled organic wastes, must be fulfilled. Once these two conditions are met, solving the other issues, as the one correlated to the stability of the polymer, would be downhill.

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Metagenomics Analysis of Extremophiles and Its Potential Use in Industrial Waste Water Treatment

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Abstract Industrial waste water treatment is significant in managing the water crisis being faced globally. Though number of physical and chemical technologies have been developed for waste water treatment, there are challenges being faced with respect to the cost of implementation and removal of pollutants. Use of enzymes from mesophilic organisms for the treatment of toxic pollutants is cost-effective but limited due to their narrow range of stability. Alternatively, enzymes from extremophiles show a broader range of stability under extreme environmental conditions and can aid in effective biodegradation of heavy metals. The recent advances in metagenomics and meta transcriptomics analysis gives information on operational responses of these extremophiles to environmental disturbances which is crucial in optimizing waste water treatment processes on a large scale in bioreactors. This chapter gives insight on how these new and advanced approaches can result in design of novel enzymes for potential application in waste water treatment.

Keywords Waste water treatment · Enzymatic treatment · Metagenomics · Extremophiles · Meta transcriptomics

1 Introduction

The remarkable ability of certain organisms to thrive under extreme environmental conditions make them very attractive prospect of their usage for various biotechnological applications. These are classified as extremophiles having the ability to survive at extreme conditions such as high or low temperature (Elleuche et al. 2015), acidic or basic pH (Aguilera 2013; Luís et al. 2022; Qiu et al. 2021; Cavicchioli et al. 2002; Shah 2021a, b; Struvay and Feller 2012) high or low pressure (Ichiye 2018; Jin et al. 2019; Canganella and Wiegel 2011; Qiu et al. 2021). Predominantly, microbes especially various types of bacteria have this ability to survive under extreme conditions

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(Qiu et al. 2021; Fenice et al. 2021; Shah 2020; Van den Burg 2003; Elleuche et al. 2015). These extremophiles provide unique opportunity of exploiting their proteins or enzymes for various applications (Elleuche et al. 2015) as these products can function in suboptimal conditions such as extremes of temperatures, pH and toxicity of the reaction conditions. Unfortunately, most of the extremophiles are not culturable and very few of them actually grow into viable organisms in the lab. This makes possible loss of certain important enzymes or proteins that may have potential utility for biotechnological applications. Metagenomics is an approach of identifying the genome features of organisms especially microbes that are not culturable (Cowan et al. 2015; Escuder-Rodríguez et al. 2018; Verma et al. 2021; Liu et al. 2021). The traditional metagenomic approach involves isolation of the DNA from a given sample that is representative of all organisms in that sample, fragmentation of DNA, adapters ligation and cloning into suitable vectors for creation of genomic library and sequencing of the library (clones) and annotation or identifying the genomes by assembling them to reference genomes or homologous searching against genome databases. This process is tedious and lengthy in terms of time, labour and cost. However, with the advent of next generation sequencing, the DNA sequencing is straightforward after adapter ligation to DNA fragments, it is fast and less expensive (Fig. 1). After high throughput sequencing, obtained sequences can be assembled to many genomes as there is availability of many reference genomes and very efficient sequences assembling bioinformatics tools. From this metagenomic approach, it is possible to identify enzymes or protein products that are capable of functioning normally under sub optimal conditions.

Water is the universal solvent and many large-scale industries require constant supply of water for various reaction conditions and treatment. Unfortunately, the by-product of the industrial process is the release of large amount of spent water with enormous amount of toxic chemicals and other undesirable entities. As the release of

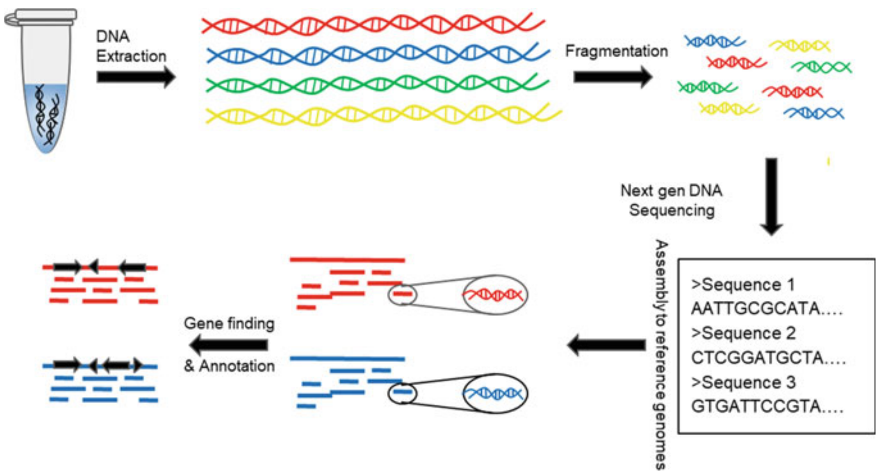

Fig. 1 Metagenomics approach for using next generation sequencing

untreated industrial waste water into the surrounding environment is prohibited by industrial regulations that are in place, various methods of treating the industrial waste water already are under use. However, many of these methods suffer from inherent issues and not cost effective. Usage of biological agents is actively being considered and are being utilized or under consideration for use (Sharma et al. 2021). As the industrial water is stuffed with lot of toxic impurities, usually at high temperature with pH extremes, most of the biological agents or protein products such as enzymes may not actively function and the process could be slow and expensive. In this context, as extremophiles thrive under extremes of temperature, pH and salinity, either themselves or their products such as proteins and enzymes make an attractive prospect for treating industrial waste water. In this chapter, we provide the utility of extremophiles and their products, identification of them by metagenomic approach and their usage in the treatment of industrial waste water.

2 Extremophiles and Their Characteristic Features

Extremophiles are mostly prokaryotic microorganisms that thrive in extreme conditions. The conditions in which the existence of life seems impossible are the places where extremophiles lives. These organisms can sustain and are viable in places that impose high pressure, temperature and pH. Some of them survive using the nutrients that are considered toxic and some can withstand high amounts of radiation and salinity. There are few more that can survive vacuum and extremely xeric conditions. As contrast to extremotophiles, extremotolerants can tolerate extreme condition for certain period of time but they grow optimally only at normal conditions. They are sometimes called moderate extremophiles. Sometimes extremophiles have shown to utilize more than one extreme condition to survive. These are called as poly extremophiles. For instance, the microorganisms that dwell on deep sea floor and trenches live under extreme hydro pressure and cold temperature. The organisms have successfully adapted their structure and metabolism to these harsh conditions to their advantage. These characters of adaptation can be harvested for working in areas which have potentially a high risk involved like radioactive sites, industrial waste management sites, mines, etc. Extremophiles has been elaborately reviewed with reference to their physiology and genetics in the past (Seckbach and Stan-Lotter 2013).

2.1 Organisms

Extremophiles mostly consist of Archaean microorganisms. They also include eukaryotes such as protozoa, algae, fungi, bacteria and even some multicellular organisms.

Archean

Archaean's being obligate anaerobes can live in oxygen free environment. Their adaptability makes them flourish in almost all of the extreme conditions that are known. They grow in the most acidic, basic, thermophilic and halophilic environments. *Methanopyrus kandleri* strain 116 (Takai et al. 2008) is a methanogen, that grows in deep sea can be cultured at high temperature of about 122 °C and high pressure of 20 MPa.

Protozoa

These being mostly parasitic, are found with algae and bacteria that are present in hypersaline conditions. For example, *Trachelocerca conifer, Metacystis truncata* & *Chilophrya utahensis* are found in the brackish lagoons. Algae-Microalgae sustain and live in most of the extreme conditions. *Oscillatoria terebriformis* is the blue-green algae found in hot springs, at about 70 °C. The temperature tolerance of these algae is due to the presence of homo polar bonds in their proteins.

Fungi

Eutypella sp. D-1 (Lu et al. 2014) have been isolated from soil of high altitude Arctic region. *Cladosporium sphaeospermum* showed increased viability in the Chernobyl Nuclear Power plant (Dadachova et al. 2007). *Cryomyces antarcticus* is the toughest eukaryotic organism, known as black fungi, is known to survive solar radiation, desiccation, extreme polar coldness and dryness (Onofri et al. 2007).

Bacteria

These are the most versatile among all the other extremophiles. *Serpentinomonas sp. B1* can grow in highly basic pH of 11–12.5 (Suzuki et al. 2014) While *Halarsenatibacter silvermanii SLAS-1* uses extreme salinity of 35% of Soda Lake in USA and grows, it is *Planococcus halocryophilus Or1* that survive a freezing temperature of −15 °C. *Pyrococcus yayanosii* (Birrien et al. 2011), an obligate barophile, grows on ocean floor, at a pressure of 50–52 MPa. *Deinococcus radiodurans* can survive grave radiation exposure of 5000 Gy without losing viability. It is known as the world's toughest bacterium as it can live even after exposure to extreme cold, radioactive rays, desiccation, acidic condition and also in vacuum.

Multicellular Organisms

Tardigrades, one of the most interesting species of multicellular organisms, are actually extremophiles. Their presence is reported in hot springs, deep sea, polar regions and also in outer space.

2.2 *Characteristics and Adaptations*

Acidophiles

Acidophiles are those that thrive in environment with extremely acidic pH. Some of the most acidic environments found on earth are at the mining sites and geothermal springs. For the extremophiles to survive in the extremely acidic pH (nearing 0), the cytoplasmic pH at around 6.0 should be maintained. This is achieved by increasing the activity of the pathway that pushes the excess cytoplasmic protons out of the cell or by consuming it. The pathways that express the proteins for outward proton pumping is active in these organisms (Krulwich and Pandan 2011). They also keep the organic acid from moving out of the cells by degrading them within the cytoplasm.

Alkaliphiles

Alkaliphiles are the extremophiles that live in extremely basic pH. The Soda Lake in USA and Lake Turkana in Kenya are major alkali lakes in the world. With pH range of 8.5–10, these lakes have a unique ecological condition. The organisms that thrive in these conditions, the alkaline challenges are faced by enhancing the Na^+/H^+ antiporter activity. These antiporter proteins also hold a pH sensor domain that results in considerably large fold increase in ion exchange. They can also secrete the organic acids like acetic acid and lactic acid to change the immediate surrounding pH (Zhang et al. 2016).

Halophiles

Halophiles are those extremophiles that survive high salt concentration. *Salinibacter ruber* and *Halobacterium salinarum* are among the extreme halophiles that grow around 20–23% salinity ranges of The Great Salt Lake. To escape the loss of water due to high salt concentration, these organisms have developed two approaches. Firstly, they can equilibrate their salt concentration to reach the same level as that of external environment by salt accumulation. These involves the action of Cl^-/K^+ pumps, rhodopsins (Adamiak et al. 2015). The organisms accumulate K^+ or Cl^- in their cytoplasm. Secondly, they produce organic solutes known as osmolytes from external environment that protects the proteins from denaturation (Saum and Müller 2008).

Thermophiles

Thermophiles are those that flourish in very high temperatures. Being one of the most important physical attributes for an organism to sustain, thermophiles breach the human boundaries and grow in some of the most hostile temperature conditions. The minimum temperature at which the microbes can live is reported by bacteria *Deinococcus geothermalis* DSM1130 at −25 °C (Frösler et al. 2017) and the maximum by archaea *Geogemma barossii* at 130 °C (Kashefi and Loveley 2003). The proteins necessary for their survival have shown to be adapted by two means. The archaean's show a structure-based stability as they have evolved from a hyperthermophilic habitat. Whereas the bacteria show sequence-based stability which is achieved by less compaction and additional salt bridges (Berezovsky and Shakhnovich 2005).

Psychrophiles

Psychrophiles are those that are opposed to thermophiles and survive in very low temperature. They have adapted membrane fatty acids with LC-PUFAs in them which serves as antioxidant. They have polar carotenoids as membrane pigments that increases fluidity of the membrane. The anti-freeze proteins (also called ice structuring or thermal hysteresis proteins) are non-colligative biological antifreezes. These can bind to ice and inhibit ice growth and recrystallization, thereby decreasing the freezing point resulting in reduced ice growth (Voets 2017). They prevent ice recrystallization as they keep a liquid environment around the cell (Raymond). They are also equipped with anti-nucleating proteins, a large membrane bound protein, which initiates heterogeneous ice formation (Lorv et al. 2014). Biosurfactant like glycolipids and phospholipids produced in these organisms helps in movement across the cells. They have very effective and efficient DNA/RNA chaperons that prevent misfolding and stabilize the DNA and RNA secondary structure (Collins and Margesin 2019).

Radiophiles

Radiophiles, as the name suggests, grows well in radioactive contaminated soils. The two types of radiation that affect the functioning of the cell are ionizing and non-ionizing radiations. The ionizing radiations, like gamma rays, causes double stranded breaks in the DNA and also damage proteins and lipids. To overcome these adversaries, the microorganism has developed precise and fast DNA repair mechanism (Pavlopoulou et al. 2016) and condensed nucleoid. They prevent protein damage by suppressing ROS production cell cleaning functions and selective protein protection. In case of non-ionizing radiations like UV rays, the DNA damage is subtle, by pyrimidine dimer formation causing photolesions. They have photoreactivation genes and numerous other repair strategies that help to overcome the damage (Jones and Baxter 2017). They also employ gene duplication and carotenoids and superoxide dismutases formation for their protection from continuous exposure to radiation.

Barophiles

Barophiles are the organisms that live under high pressure. They are also called as pzeiophiles. They colonize and grow under high hydrostatic pressure (HHP), usually in great depths of the ocean. The cytoplasmic membrane in these organism gets compacted and becomes impervious to water. To increase the fluidity of the membrane, unsaturated fatty acids are packed more. The porins like OmpH/L and proteins ToxR/S act as pressure sensors in these organisms. Their ribosomes are stabilized by extended loops at regions (Oger and Jebbar 2010). Barophiles also includes organisms that survive in low pressures present on mountains and outer space, with a pressure range of 0.0033–10^{-13} MPa. They develop biofilms around them or undergo spore formation to protect itself (Horneck et al. 2010).

Metallophiles

Metallophiles which can survive in high concentration of heavy metals. Most of these are chemolithoautotrophs. They are found in acidic environments especially in the mines, industrially polluted sites, volcanic eruption sites and the hydrothermal vents. They use heavy metals like cadmium, cobalt, copper, lead, mercury, nickel, zinc and so on for their survival. Their ability to solubilize metals has been successfully used in bio mining applications (Rohwerder et al. 2003). These organisms produce biofilms around their consortium which protects the organism layer beneath and also helps in horizontal gene transfer among them. They have a capsule that protects cell and an efficient proton efflux system along biomolecules repair mechanisms allows these organisms survive the harsh environment (Hu et al. 2020).

But not all the extremophiles that have been isolated from these harshest environments can be cultured. Isolation and culturing of extremophiles have completely different challenges. Isolating and identifying an extremophile is easier. But when it comes to culturing, the organisms that survive at the extreme ends of the nutrition or physical condition spectra, have been hard to culture.

3 Bioprospecting of Extremozymes in Industrial Wastewater treatment

Industrial waste water has high percentage of heavy metals and other toxic compounds which has to be treated before discharging to landfills and water bodies or reusing for agriculture and industrial processes. Conventionally, a series of physical and chemical techniques are followed in industries for waste water treatment to meet the standards required before its discharge or reuse. But these methods have several limitations which includes high expenditure, production of by-products during the treatment process which could be harmful and not so adequate heavy metals removal efficiency. The alternative approach which minimises these disadvantages is enzymatic treatment. Enzymes have unique properties such as high specificity and stability, faster reaction by reducing the activation energy and relatively low process costs. Enzymes obtained from extremophiles are known as extremozymes and they have the potential to contribute significantly to the global enzyme market in the near future. The benefits of using extremozymes is that they can catalyse reactions even in harsh environment. However, the challenge is to create such harsh conditions in the industrial laboratories to grow extremophiles for production of extremozymes. Additionally, the biomass yield of extremophiles is very low relative to mesophylls (Sysoev et al. 2021). Due to all these hurdles, there is very little research with respect to structure, function, properties and applications of extremozymes.

3.1 Extremozymes Having Potential in Industrial Waste Water Treatment

Some of the extremozymes known to have capacity to degrade and remove heavy metals and toxic compounds from waste water are discussed below.

Nitrilase

Nitrilases have wide applications concerning compound exterior surface changes, sewage treatment and formation of carboxylic acids. Nitrilase converts nitrile compounds to carboxylic acid and releasing ammonia by acting on carbon–nitrogen triple bond. Extremozyme Nitrilases have been isolated from wide variety of bacteria, fungi, yeast and plants. Extremozyme Nitrilases relatively have faster biotransformation, high substrate solubility, and reduced viscosity and contamination (Gunjal Aparna et al. 2021).

Peroxidases

These enzymes degrade by oxidizing phenolic compounds, polychlorinated biphenyls (PCBs) compounds, hydrocarbons and dyes. Lignin peroxidases and manganese peroxidases are commonly used enzymes which act by breakup of alpha and beta carbon bonds, oxidation of phenolic groups and benzyl alcohols and hydroxylation of benzylic methylene groups. Lignin peroxidases requires hydrogen peroxide for its catalytic reactions. Manganese peroxidases accelerates the conversion process of Mn^{2+} to Mn^{3+} (Gunjal Aparna et al. 2021).

Lipases

Lipases are utilized in the biodegradation of oil, proteins and salts present in waste water from dairy and tannery industries (Gunjal Aparna et al. 2021).

Laccases

Laccase belongs to class of oxidoreductase enzymes. Extremozyme laccase are very stable in extracellular fluids. Some investigations observed the use of laccase in degradation of polymers esp. polycyclic aromatic hydrocarbons (PAHs). PAHs accumulate as a result of incomplete combustion of organic matter and automobile exhausts. They are extremely detrimental due to their carcinogenic properties. Laccase catalyse by oxidation or decarboxylation of substrates which can be phenols, phenolic compounds or lignin and reducing oxygen to water and carbon dioxide. The main source of laccases are white rot fungi and molds. Furthermore, laccases can degrade xenobiotic and non-phenolic compounds. Another important application of extremozyme laccase from alkali-halotolerant bacteria is in degradation of azo dyes in textile industry waste waters which is a threat to aquatic life and human beings (Gunjal Aparna et al. 2021).

Pectinases

Pectin is abundantly present in fruits. Apparently, waste water from fruit processing industries has high concentration of pectin as by-product. This in turn can affect treatment as pectin can produce methane which can interfere in activated sludge process. Studies have shown Alkalophilic *Bacillus* spp. produces pectinase enzyme which is used for pre-treatment of waste water to remove pectin before water enters the activated sludge tank (Gunjal Aparna et al. 2021).

Catalase

Hydrogen peroxide is used often in textile and semiconductor industries. It can cause severe damage to the existence of marine life if waste water containing hydrogen peroxide is discharged without treatment to water bodies. Catalase enzymes effectively removes hydrogen peroxide from effluents (Gunjal Aparna et al. 2021).

Cellulases

Cellulases from alkaliphilic organism *Bacillus* sp. finds applications in textile industry where indigo dyes are used as colouring agent. The waste dye gets released into effluents. Cellulase enzyme help in removal of stains and dyes from waste water.

There are three groups of cellulases which acts in different ways. Endoglucanases converts cellulose fiber into free chain ends, exoglucanase separates cellobiose units and β-glucosidase forms glucose (Gunjal Aparna et al. 2021).

Esterases

The esterases belong to the hydrolase group and hydrolases ester containing compounds such as carbamates, organophosphates etc. and used in degradation of xenobiotic and various other toxic compounds like cypermethrin, sulfosulfuron and fipronil. The esterases extremozyme are isolated from the thermophilic microorganisms (Gunjal Aparna et al. 2021).

Tyrosinases

Effluents from coal, plastic, resin manufacturing, and petroleum refining industries has huge content of harmful phenolic compounds which can hinder the activated sludge process. These enzymes catalyse oxidization of phenols and convert to useful products like amino acids (Gunjal Aparna et al. 2021).

Dioxygenases

Dioxygenases oxidizes aromatic compounds into aliphatic products. They find applications in detoxification in response to oil spills in oceans (Gunjal Aparna et al. 2021).

Monooxygenases

Monooxygenases are also used for biotransformation of aromatic compounds and hydrocarbons. They act by stereo selectivity on different types of substrates which are applied as reducing agents. One such commercial enzyme is methane monooxygenases. (Gunjal Aparna et al. 2021).

4 Metagenomics Analysis

The extremity of survival conditions of organisms has been expanding ever since man began to isolate and culture them. The microorganisms were isolated from every possible habitat on Earth and from space. They were cultured on different growth enrichment media to get pure cultures and finally identified them by various genomic studies. This led to flow of information regarding a specific organism and its genomic content. The cultured organisms were also used for proteome analysis and thereby its structural and functional uniqueness. But the organisms that survive at the extreme end of the physicochemical spectra were still left out as they were very hard to culture. There were communities of organisms that grew in close proximities forming a complex consortium that could adapt instantly to changing environments. Studying these microorganisms was a distant vision until genome sequencing was developed and used in the study of genomes.

The idea of "metagenomics" was first conceptualized by Handelsman et al. (1998), where they envisioned the idea of sequencing of collection of genes from a milieu and study them in a way that was similar to that of study of a single genome. In Chen and Pachter (2005), Unviersity of California researchers, defined metagenomics as "the application of modern genomics technique without the need for isolation and lab cultivation of individual species". Metagenomics uses shot gun or PCR based genome sequencing techniques to analyze a collection of genes of an entire community of organisms that dwells in a particular environment.

Metagenomics involves two primary studies. It begins with isolation of metagenomic DNA followed by PCR amplification of molecular markers with taxonomic values. If it is a bacterial genome 16S rRNA gene amplification is done and if fungi then 18S rRNA gene amplification is accomplished. The results are carefully analyzed to construct library. This involves reading short metagenomic sequences and join them to form contigs which in turn are clustered in a process called binning to form classes. The sequences thus formed are analyzed by either simple or multiple alignment method. The relationship between the protein and the source family is built. And finally, protein structure prediction and phylogenetic analysis is carried out. The metagenomics analysis is elaborately studied in the recent past with new and sophisticated software which can be read in papers by Prayogo et al. 2020, Garlapati et al. 2019, Lorenz and Eck (2005).

4.1 Structural Metagenomics

Structural metagenomics involves the study of the characters of the microbial community. The relationship between components in the community, helps in understanding about the ecology and biological functioning of the community. The methods consist of assembly, binning and taxonomic profiling via community analysis (Jimenez et al. 2012).

4.2 Functional Metagenomics

Functional metagenomics concentrate on the genes that code for a particular protein and finding its application in the industry. The protein structure predicted after metagenomic analysis is used for the characterization of the protein like its activity, optimum temperature and pH, etc.

4.3 DNA Extraction

Metagenomic analysis needs entire genetic compliment to be cloned to construct libraries. For this process the need for intact DNA is essential. Therefore, DNA extraction and purification is a primary and crucial step which involves careful sampling and minimum transportation time (Felczykowska et al. 2015).

Water Habitat

Seas and oceans that covers majority of earth are also the source of some extreme microbes that can be beneficial for man. There have metagenomics studies mostly on the surface water inhabiting micro-organisms as underground water with oligotrophic environment shows less diversity. Most of the projects are dedicated to marine water environment, but there is also focus on the lagoons, lakes, river beds, hot water springs and also frozen water that is in the form of ice.

Concentration of the sample is important to get desirable amount of DNA. The large volumes of water should be centrifuged to acquire sufficient biomass. The water can or/and should be filtered using different flow filter system equipped with filters of appropriate pore size to retain the target microbes. The cell lysis can be accomplished using enzymatic, detergent, high temperature or mechanical treatment. The pre-requisite of sequencing is that the sample should be as pure as possible. Thus, after extracting DNA from water, it is important to remove all the chemical and enzymatic contaminants. C-TAB has shown good purification effects on the isolated DNA (Ranjan et al. 2005). Sometimes even after concentrating large volumes of water, amount of DNA extracted will be much smaller than expected. In such a scenario, DNA can be amplified using linker amplified shotgun library (LASL) (Kim and Bae 2011) or by multiple displacement amplification using random hexamers and Φ29 polymerase, a bacteriophage DNA polymerase.

Soil Habitat

One of the most challenging habitats, soil, has highest concentrations of microbes. There are commercially available DNA isolation kits for the process. But it has been reported that manual isolation gives substantial quality DNA (Tanveer et al. 2016). The extraction from soil can be direct wherein the DNA is isolated in situ (Robe et al. 2003) or indirect where the microbes are segregated from the soil first and then the DNA is isolated (Hu et al. 2010).

Both in direct and indirect extraction, the access to the nucleic acid come after the cell lysis. The means by which the cell lysis takes place may differ from source to source. Some of them require physical disruption like bead beating sonication, vortexing, homogenization, thermal treatments, etc. In case of direct extraction, the cell lysis buffer is added to the soil sample and physical treatments are placed to act after which the soil aggregates are separated. In case of indirect extraction, the microbes are first separated from the soil and then the cell lysis is done. Soil is separated from the sample using centrifugation or density gradient centrifugation.

Centrifugation is employed to separate the heavier soil impurities at low speed first and then to sediment the microbial community from the supernatant at higher speed. Density gradient centrifugation is a less efficient alternate to separate soil from the community. It is used in the case of very challenging high clay soil. The lysis buffer used for extraction contains chemicals like SDS, PEG, sodium deoxycholate, etc. Purification involves complete removal of soil and humic acid. The charge characteristics of humic acids are similar to DNA, as a result gets precipitated along with it. Humic acid can be quantified with UV absorbance at 230 nm as DNA shows absorbance at 260 nm. Purification can be accomplished using precipitation with potassium acetate, isopropanol and ethanol. To get high quality purified nucleic acid sample, the purification can be continued using Sephadex gel filtration or ion exchange chromatography. Sometimes Cesium chloride gradient centrifugation is done to isolate a relatively pure product.

Sludge Habitat

Waste water milieu hosts diverse microbes that are of much interest and significance. The first reason being the health of humans depends on safe drinking water and secondly to understand function of the microbes in foaming, nitrification, etc. (Yadav and Kapley 2019). The waste water contains different pollutants like less toxic domestic sewage and toxic wastes from the industries. To carry out a successful DNA extraction these pollutants should be removed. Larger insoluble impurities are removed by mechanical separation. In the second step the dissolved organic compounds are removed by biological treatments with the help of microorganisms. In the third step the inorganic waste, mainly nitrates and phosphates produced during second step, are removed. Alternately, the commercial kits for extraction of DNA from soil can also be used in DNA preparation (Arumugam et al. 2021).

Human Body Microbiome

The most common human microbiome samples used in metagenomic analysis is the human faeces. To extract DNA, modified cetyltrimethylammonium bromide–polyethylene glycol (CTAB) phenol:chloroform extraction protocol is mostly used. The CTAB is used along with phenol:chloroform to lyse the cells and separate protein from the sample in a bead beating process. Chloroform, linear acrylamide and PEG are used for purification of the DNA (Sui et al. 2020).

4.4 16S rRNA Sequencing

Sanger sequencing is the conclusive sequencing approaches that can be used for the analysis. But because of its labour-intensive process and high cost, newer and much cheaper Next-Generation Sequencing is being used. The Illumina/Solexa and 454/Roche are the most predominantly used systems (Shuikan et al. 2019).

454/Roche clonally amplifies the DNA fragments attached to beads by emulsion polymerase chain reaction. This is then placed in picotitre plate and sequenced in parallel pyrosequencing that is, all the four deoxy ribonucleotides which gets incorporated by DNA polymerase when complementary. The polymerization releases pyrophosohates resulting in light beam which is measured. A charged coupled device camera converts the light to the sequence of the template. In the Illumina/Solexa NGS (Pérez-Cobas et al. 2020), random DNA fragments are immobilized on a solid surface. Then PCR amplification is done and the sequences are read. This is a highly efficient, fast system with yields as high as 60Gbp.

4.5 Assembly

In this step, based on the desired outcome like need for functional information or determining genome, the short sequence reads are assembled to develop longer contigs. Assembly of sequences can be either reference based or de novo. The reference-based assembly can used when the sequences in the contigs are similar to the datasets that are already available. De novo assembly is put to use when the conserved sequences do not match to the existing datasets. The de novo approach works with either OLC graphs or de-bruijn graph. With respect to input reads, OLC graph provides more information than de-bruijn, but they also require more computational resources. In case of de-bruijn, the graphs are simpler and more arguments can be added to analyze the contigs more efficiently (Rizzi et al. 2019). It is to be noted that de novo type of assembly requires complex computational strategies along with expertise in the field.

4.6 Binning

The organization of the contigs into different bins depending on its composition and gene present is termed as binning. Taxonomic dependent binning relies on the fact that the genomes contain conserved set of nucleotides that can be exploited to segregate the sequences. But in case of unknown DNA fragments the similarity is searched for the genes encoded by the genome independently, without any reference, to classify them. This is called as taxonomic independent binning or genome binning. Genome binning can be either based on the assumption that features like essential single copy genes, %G + C, and nucleotide frequency are similar in same genome (Sangwan et al. 2016) or on the hypothesis that abundance of sequences of same genome have parallel abundance in the sample taken and sequences of same species have similar abundance in multiple samples (Sedlar et al. 2017), or a hybrid of both the strategies (Alneberg et al. 2014). There are numerous algorithms that are available to cluster the sequences into respective genomes that are discussed by Yue et al. (2020).

4.7 Annotation

The identification of the genes, which is feature prediction, is structural annotation, and it's supposed function done to predict the taxonomic neighbour comprises functional annotation. Structural annotations need the identification of the reading frames, coding and non-coding regions, start/stop codons, regulatory motifs and splicing sites. Depending on the outcome of the structural annotation we can predict the functional annotation of a gene like its biochemical and biological functions, its expression and the pathways that are regulated by the products. The tools required for annotation can be studied in the reviews given by Dong and Strous (2019), Tamames et al. (2019).

Every stage in the metagenomic analysis requires efficiency and precision. The loss of DNA due to fragmentation or the process itself may result in inaccuracy of the sequence that is read in 16 s rRNA sequencing. Shorter reads and contigs are not entertained during the binning process. Most of the work after DNA extraction and amplification are completed with the computational algorithm and software. But the in-depth familiarity about the sequences, genomes and thereby its lineage is very much essential for the successful completion of the analysis. Although these analyses show optimistic results, it is worth the hardship only if there is any productive outcome like industrial application. The sequenced genes upon use should yield a product that can be used in industries to increase yield or decrease the environmental damage caused by the industrial activity. Research in waste water management from commercial, industrial as well as domestic areas are need of the hour. The metagenomic analysis of the extremophiles and/or their gene products that can clean the waste water is necessary to stop the already depleting fresh water reservoir from being unable to use.

5 Optimization of Waste Water Treatment Using Metagenomics and Meta Transcriptomic Analysis

Waste water treatment plants (WWTP) are essential for efficient management of water resources. The efficiency of these plants depends on the type of treatment being carried out (Ma et al. 2011; Saikaly et al. 2005; Tchobanoglous et al. 2003).

The activated sludge aeration basin is the heart of WWTP‘s and its functionality is dependent on a wide range of conditions. One of the major reasons which effect the overall waste water treatment process is the change in microbial population in the activated sludge basin during different stages of treatment & operational parameters. The variation in the population of the microorganisms is also due to the varying environmental/climatic conditions (Saikaly et al. 2005). Hence, an understanding of the varied activities of the microbiome present during the different processes of waste water treatment would aid in developing an efficient treatment strategy.

Traditionally, culture-based assays are used to detect the microorganisms, however, they are not highly efficient and fail to identify the pathogenic organisms at low concentrations and are time consuming. Second generation sequencing (SGS) technologies help in detecting, identifying and quantifying population of microbes including those which are un-culturable by traditional methods. SGS like 16S r DNA sequencing and whole metagenome sequencing techniques are widely being used for determination of microbial populations in waste water treatment plants. Ilumina platforms and Pyrosequencing techniques (high throughput) are widely adapted for SGS due to their low error rates and greater data output compared to other platforms. Sanapareedy et al. (2009), Zhang et al. (2012) have reported the use of pyro sequencing techniques to determine the microbial population in the activated sludge of waste water treatment plants.

Buettner and Noll (2018) reported the various microbial communities present in anaerobic digesters of biogas plants and sewage treatment plants (STP’s) using 16 S rDNA sequencing. They reported a greater diversity of microbial population in STP than in the anaerobic digesters. El Chakhtoura et al. (2015) reported the variation in microbial population within the water distribution network by using 16S r DNA sequencing methods. They observed an abundance of rare taxa Viz, Nitrospirae, Acidobacteria and Gemmatimonadetes.

16S rDNA encodes for the highly conserved 16S RNA subunit of bacterial and archaeal ribosomes and the phylogeny of the organism can be established based on the degree of similarities of these sequences. The hyper variable regions (HVR) flanked by conserved sequences present in the 16S rDNA can be easily amplified and identified by PCR. The different groups of microorganisms can be identified based on the composition of the HVR and the number of reads per group gives the relative abundance of those microbes in a given sample. However, the accuracy of identification and classification by this method decreases at species level. Taxonomic assignment at species level is therefore recommended for those HVR sequences that match exactly with a reference data base. Identification at species level with conserved gene markers and indels can also be also done with species specific PCR after 16S rDNA sequencing.

An alternative to 16S rDNA sequencing is whole-metagenome sequencing (WMS). The WMS sequences do not require primers and are taken from the fragmented genomes of the entire population. Hence can be used to identify uncharacterized sequences unlike in 16S rDNA sequencing. WMS can also be used for identification of metabolic pathways of functional significance.

Metagenomic analysis enumerates the entire population of the organism's present and can aid in identifying pathogenic microbial species, antibiotic resistance genes, microbial species which hinder the treatment process like blooms of mycolic acid producing bacteria which result in increased foaming. Further comparative analysis of metagenomic and meta-transcriptomic datasets could help in determining the relative metabolic activities of the different microbial populations. Several researchers have used the metagenomic approaches to study the changes in microbial populations in anaerobic sludge digesters. They have identified the changes in microbial community at functional level. WMS analysis can give insight into the overall population of microbes at species level as well as the metabolic and functional activities involving the various interactions of the microbial population at a given time. WMS does not involve any amplification bias and can be used for detection and quantification of pathogens and other microorganisms of low abundance.

6 Design of Novel Enzymes from Extreme Environments Using Metagenomic Analysis for Effective Bioremediation

To survive in extreme ecological niches contaminated with pesticides, aromatic hydrocarbons, heavy metals and nuclear wastes, microorganisms have developed several physiological adaptations. These microorganisms are potential source of enzymes for environmental bioremediation. The scanty information on the factors influencing the microbial growth under stressed environments is the major bottleneck for the isolation of these novel biomolecules from these extreme habitats. For the effective utilization of novel biomolecules for bioremediation depends on the deeper understanding on the microbial physiology, metabolic capabilities, factors influencing the composition and role of indigenous microorganism. Metagenomics is an emerging approach used to study in detail structure, physiology and metabolic processes of these novel microorganisms. The metagenomics studies in conjunction with high-throughput sequencing technologies are used for the isolation and characterization of novel biomolecules used in environmental bioremediation.

As more than 99% of microorganisms in the environment are uncultivable, substantial portion of microbial community has remained unexplored for their potential applications. The new method of study has been used for the identification of the novel microbial communities based on the 5S and 16S rRNA gene without culturing the microorganism. The information required for culturing of the uncultivable microorganisms is obtained from the sequencing of 16S rRNA gene. The metagenomics approach whereby the extracted whole DNA from the sample is sequenced using the various next generation sequencing platforms such as SOLiD, Oxford NanoPore, Illumina and PacBio has revolutionized the studies in search of novel biomolecules (Handelsman et al. 1998). The insights given by the metagenome analysis of indigenous microbes can be used for designing the optimum conditions required for the isolation of uncultured potential microbes with novel biomolecules (Lorenz et al. 2002; Prosser 2015). Both gene centric and genome centric metagenomics approach is used to find the presence of new taxa with metabolic and functional genes and to reconstruct the complete genome respectively from the sequence data obtained from the environmental DNA.

In addition to acceleration of in situ bioremediation using metagenomics, rate of decontamination is enhanced either by bioaugmentation with the addition of whole cells or by biostimulation with the addition of rate limiting nutrients (Nikolopoulou and Kalogerakis 2009). The molecular tools based phylogenetic studies revealed that representation of cultured bacteria is only <1% of the bacterial diversity and the great majority of the microorganisms are unculturables or yet to be cultured which can be harnessed for the isolation of novel enzymes with the right culture conditions. To tap the substantial reservoir of unseen natural diversity containing microbial community with potential novel enzymes, collective genomes of all the organisms of that particular habitat is used for the metagenomics approach where the analysis of the 16S rRNA gene reveals the diversity, physiology and metabolic requirements of microbial communities of a given habitat. Currently the hypervariable regions (V1–V9) reflecting the significant sequence variation among bacteria are used for sequencing and identification by the researchers. Moreover, the structural and physiological information obtained from the metagenomics analysis of the indigenous microbes can be used for designing the culture conditions including the media for the isolation of microorganisms with novel enzymes (Singh and Gabani 2011; Jeon et al. 2009).

Metagenomic approach involves the extraction of DNA from the environmental sample, restriction digestion, cloning of DNA fragments in a suitable vector (plasmid, cosmid, fosmid or BAC vectors), transformation of the engineered vector into a suitable host followed by the screening of the clones in a DNA library for a particular function using either "function based" or "sequence based" approach. The function-based screening involves selection of clones expressing desired traits followed by the characterization of genes and encoded products. The sequence-based screening is the widely accepted method which involves the generation of metagenomics DNA library, sequencing, assembly of the raw data into longer contigs and identification of the protein sequences by aligning database against reference databases such as NCBI-NR or NCBIRefseq. Further, this database can be used for gene synthesis, codon optimization and biochemical characterization of identified enzymes. With the advent of next generation sequencing (NGS) in conjunction with bioinformatics tools, sequence-based screening has facilitated the isolation, identification and characterization of several novel enzymes of industrial importance (Knietsch et al. 2003).

Using function-based approach, the screening of metagenomic libraries enabled in the identification of genes encoding novel lipases, proteases, novel phosphodiesterase, alcohol oxidoreductase, cellulase, chitinase, dehydratase, β-lactamase and amylases.

As the environmental pollution is a serious global concern, several microorganisms especially bacteria which produce novel catabolic enzymes are used for bioremediation to transform or degrade the pollutants. The metagenomics is a promising field which can be effectively used to access the untapped microbial resources for effective bioremediation. Whereas the earlier microbiological processes for the identification of the novel molecule was labour and cost intensive, metagenomics is time saving and cost-effective technology for the same. The high-throughput sequencing technology and functional screening of metagenomics libraries immensely help the mankind for the detection of novel biocatalysts for effective bioremediation. The huge data set obtained from the metagenomic analysis can aid in discovering the potentially novel biomolecules.

7 Conclusion

Biological waste water treatment plants host a diverse population of microorganisms which is dynamic in nature. The variations in the microbial consortia are dependent on the environmental and process conditions occurring during the different stages of waste water treatment. Integrated omics technologies comprising of metagenomic analysis, transcriptomic analysis and metabolomic analysis has an immense application in the identification and characterization of these microbial communities. In recent years, these technologies have been used to understand the diversity in microbial population during waste water treatments. The knowledge of this variation in the microbial consortia at different stages of waste water treatment can help in designing effective treatment strategies as well as help in identifying pathogenic microorganisms and antimicrobial resistance over a broad range.

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Prospects of Nanobioremediation as a Sustainable and Eco-Friendly Technology in Separation of Heavy Metals From Industrial Wastewater

Prathibha Narayanan, S. Divijendra Natha Reddy, and Praphulla Rao

Abstract Rapid industrial development and discharge of effluents into rivers has polluted water with heavy metals and other toxic matter. Most of the conventional techniques for waste water treatment are known to cause harmful impact on environment by releasing hazardous components. Some industries use bioremediation to remove heavy metals which is eco-friendly but ineffective where the environment itself is toxic to microorganisms due to presence of chemicals and non-biodegradable metals. Advanced nanotechnology such as nano-adsorption and nanomembrane, is an emerging field to treat effluents with high efficiency though it's not cost effective and eco-friendly. The solution to these problems can be integrating bioremediation with nanotechnology. In this chapter, the structure and characteristics of nanomaterials are discussed. Additionally, the chapter also highlights the integration of microbiology and nanotechnology in two ways: Firstly, in green synthesis of nanoparticles which is less expensive and sustainable and secondly, coating nanoparticles in microbes for effective remediation.

Keywords Nanotechnology · Bioremediation · Nanobioremediation · Industrial waste water treatment · Heavy metals · Green synthesis

1 Introduction

The sustainability of human civilization depends on the judicious usage of natural resources such as water. With the advent of rapid industrialization during last century and usage of water as the universal solvent for many industrial processes, it is becoming apparent to deal with the waste water being generated by many industries. As the discharge of large amounts of untreated water in to the environment from industry is not desirable and governed by many regulations, there are various process/methods that are being used for treating the waste water (Rajeshwari et al.

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2000; Ali 2012; Zheng et al. 2015; Edwards 2019; Shah 2021a, b; Singh et al. 2019; Ajiboye et al. 2021; Nazaripour et al. 2021). Most of industrial waste water contains toxic chemicals and metals that could pose problems for the environment and can cause serious health issues to the surrounding population. If left untreated and released into the surroundings, this water could contaminate and pollute the ground water and could cause chronic health issues ranging from skin diseases to cancer. Hence the removal of metals and other toxicants from the waste water is an urgent priority. There are many technological processes that utilize chemical or biological agents for industrial water remediation. Conventional physicochemical methods are being used for treating the water to remove toxic metals and chemicals (Shahedi et al.2020; Shah 2020; Gunatilake 2015; Zawierucha et al. 2016; Edwards 2019; Crini and Lichtfouse 2019; Mao et al. 2021). However, these methods suffer from being cost intensive and cannot be adapted universally for all kinds of industrial waste water treatment (Crini and Lichtfouse 2019). During last few decades, with the better understanding of microorganisms spanning all life forms, their utility as a bioremediation agent has been actively explored as the process of bioremediations seems to be natural and cost effective. There are many microorganisms or their products that are being used or exploited for removing organic and non-organic components such as metals from the industrial waste water (Rajeshwari et al. 2000; Crini and Lichtfouse 2019; Sharma et al. 2021). Although bioremediation is successful in many instances, its use for large scale or complete remediation or control over microbes is still debatable. Nanotechnology is relatively modern field having implications across various spectrum of sciences, engineering and medicine (Rajeshwari et al. 2000; Sawhney et al. 2008; Rashidi et al. 2011; Hussain et al. 2017; Ramos et al. 2017; Crini and Lichtfouse 2019). This field has seen an explosion in development over the last couple of decades and its applications virtually touch all the fields. Recently, nanomaterials are being conceived as remediations agents for treatment of polluted water not only from industry but from other sources of human consumption (Sawhney et al. 2008; Rashidi et al. 2011; Li et al. 2015; Hussain et al. 2017; Ramos et al. 2017; Nasrollahzadeh et al. 2021).

In this chapter, we will review the nature of nanomaterials and their usage in the treatment of waste water and future perspectives of use of nanomaterials in waste water treatment.

2 Structure and Characteristic Properties of Nanomaterials

Nanotechnology, during last few decades burst in to attention due to its potential applications to all the fields of technology and engineering. Nanomaterials are expected to perform or to be used in endless potential applications due to their size ranging in nanoscale. Nano scale is defined as the range where at least one dimension of the material falls under 100 nm (nM) (Emil Roduner 2006). Because of their size, the physical and chemical properties of nanomaterials are different from the bulk materials of the same element with which they are made (Wu et al. 2020; Juh

Tzeng Lue 2007; Murty et al. 2013). One important aspect of being at nanoscale is the surface to volume ratio is more and this is being exploited for better utility or for many applications as the availability of more atoms on the surface in comparison to the bulk material. Nanomaterials comes in various shapes and forms. Based on the number of dimensions nanomaterials can be classified as zero-, one-, two- and three-dimensional nanomaterials. In case of zero all dimensions are at nano scale (nano spheres), in one dimension, two dimensions are at nanoscale and the other is outside nano scale and in two-dimensional (nanotubes, nanofibres, nanorods, nano wires), one dimension is at nano scale and the other two outside the nanoscale (nano films, nano layers). Three dimensional nanomaterials are not at nanoscale but are made up of repeating units of nanomaterials (dendrimers). Based on the composition of elements, nanomaterials can be classified as carbon based (nanofibers, nano tubes,) and metal based such as gold and silver nanoparticles and TiO_2 oxide nanoparticles. The physical and chemical properties of nanomaterials are different from the corresponding bulk material. Catalytic activity of some nanomaterials is better as the surface to volume ratio is more(ref). They possess better electrical conductivity in case of ceramics and magnetic composite nanomaterials while metal nanomaterials may have increased resistance. Nanomatrials generally have increased magnetic activity and also behave as super paramagnetic material. Some metal nanomaterials show marked toughness, ductility and plasticity (Wu et al. 2020; nanotechnology and 2007 2007). Some nanomaterials are characterized by shift in optical absorption, fluorescence and increased quantum efficiency. When it comes to biological applications or in medicine, nanomaterials are proved to be better in overcoming biological barriers such as cell membranes and also shown to be more biocompatible (Hu et al. 2006, 2017; Kyriakides et al. 2021). Because of changes in physical and chemical properties at nano scale, nanomaterials are being exploited for their usage in purification of water either from natural sources or from the sources of contamination such as an industry.

3 The Science Behind Nanobioremediation

Industrial harmful contaminants have distinct physical and chemical properties and each contaminant interacts in a different manner with environmental parameters. Due to this the conventional techniques to treat waste water is not so effective. Bioremediation is used in many industries for their low cost and varied applications Recently in several investigations, nanomaterials have been used in association with bioremediation technologies which prevents formation of dangerous by-products as well as accelerates the process of degradation or removal of contaminants (Vázquez-Núñez et al. 2020). Using nanomaterials for bioremediation has many benefits. Because of its nanosize, the material will have high surface area which increases reactivity with surrounding components. They require less activation energy for reactions. Nanomaterials have the advantage of exhibiting surface plasmon resonance which can be applied in detecting toxic substances. Due to small size, nanomaterials have the potential to reach deep into contamination zones. Also, oxide coating with nanomaterials can increase reactivity to a large extent. Some metal nanoparticles can function as biocatalysts. For example, few studies revealed Palladium nanoparticles which are coated on the cell walls of *Shewanella oneidensis* gets charged with radicals in the presence of substrates which act as electron donors such as acetate, hydrogen etc. When these cells coated with nanoparticles and charged with substrates come in contact with compounds containing chlorine, the radicals present in palladium removes chlorine. Immobilization of microorganisms is possible by using metal nanoparticles which are magnetic in nature. These cells have been efficiently applied in degradation or recovery of components. In one of the observations, the microorganisms treated or coated with magnetic nanoparticle effectively separated organic sulfur from fossil fuels on large scale in reactors (Rizwan et al. 2014). Interaction of nanoparticles with living organisms can lead to any outcomes like sorption, biotransformation, dissolution etc. which leads to degradation of toxic compounds (Vázquez-Núñez et al. 2020). Nanoparticles can either inhibit or activate the metabolism of living organisms participating in bioremediation. Therefore, it is very essential to confirm the effects of nanoparticles with living organisms before its application in remediation processes. Some of the parameters that needs to be studied are nanoparticles size and shape, surface area, chemical properties of both toxic components and nanoparticles, nature and type of organism, growth media, pH, temperature etc. (Tan et al. 2018). Due to involvement of so many factors, it is very difficult to study their individual effects. There are not many research studies done which establish the relationship between these parameters. Sorption studies are one of the significant methods in nanobioremediation. Contaminant removal either can be through adsorption which is surface phenomena or through absorption where the toxic compound penetrates the nanoparticle and gets separated as a solution. Additionally, either the sorption can be by chemical means or by physical methods. Several research has been performed on adsorption isotherms, thermodynamics, and kinetic studies to understand the type of adsorption processes and interaction between nanoparticles and contaminants. Nanoparticles can degrade contaminants by photocatalytic processes. In some cases, enzymes produced by living organisms degrade pollutants (Vázquez-Núñez et al. 2020).

4 Formation of Nanoparticles Using Natural Sources

Synthesis of nanoparticles naturally using microorganisms and plants is cost effective, safe and eco-friendly. Not all organisms can produce metal nanoparticles. Formation of nanoparticles from organisms is natural and can be in any two ways: First method is by bio-reduction where metal nanoparticles are produced by reduction using biological tools. In this method, metal ions are reduced and enzymes are oxidized. Nanoparticles can be then recovered from the contaminated samples. The second method is bio-sorption where metal ions from polluted locations are bound to the cell walls of organisms or the organisms synthesize peptides which assemble into suitable nanoparticles (Zhang et al. 2020).

4.1 Sources for Synthesis of Nanoparticles

Bacteria

Several species of bacteria are widely studied to have the capacity to produce nanoparticles by reducing metal ions. It is relatively easy to engineer bacteria as per the requirements for the synthesis of nanoparticles (Singh et al. 2018). Some bacteria known for production of silver nanoparticles are *Escherichia coli*, *Lactobacillus casei*, *Bacillus sp.*, *Pseudomonas sp.*, *Enterobacter cloacae*, *Corynebacterium* sp. and *Shewanella oneidensis.* Gold nanoparticles production from bacteria are comparatively complex and few bacterial species recognised includes *Shewanella alga, Escherichia coli, Bacillus sp.*, *Desulfovibrio desulfuricans*, and *Rhodopseudomonas capsulate.*

Synthesis of palladium has been studied for *Escherichia coli* and Psedomonas cells. Reports claim that copper nanoparticles from *Morganella morganii* need to be stabilized immediately after their formation since copper metal is usually unstable and readily gets oxidized to form cupric oxide. Copper nanomaterials accumulates extracellularly as a result of intracellular uptake of copper ions which is then enzymatically reduced (Zhang et al. 2020). Nanomaterials formed from different strains of bacteria vary in size, shape, morphology and time duration for their synthesis.

Fungi

Fungi are found to be better sources relative to bacteria for production of metal and metal oxide nanoparticles in view of the fact that many intracellular enzymes, proteins and reducing agents are present on their cell surfaces which aid in the mechanism. The process is cost effective and efficient resulting in large amount of nanoparticles of well-defined morphologies (Singh et al. 2018). *Fusarium oxysporum, Aspergillus fumigatus and Trichoderma reesei* have been identified to produce silver nanoparticles extracellularly. Since *Trichoderma reesei* morphology is extensively studied, there is advantage of manipulating them for the nanoparticle synthesis. *Aspergillus fumigatus* produce silver nanoparticles within minutes of exposure. White rot fungi form silver nanoparticles intracellularly. Although not much work is done on production of gold nanoparticles form fungi, but there are studies reported on the formation of gold nanoparticles from *Verticillium* species by reducing Tetrachloroaurate ($AuCl_4$) ions externally on the mycelium (Zhang et al. 2020).

Few research work reveals synthesis of single platinum nanoparticles (PtNPs) intracellularly by *Neurospora crassa. Fusarium oxysporum* also synthesizes PtNPs both extra and intracellularly. Some fungi such as *Fusarium oxysporum* and *Verticillium* sp. produce metal oxides like magnetite nanoparticles (MaNPs) intracellularly (Zhang et al. 2020).

Fungal mycelia have relatively larger surface area. In addition, for large scale production and purification process use of fungal species has been proven efficient and less expensive through investigations. Furthermore, enormous number of proteins and enzymes are formed in fungi, which are required for high production of nanoparticles. However, most of the fungi are pathogenic and might be a concern (Zhang et al. 2020).

Yeasts

Like fungi, yeasts also have large surface area to their advantage. They synthesize metal nanoparticles through various methods such as precipitation, sorption, sequestration etc. Most of the nanoparticles are produced intracellularly by yeasts. For example, *Candida glabrata* produces CdS quantum dots intracellularly in the presence of cadmium salts. Also, *Torulopsis* sp. in the presence of lead ions forms PbS quantum dots and *Pichia jadinii* is known to produce gold nanoparticles intracellularly (Zhang et al. 2020).

Plants

Plants have the tendency to store heavy metals. In addition, plants have varied biomolecules such as carbohydrates, proteins, fats, enzymes etc. and phytochemicals like sugars, ketones, aldehydes, carboxylic acids, flavonoids and terpenoids, which play a key role in reduction of metal ions to nanoparticles and makes them one of the best sources with regards to ease of process, faster synthesis, stability and cost. Many plants have been studied over the past few years for production of nanoparticles which includes *Aloe barbadensis* (Aloe vera), *Azadirachta indica* (Neem), *Avena sativa* (Oats)*, Osimum sanctum (*Tulsi*), Coriandrum sativum* (coriander) and *Cymbopogon flexuosus* (lemon grass) for synthesis of silver and gold nanoparticles to name a few. Other metal nanoparticles like zinc, cobalt, nickel and copper are found to be synthesized from *Brassica juncea* (mustard), *Helianthus annuus* (sunflower), *Coriandrum sativum* (coriander), *Hibiscus rosa-sinensis* (China rose), *Camellia sinensis* (green tea), *Acalypha indica* (copper leaf) and *Aloe barbadensis* (Aloe vera). Most of the metal and metal oxides nanoparticles are produced by reduction of metal salt ions accumulated in plants. For example, flavonoids reduce metal ions into metal nanoparticles by transformation of enol to the keto forms. Studies show that conversion of enol- to keto is the key mechanism in the formation of silver nanoparticles from *Ocimum basilicum* (sweet basil) extracts. Sugars like glucose also participate in metal nanoparticles synthesis with different size and shapes. Also, proteins comprising of amino acids reduce the metal ions to form nanoparticles. Some investigations reveal that few amino acids are capable of binding with silver ions and hence produce silver nanoparticles. Furthermore all the naturally occurring amino acids were examined and tested to study their reduction process of gold ions (Singh et al. 2018).

Plants used for metal nanoparticle synthesis differ in their process times and form nanoparticles of various shapes and sizes. *Jatroa curcas* extracts synthesize homogenous silver nanoparticles of small sizes from silver nitrate ($AgNO_3$) salt in about 4 h. On contrary, *Acalypha indica* leaves form homogeneous silver nanoparticles of slightly bigger sizes (Singh et al. 2018). *Medicago sativa* seeds were found very effective in the synthesis of silver nanoparticles as the process was faster and took less than an hour and more than 90% of silver ions reduced. The temperature requirement is also low to enable action of enzymes. The nanoparticles were triangular or spherical with a rather heterogeneous size range (Zhang et al. 2020). In another study, silver and gold nanoparticles were formed from a single component such as phyllanthin isolated from the plant *Phyllanthus amarus*, rather than using whole plant or plant parts. Concentrations of phyllanthin highly influenced the shapes of nanoparticles. For instance, phyllanthin added in large amounts resulted in spherical nanoparticles (Singh et al. 2018).

Some extracts from plants like *Anacardium occidentale* (cashew nut), *Azadirachta indica* (neem), *Swieteni amahagony* (mahogany) and vegetable extracts (Zhang et al. 2020) have been reported to synthesize bimetallic silver and gold nanoparticles.

Algae

Cyanobacteria or blue green algae, such as *C. vulgaris,* and *S. Platensis L. majuscule*, and *C. prolifera*, are yet another cost-effective sources for green synthesis of nanoparticles. Recently, synthesis of gold metal nanoparticles by reduction of Au^{3+} ions to gold oxide (AuO) using cyanobacteria *S. platensis* proteins, have been reported (Aman Gour et al. 2019).

Viruses

The capsid proteins on the surface of virus make them highly reactive towards metals leading to formation of nanoparticles. For example, Tobacco mosaic virus (TMV) has close to 2000 capsid proteins on their surface which reacts with silver or gold ions in addition to plant extracts of *Nicotiana benthamiana* (Round-leaved native tobacco) or *Hordeum vulgare* (Barley) (Zhang et al. 2020). The synthesized nanoparticles were relatively smaller in size.

4.2 Solvents

Water is the most commonly used solvent for nanoparticle synthesis as it is relatively cheaper and readily available (Shanker et al. 2016). For example, gold and silver nanoparticles are produced from gallic acid prepared in an aqueous medium. Furthermore, oxidation of gold nanoparticles by the oxygen available in the water, increases its efficiency and reactivity and enhances its role in bioremediation (Singh et al. 2018).

4.3 Factors Modulating Formation of Metal Nanoparticles

The size and shape of nanoparticles depends on various parameters such as reaction time, reactant concentrations, pH, and temperature. These factors need to be optimized for the synthesis of nanoparticles of desired morphology.

pH

pH of the reaction medium is very important factor and alters the size and shape of nanoparticles. For example, rod-shaped gold nanoparticles of size in the range 25-85 nm were formed from biomass of *Avena sativa* (Oat) at acidic pH of 2. These gold nanoparticles were relatively smaller (5–20 nm) and nucleation was comparatively good when pH was maintained at 3 or 4. Slight alkaline conditions (pH > 5) are required for synthesis of silver nanoparticles from *Cinnamon zeylanicum* extracts which are homogenous and spherical in nature (Zhang et al. 2020).

Reactant Concentration

The presence and concentration of phytochemicals and other bio-components in the extract determine the size and shape of the nanoparticles. For example, the growth of gold and silver nanomaterials formed from extracts of *Cinnamomum camphora* (camphor) were reported to be influenced by the quantity of biomass in the reaction mixture. Also, addition of chloroauric acid to *Aloe vera* leaf extract led to spherical nanoparticles rather than triangular plates. It was also reported that the size of the nanoparticles can be manipulated by varying the concentrations of the substrates. In another similar study, varied shapes of silver nanoparticles including the desired spherical profiles, were synthesized by altering the concentration of *Plectranthu samboinicus* extract in the process mixture (Zhang et al. 2020).

Reaction Time

Yet another factor influencing morphological characteristics of the nanoparticles is the reaction time. A research investigation observed formation of spherical silver nanoparticles of average size 12 nm from *Anana scomosus* (Pineapple) extract and $AgNO_3$, within 2 min of time showing a rapid color change. *Chenopodium album* leaf extract produced silver and gold nanoparticles within 15–20 min of the reaction and the process persisted for more than 2 h. In another study, it was revealed that different sizes of nanoparticles formed from *Azadirachta indica* leaf extract and silver nitrate (ranging 10–35 nm) resulted in an increase in reaction time from half an hour to more than 4 h (Zhang et al. 2020).

Reaction Temperature

The temperature modulates the structure, size, and productivity of nanoparticles synthesized from different plants. One of the experimental studies reported that temperature had reciprocal effects on the size of nanoparticles synthesized from the *Citrus sinensis* (sweet orange) extracts from its rind. The nanoparticle size decreased with an increase in temperature. Also, in few other studies it was noted that the temperature altered shape of gold nanoparticles produced using *Avena sativa* (oat). The gold nanoparticles formed at lower temperature were spherical compared to nanoparticles at higher temperatures which exhibited non-spherical patterns. Process temperature conditions also affects productivity like it was noticed in few observations that the yield of platinum nanoparticles synthesized extracellularly, was around 5.66 mg l^{-1} which varied with temperature (Zhang et al. 2020).

4.4 Identification and Removal of Heavy Metal Ions from Waste Water

Industrial development has led to accumulation of contaminants and pollutants in soil, air and water. Industrial effluents especially are discharged into water bodies. Most of the effluents include harmful heavy metals like copper, lead and cadmium which are detrimental even at low concentrations. There are several methods for identifying these pollutants but these conventional techniques are not cost effective, consume lot of time and also require skilled and experienced personnel. Use of metal nanoparticles has many advantages due to its size and optical properties like the process is simple, relatively cheaper and has high sensitivity to detect toxic heavy metals even at low concentrations. In one of the investigations reported, silver nanoparticles were synthesized from different plant sources which were sensitive in identification of heavy metals based on their plant source. For example, silver nanoparticles prepared from mango leaves displayed calorimetric sensing selectively for lead and mercury ions. Another significant pollutant from industries like textile, plastic, leather, paper and food are coloured dyes. Dyes are important component used in manufacturing process in textile industries. About 10–15% of dyes are discharged as wastes into water bodies. These are very dangerous as they increase turbidity in water thereby preventing penetration of sunlight which can be fatal for marine life. For removal of dyes from water, usually metal oxide nanoparticles like zinc oxide (ZnO), titanium dioxide (TiO_2), and copper oxide (CuO) with photocatalytic properties are used for their high surface area. High surface area increases absorption of reactive dyes at low concentrations as nanoparticles have large number of reactive sites available due to high surface energies. Furthermore, very less quantity of these nanoparticles is required for large mass of effluents (Singh et al. 2018).

5 Coating of Nanomaterials Inside Microbes for Effective Bioremediation

Nanomaterials can be potentially used for treating industrial wastewater and ground water. Nanomaterials can treat the wastewater by means of adsorption, photocatalysis, disinfection and membrane application. Adsorption is a common mechanism used with nanoparticles to treat the wastewater. Properties such as high surface area, selective adsorption sites, short diffusion distance between particles, alterable surface chemistry, reusability and so on gives the advantage of nanoparticles in wastewater treatment. Most commonly, heavy metals, arsenic, chromium, mercury phosphates, organic compounds, PAHs, DDT are adsorbed by nanoparticles. Organic compounds, volatile organic carbon, azo dye, congo red dye and so forth are removed from wastewater by means of photocatalytic oxidation. Nanoparticles with high photocatalytic activity such as TiO_2, ZnO, iron oxide nanoparticles are used in this technique. Nanoscale zero-valent iron (nZVI), nanoscale calcium peroxide nanoparticles are used to treat wastewater with halogenated organic compounds, metals, nitrate, arsenate, oil, PAH, PCB by means of redox reactions. Some of the nanoparticles have strong antimicrobial properties like silver and TiO_2 nanoparticies that can be used to disinfect the waste water. These nanoparticles have low toxicity, high chemical stability and reusability. Several research studies have confirmed use of nanoparticles of metal and metal oxides such as silver, TiO_2, Zeolites and CNTs that exhibit strong hydrophilicity, high mechanical and chemical stability, high permeability and selectivity, can be embedded to the membranes to increase their effectiveness in purification of the wastewater (Yadav et al. 2017).

Few investigations observed that Palladium, Pd(0) nanoparticles can be used for removal of chlorine. The cell wall and inside the cytoplasm of *Shewanella oneidensis* were coated with Pd(0) nanoparticles which gets charged on reaction with suitable substrates The protons present in these charged Pd(0) coated cells when exposed to chlorine rich mixtures, react with phencyclidine, leading to the separation of the chlorine molecule.

Sarioglu et al. (2017) studied the dye removal capacity of bacterial cells encapsulated within electrospun nanofibrous webs. Commercially available strain of *Pseudomonas aeruginosa* was immobilized using polymers like polyvinyl alcohol (PVA) and polyethylene oxide (PEO). The extent of encapsulation and the viability of bacteria were examined using advanced and modern tools and techniques. It was reported that bacteria encapsulated in PEO web showed higher methylene blue dye removal efficiency which could be due to better accessibility of bacteria trapped in the nanofibrous webs. Studies reported that the encapsulated bacterial cells remained alive for about a couple of months when the webs were stored at 4 °C and thus could be preferred to lyophilized cells which selective culture media and space for storage. In addition, some research reveal that the electrospun nanofibrous webs containing viable cells find scope in bioremediation of water systems.

The electrospun cyclodextrin fibers (CD-F) manufactured by electrospinning technique has been used in few studies as carrier matrix and feed for encapsulation of bacteria which can be effectively applied for bioremediation purposes. N.O San Keskin et al. (2018) reported in his findings that electrospinning process is simple and easy for immobilization of bacteria into CD-F matrix and is widely applied in wastewater treatment. CD-F are not toxic and hence display cell viability for more than a weeks of time when stored at 4 °C. The observations were promising for nanobioremediation as the bacteria encapsulated with CD-F showed high heavy metals and dye removal efficiencies showing results as $70 \pm 0.2\%$, $58 \pm 1.4\%$ and $82 \pm 0.8\%$ for Nickel, chromium and reactive dye, RB5, respectively. Added advantage is bacteria can consume CD as an extra carbon source when primary carbon source depletes thereby increasing their growth rate.

6 Conclusion

Over the last few years research studies on green synthesis of metal and metal oxide nanoparticles has increased due to its various advantages. Among the numerous sources, plant extracts have been observed to have significant roles as stabilizing and reducing agents which under optimized reaction conditions can lead to formation of nanoparticles with desired sizes, shapes, and other morphological characteristics. Nanoparticles play a vital role in degradation of heavy metals in waste water. Nanoparticles on one hand degrades waste that might be toxic to microorganisms and on the contrary, improves performance of microorganisms in remediation of harmful substances. Though studies have investigated the inter-relation between nanoparticles and microorganisms in degradation of harmful substances in batch experiments, but the findings are not sufficient to comprehend the synergetic effect of nanoparticles and microorganisms during a nanobioremediation process and its applications. Also, there is no safety data available that indicates the long-term effects on use of nanoparticles with microorganisms. Hence, there is lot of scope in this field to explore and research and apply nanobioremediation technologies in varied applications following the regulatory framework and safety guidelines.

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Nanotechnology for Bioremediation of Industrial Wastewater Treatment

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Abstract At an ongoing pace, the population of the world will increase manifold. The population explosion and global warming will impact the available useful water for drinking. Simultaneously to fulfil the demand of other sectors like industries and agriculture there will be pressure on water availability on earth. Due to human activity, various pollutants are released from the industrial sector. Quick attention is needed towards the treatment of the wastewater before it gets released into the environment to maintain the quality and purity of water. For the treatment of wastewater, various methodologies have developed in the last few years. One of the most interesting ways to treat wastewater is the use of nano bioremediation. The present book chapter enlightens the use of nanotechnology-based bioremediation for wastewater treatment. Nanobioremediation is a method or process in which a combination of biology and nanotechnology is used to remove heavy metal pollutants from wastewater and make water clean. The removal of environmental contaminants (such as heavy metals, organic and inorganic pollutants) from contaminated sites using nanoparticles/nanomaterials formed by plants, fungi, and bacteria with the help of nanotechnology is called Nano bioremediation. In traditional ways using either nanotechnology-based or biological-based methods, these

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both are capable of removal of toxic metals from the environment. Using nanoparticles along with microorganisms acts synergistically for the removal of pollutants. Sometimes a single enzyme is also effective for bioremediation but the enzyme is not stable. Using functionalized nanoparticles will provide stability and improve bioremediation speed.

Keywords Nanotechnology · Nano bioremediation · Industrial wastewater treatment · Environmental science

1 Introduction of Nano Bioremediation for Industrial Wastewater Treatment

The need for water is just as important as those for food, shelter, and clothes for human beings. Water is indispensable to our existence and life originated here without it, we cannot exist. While water is a natural resource, its availability is limited. According to reports, one of the six individuals has difficulty getting fresh water. It is estimated that until 2025, around 50% of the global population will suffer from water-related issues (Aguilar-Pérez et al. 2021). Clean water is essential for all creatures on the planet. Water access can impact the environment and climate change if there is no proper access. Lack of water will affect biodiversity and destroy habitats in addition to causing landscape erosion (Hashem 2014). With the increase in population, rapid commercialization, and less precipitation, hazardous chemicals have spread across the earth's surface and are affecting the groundwater system. As a result, ecosystems have undergone drastic changes (Zekic et al. 2018). The scarcity of pure water is the primary problem throughout the world, so there is a need to explore new methods and technologies for cleaning water. One method is to use nanotechnology to clean wastewater. During the past few years, water pollution has received a lot of attention. By providing quality water, people will be able to satisfy their current needs for water (Pandey 2018; Shah 2020a, b).

Nanoscience and nanotechnology are experiencing a new era of opportunities due to technological developments. Nanoscience is a branch of science that deals with molecules that have at least one dimension smaller than 100 nm. Even though nanoparticles are made from metal ions, they behave differently from metal ions (Rizwan and Ahmed 2018).

This chapter mainly addressed nanotechnology-based solutions for bioremediating wastewater. The goal of this chapter is to provide an introduction to bioremediation, different methods of bioremediation, nanobioremediation, and different kinds of nanoparticles used in bioremediation, their role, drawbacks, and conclusion.

Before we look at nanotechnology-based wastewater treatment, we should get a better understanding of water pollutants, their sources, types, and effects, as well as the methods used to remove contaminants from water.

1.1 The Main Source of Water Pollutants

Chemical and biotechnological industries produce a variety of useful products. There is no doubt that industries produce inevitable products, but they are also producing products which are ultimately responsible for a source of pollution. Due to various industrial activities, toxic chemicals are released into water.

Here are a few examples of pollutants released by different industries. They can be classified as organic or inorganic. Chlorinated phenols, phthalic esters, persistent organic pollutants (POPs), azo dyes, petroleum hydrocarbons, pesticides, etc., are among the most common organic pollutants. In contrast, inorganic pollutants include heavy metals such as aluminum (Al), cadmium (Cd), nickel (Ni), chromium (Cr), lead (Pb), and mercury (Hg) (Hussain et al. 2016; Bharagava et al. 2020).

Organic Pollutant: Their Sources, and Effects

Chlorinated Phenols

Chlorinated phenols is a major constituent released by coking plants, oil refineries, resin manufacturing, paper and pulp industries. They are recalcitrant and toxic, and the level of chlorination determines their toxicity (Annachhatre and Gheewala 1996; Shah 2021a, b).

Phthalic Esters

They are a class of organic refractory plasticizers used in various products such as plastic roofing, piping, gaskets, medical equipment, rainwear, plastic film. The phthalic ester in plastics is responsible for its flexibility. More than one million tons of phthalic ester is used in the manufacture of plastic in China. It has a serious effect on human health as these compounds are mutagenic and affect the embryonic development of the foetus (He et al. 2015).

Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) consist of different chemical groups that require attention. Polychlorinated dibenzo-p-dioxins, dibenzo-furans (PCDD/Fs), polychlorinated biphenyls (PCBs), and heptachloride present in pesticides require special attention. Forest fire and volcanic activity are main natural sources of POPs such as dibenzofurans and dioxins. POPs can also be produced by furnaces, incinerators, power plants, and heating systems used in industrial sites. Various countries have banned this type of chemical in recent years. These are organic compounds that are harmful to the environment. POPs are mainly non-biodegradable and lipophilic. It affects the respiratory, circulatory, and digestive systems of the human body (Alshemmari 2021).

Azo Dyes

In many industries, dyes are used as colouring compounds. Colour-producing compounds are classified according to their use and chromophore group. Phytocyanine dyes, anthraquinone dyes, azo dyes and phthalocyanine dyes are chromophore-containing dyes. An Azo dye has a special azo bond responsible for absorbing light in visible wavelength regions.

More than 3000 types of dyes are available on the market. Each year, more than 2.8 tons of dyes are added to the water system. The reason why so many dyes are available is that they are chemically stable and easy to synthesize. A number of industries use azo dyes, including food, paper, leather, cosmetics, textiles, pharmaceuticals. According to the survey, the dye which is used in the dyeing process is not completely utilized, about 10% of the dye is not utilized and unutilized dye discharges into water bodies. These azo dyes released from effluent have many side effects. It damages the cells of the organism, the rate of photosynthesis is reduced, it leads to a decrease in oxygen content which affects the flora and fauna of water bodies. Mainly azo dye consists of a nitro group which is responsible for mutation. When dye is released from industrial water, it breaks down and forms a new kind of toxic product. Such products are mutagenic and carcinogenic. Several derivatives of azo dye after breakdown are o-toluidine, 1,4-phenylenediamine, and 3-methoxy-4-aminoazobenzene which are carcinogenic. According to a study, unsulfonated azo dyes are more toxic and mutagenic than sulfonated azo dyes (Singh and Singh 2017).

Petroleum Hydrocarbons

The oil industry releases wastewater that contains a lot of hydrocarbons, ethylbenzene, benzene, xylene, toluene, small amounts of oxygen, sulfur, nitrogen, and heavy metals. The presence of these hydrocarbons in terrestrial systems or on land changes the original composition of soil and affects the growth of plants. Through different transport processes occurring in nature, petroleum hydrocarbons eventually end up in the sea and pollute marine ecosystems. As a result of fuel spills, large environmental losses occurred in cold regions and threatened the health of organisms. These large numbers of hydrocarbons are responsible for damage to the central nervous system and also lead to suppression of bone marrow activity. In order to remove this kind of contamination from contaminated sites, nanoparticle-based tools can be used (Mohammadi et al. 2020).

Inorganic Pollutants

Industrial wastewater from different sources contain different kinds of heavy metals, such as copper, cobalt, cadmium, chromium, lead, iron, zinc, nickel, and magnesium. Metals such as manganese, copper, zinc, nickel, cadmium, mercury, lead, aluminium, chromium, iron, and cobalt require immediate attention, according to the WHO.

Many countries are concerned about the release of metals or heavy metals into industrial water because these metals or heavy metals persist in the environment and lead to bioaccumulation. Their non-biodegradable nature makes them responsible for continuous transfer into the next generation if they bioaccumulate in organisms. Radioactive metals can cause harm to the human body if they accumulate in the body (Bernard 2008; Assi et al. 2016; Hussain et al. 2016; Koul and Taak 2018a; Magrì et al. 2018).

Here, we will discuss different types of metals, their sources, and their effects on the environment.

Zinc

Industries like coal mining, steel processing, and waste combustion emit zinc. A direct accumulation of zinc causes diarrhoea and gastrointestinal distress, while inhalation of zinc fumes causes fever.

Nickel

Fossil fuel combustion, metal plating industries, nickel mining, electroplating fumes from alloys used in welding and brazing, and nickel-refining industries release nickel. Nickel acts as a carcinogen and causes respiratory tract disease.

Mercury

In paper and pulp manufacturing, gold extraction, chloro-alkali operations, smelting operations, and amalgam tooth fillings, mercury is released. It leads to kidney damage. As a result of chronic poisoning, the blood's ability to carry oxygen is reduced. Mercury can also cause nutritional disturbances, excessive irritation of tissues, loss of appetite, gingivitis, and excessive salivation. Exposure to mercury fumes may cause respiratory problems.

Manganese

Steel and welding industries release large amounts of manganese. Consequently, neurotological disease is caused by this metal. Poisoning can lead to irritability and speech problems.

Lead

Rubber production, glass polishing, jewellery making, lead-glazed plastic manufacturing, stained glass crafting, pottery, and painting make up this industry. Lead affects many different organs of the body. The toxins not only damage organs, but also affect cellular mechanisms and enzymes.

Iron

It includes Hematite mining, metal industries, and welding. Lung silicosis is caused by iron fume accumulation in heavy fumes.

Copper

Copper emissions are mainly caused by welding, copper mining, and metal fumes from smelting operations. Copper causes Wilson's disease and anaemia as well as higher accumulation of copper in the body. Aside from kidney and liver damage, other vital organs can also be harmed and it affects digestion also. At high levels, sulfate and copper induce necrosis and kill the organism.

Cobalt

Paints and cement are the main industries that use cobalt. At low concentrations, it causes respiratory problems such as irritation of the respiratory tract, while at high concentrations, it may cause pneumoconiosis.

Chromium

Chromium is mainly used in paint and colouring companies. Moreover, it is released in the manufacturing of chemical and refractory materials, cement manufacturing, chrome plating, ferrochrome manufacturing, metal finishing industries, combustion of fossil fuels, tanneries, and textile plants. It damages kidneys when at low concentrations, while causing respiratory chromogenic substances when at high concentrations, which are mostly carcinogenic and also cause allergies.

Cadmium

Cadmium is released by several industries, including the automotive and aircraft industries, alloys' production, metallurgy processing, nickel–cadmium battery manufacturing industries, paint industries, plastic industries mining, and textile printing industries. Inhalation and ingestion can cause acute and chronic toxicity. A number of metabolic pathways are affected by Cd, including alcohol dehydrogenase, pyruvate dehydrogenase, pyruvate decarboxylase, delta-aminolevulinic acid dehydratase, arylsulfatase, and lipoamide dehydrogenase.

Aluminium

The production of aluminum alloys, the pharmaceutical industry, and packaging units release aluminium. Humans can develop lung fibrosis from the fumes from these sources. Fumes from these sources can cause lung fibrosis and osteomalacia. Large accumulations in bones can cause lung fibrosis.

Contaminated water also contains microorganisms in addition to organic and inorganic pollutants. These microorganisms are present in wastewater from the healthcare industry because of improperly sterilized medical swabs, needles, and bandages. In normal water bodies, unsterilized tools make the water contaminated.

1.2 The Science Behind the Use of Nanomaterials in Bioremediation

The goal of remediation is to remove toxic, heavy metal, and harmful waste from the environment. The three main methods of environmental remediation are physical, chemical, and biological (Singh et al. 2020).

Remediation of the environment usually involves chemical methods such as reduction, chemical washing, chelating flushing, ion exchange, electrowinning, adsorption, membrane filtration, and concentration. There are a number of biotechnology methods that remove pollutants from the environment, including rhizofiltration, bioslurping, windrows, biosorption, land farming, bioplies, bioventing, bioleaching bioreactors, biosparging, composting, bioaugmentation, biostimulation phytoremediation, and mycoremediation. Among these three major processes, biological processes are the most commonly used because they do not involve toxic chemicals or energy consumption. In most cases, bioremediation does not require the use of external chemicals, as the process relies on naturally occurring plants and microorganisms (Yadav et al. 2017).

Bioremediation can be classified into two major categories: in-situ and ex-situ. In situ bioremediation occurs at the original site where contaminated and toxic substances were excavated before being treated. As a result, it is faster, easier, and more effective than ex-situ bioremediation (Singh et al. 2020).

In soil, there are bacteria capable of degrading soil chemicals. These bacteria are called "bugs". These tiny bugs are often found in oil spills and gasoline. Microbes can digest chemicals and then convert them into harmless forms, such as water and carbon dioxide. The process occurs in two different ways. As an initial step, it is necessary to manipulate parameters such as nutrient levels, temperature, and oxygen ions in order to increase the ability of microorganisms to digest harmful metals. Adding microorganisms to contaminated sites is the second method, which is not the most common. The two methods serve the same purpose, however (Yadav et al. 2017).

In bioremediation, pollutants are removed from the environment. Nanobioremediation involves removing pollutants from the environment using nanoparticle-based solutions made from plants, fungi, or bacteria.

In their talk, Richard Feynman introduced the concept of nanotechnology. In a talk titled 'There's Plenty of Room at the Bottom,' he did not use the term nanotechnology directly. Nanotechnology is a milestone in the development of new branches of science. Nanotechnology creates new materials by manipulating materials at very small scales. Reduced metal ions have completely different properties than their original forms. Nanoparticles or nanomaterials differ in two important ways; the first is the surface area ratio, and the second is the quantum effect. In order to effectively remove contaminants, both of these factors are necessary. Quantum effects accelerate response time, and when there is a large surface area, the interaction is greater (Mourdikoudis et al. 2018; Rizwan and Ahmed 2018). Based on its applications, nanotechnology can be classified into many branches. In wastewater treatment, a variety of nanomaterials are used. In order to remove pollutant size dependence, nanoparticles play a vital role. Nanoparticles possess special properties that make them suitable for use in different methods of removing contaminants.

Another sub-branch of nanotechnology is green nanotechnology. Eco-friendly nanoparticles and nanoproducts can be made with green nanotechnology. Nanoparticles are most efficient at removing contaminants, which requires fewer resources, in wastewater treatment. These simple, cost-effective processes can be used to remove contaminants from wastewater (Alshemmari 2021).

Nanotechnology-based solutions are widely used in bioremediation because they can be easily synthesized and are safe. In addition, nanoparticles increase the efficiency and speed of bioremediation.

The following examples illustrate how bioremediation works. Phytoremediation involves using plants to eliminate toxic pollutants. Nanoparticles functionalized with specific enzymes will produce the greatest productivity. The procedure is essentially very straightforward. Plants cannot degrade large molecules such as oligosaccharides and large chain hydrocarbons. Plants degrade these complex molecules into simple molecules, which are then degraded by nanoparticles. With this method, bioremediation will be more effective if it is combined with nanoparticles. *Shewanella oneidensis* introduced into a contaminated site with Pd(0) deposition on the cell wall is another good example of nano bioremediation. As a result of their smaller size particles and semipermeable nature of the plasma membrane, these particles also enter the cytoplasm. As well as showing the presence of electron donors such as hydrogen acetate, it will also charge and show the presence of chlorine in water bodies. In addition to acting as indicators, they degrade chlorine (Singh et al. 2020).

1.3 Outline of the Nano Biosynthesis Mechanism Used in Bioremediation

Heavy metals can be removed from a variety of sources using nanoparticles. Nanoparticles are first synthesized using different synthesis methods such as physical, chemical, and biological, and then they are used in bioremediation. Alternatively, nanoparticles can be engineered to combine with microbes, and then these engineered nanoparticles can be used in bioremediation (Koul and Taak 2018a).

There are variety of methods and techniques that can be employed to remove toxic heavy metals from the environment. Bioremediation using nanoparticles/nanomaterials created by plants, fungi, and bacteria with nanotechnology is called nanobioremediation (NBR). There are differences between normal and nano-bioremediation. In remediation, biological agents are typically used, but when combined with nanoparticles, their efficacy is increased. NBR is the most suitable and environmentally friendly method for removing pollutants. As of now, remediation techniques are either *in-situ* or *ex-situ.* Plants, microbes, and enzymes present in organisms that use these techniques for remediation degrade heavy metals. Heavy metals are naturally absorbed by some plants through their roots; this process is known as phytoremediation (Rizwan et al. 2014). When nanoparticles made from plants, microbes, and enzymes were applied, the plant's phytoremediation efficiency was enhanced. Phytoremediation uses enzyme-functionalized nanoparticles in order to degrade long-chain hydrocarbons. Plants and microbes cannot degrade these long-chain hydrocarbons (Shah 2020a, b).

In addition to wastewater treatment, nanoparticles are also used to treat contaminated soil and air pollution (Ibrahim et al. 2016) (Fig. 1).

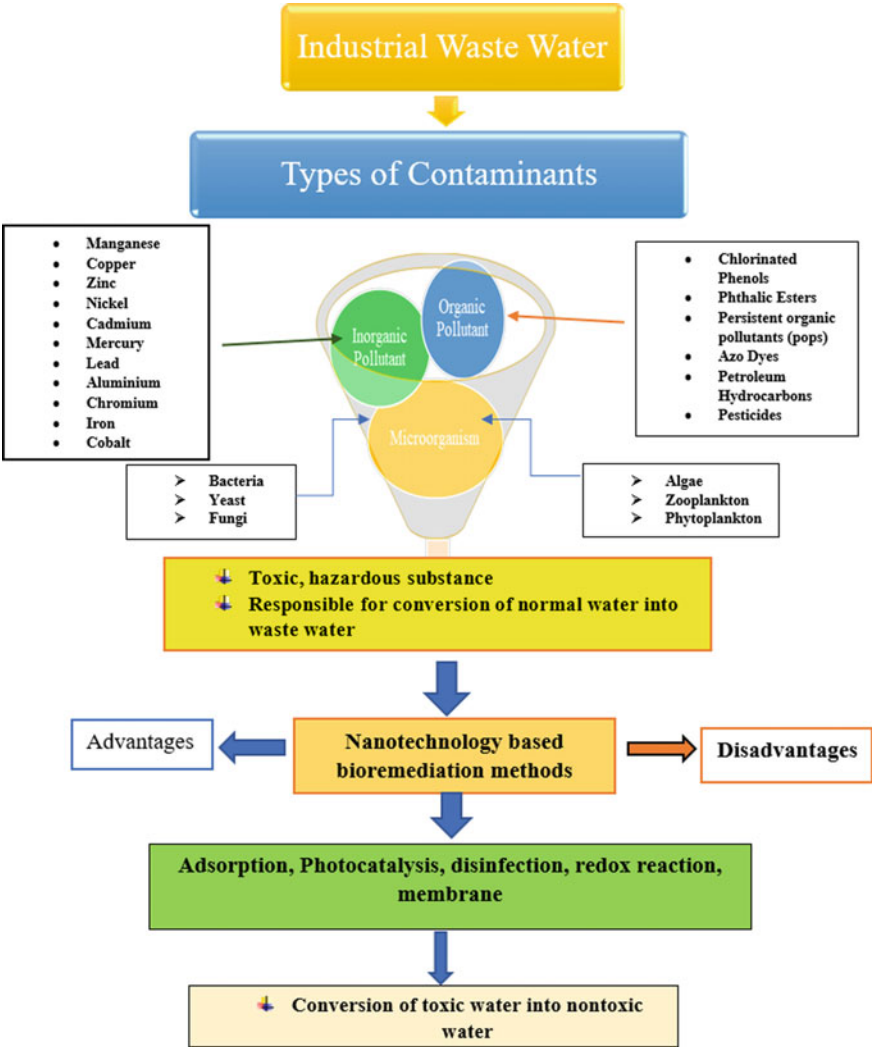

Fig. 1 Outline of bioremediation process for industrial waste water

2 The Method Used in Bioremediation of Wastewater

Several methods are available for wastewater treatment, including adsorption, photocatalytic degradation, redox reaction, disinfection and membrane-based techniques. Due to the unique properties of nanoparticles, such as strong absorption, high reactivity, and quantum size effect, these techniques are widely used (Yadav et al. 2017) (Fig. 2).

2.1 Adsorption

This is a surface-based method for removing pollutants. An adsorption process occurs when adsorbable solutes interact with solid adsorbents. Nanoparticles serve as solid adsorbents, whereas heavy metals serve as adsorbable solutes. Due to the interaction force between the adsorbent and adsorbable solute, adsorbable solute deposits on adsorbable surfaces. Adsorption stands out for wastewater treatment because of its versatility, unique properties, superior efficacy, large surface area, multiple adsorption sites, temperature-dependent modifications, specific pore sizes, unique surface chemistry, ease of handling, low cost, and the fact that it does not require pretreatment. Shape and morphology also play an important role in nanobioremediation. Because of their high absorption capacity, modified nanosorbents can sometimes become toxic (Ali and Gupta 2007).

In the removal of heavy metals, adsorbents play an important role. Nano adsorbents like activated carbon, clay minerals, and natural zeolites can also remove heavy metals, but their adsorption capacities are low, resulting in poor results. Nanoparticles with sizes of 1-100 nm are often used in bioremediation instead of traditional sorbents. Chemical, physical, and biological methods can be used to synthesize nanoadsorbents. Biosynthesis materials are widely used because they do not require toxic chemicals or high energy. Chemistry and physical synthesis nanoparticles have a well-defined shape and size, while biological synthesis nanoparticles require special

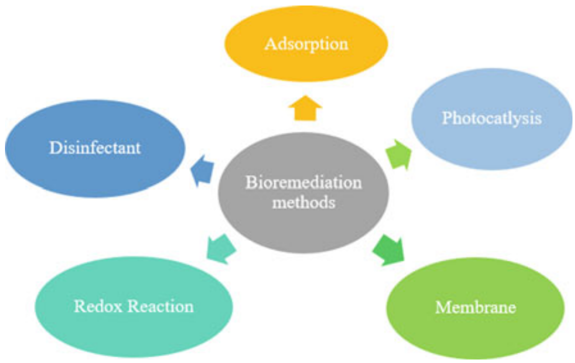

Fig. 2 Method used in Bioremediation

attention to their shape and size. In the biological synthesis of nanoadsorbents, plants such as algae, bryophytes, angiosperms, microbes, and enzymes secreted by living organisms are used as reducing agents. Functionalized nanoparticles can be synthesized intracellularly or extracellularly by secreting enzymes from the organism where they function to reduce metal ions and stabilize them (Mourdikoudis et al. 2018).

Nanoadsorbents such as nanotubes, nanowires, and quantum dots are used to treat wastewater. Nanoadsorption is currently performed with nanoparticles of zero-valent metals, metal oxides, and nanocomposites. The most commonly used nanoparticles in wastewater treatment are titanium oxides, cerium oxides, manganese oxides, nanosized zero-valent metals, ferric oxides, aluminium oxides, and magnesium oxides. Phosphate, an organic pollutant, can be selectively absorbed by cerium oxide. Metallic nanoparticles are known for their simplicity, super magnetic properties, and high adsorption capacities, which make them ideal for remediation. Besides adsorption, chemical and photodegradation methods are also used for wastewater remediation. However, metallic nanoparticles also have some disadvantages, such as their small size, which makes them less stable, causing agglomeration, and reducing their properties. Further, separation from water bodies is difficult, and higher deposition causes toxic effects on water ecosystems (Aguilar-Pérez et al. 2021).

The pharmaceutical industry uses a variety of drugs that are released into the environment. Pharmaceutical drugs can disrupt the body's homeostasis as well as the endocrine system. In addition, these products produce a large amount of free radicals, which are toxic. If these medications are used excessively, they can cause genotoxicity and immunosuppression. For instance, Ibuprofen, paracetamol, and diclofenac do not require a prescription. Paraben, which is found in many skincare products, is another pharmaceutical drug that causes environmental problems. Medically, diclofenac is used to disinfect wounds, but it produces antibiotic-resistant bacteria that cannot be killed by any antibiotic. The drug is also responsible for the emergence of drug-resistant bacteria (Sherry Davis et al. 2017).

The first report on carbon nanotubes was published by Iijima in (1991). As cylinder-shaped macromolecules, CNTs have a radius as small as a few nanometres and varying lengths. CNTS are hexagonal lattices of fullerenes with grooves. These highly hydrophobic nanoparticles are used in wastewater treatment because of their ability to entrap organic molecules due to the hydrophobic aggregation between them (Roy 2014a).

In a recent study, green-synthesised Cu nanoparticles were effective at removing three selected pharmaceutical drugs from aquatic media (Husein et al. 2019). Nanoparticles of different types are used in nanobioremediation. Metal nanoparticles of Ag, Au have good degradation properties for organic dyes (Sherry Davis et al. 2017). Bimetallic Fe/Ni nanoparticles of size 60–85 NM produced by green synthesis can remove 85.8% of triclosan from wastewater. By using biologically produced platinum and palladium nanoparticles from *D. vulgaris*, other pharmaceutical drugs, such as sulfamethoxazole, have been successfully removed. It is capable of removing 85% of the contaminants (Sherry Davis et al. 2017).

Nanoparticles can be produced using plants such as *Noaea mucronata, Euphorbia maroclada,* which can remove Pb, Cd, Zn, Cd, and Ni. Nanoparticles can also be produced by bacteria. For the removal of toxic metals, iron nanoparticles are effective.

Maghemite is a type of iron nanoparticle produced by *Actinobacter.* A study found that bacteria produce iron nanoparticles aerobically and participate in bioremediation. Additionally, thermophilic nanoparticles can be used to create magnetic nanoparticles that degrade heavy metals. nZVI nanoparticles produced from black tea, grape marcs, and vine leaves remove ibuprofen from an aqueous medium (Koul and Taak 2018a).

The orange peel powder has also been used to synthesize Fe_3O_4 nanoparticles with good adsorption capacity of cadmium from an aqueous solution. According to a comparative study of orange peel powder, chemically produced magnetic nanoparticles and orange peel-mediated magnetic nanoparticles, they have surface areas of 47.03, 76.32 and 65.19 m^2g^{-1} BET respectively (Ali 2017).

Nano clay is also used in the absorption process. In addition to removing contaminated water, these nano clays are used in soil bioremediation. Nano clay is used for the successful reduction of phosphorus, according to a report by Allophane. According to this study, phosphorus concentration reduces from 14.2 to 4.2 mg/L, a reduction of nearly 70% from the original medium (Koul and Taak 2018a).

2.2 Photocatalysis

In this process, UV light is used to oxidize organic pollutants and convert them into water and carbon dioxide as by-products. Photocatalysis can also be used to disinfect certain bacteria. During photolysis, the photocatalyst area accelerates the reaction rate without consuming it. It is an advanced oxidation process (Ibrahim et al. 2016) where nanoparticles act as photocatalysts. By absorbing UV light, photocatalysts get charged and can convert hazardous, non-biodegradable contaminants into non-hazardous, biodegradable compounds. Using light to convert contaminants has advantages and disadvantages. Reaction rates may increase or decrease as a result of light. In case of low light intensity, reaction rate and photocatalytic activity are affected. As photocatalysts, different nanoparticles are used, such as TiO_2, Si, Cd, ZnS, SnO_2, WSe_2, Fe_2O_3, WO_3, TiO_3, ZnO, etc. (Roy 2014b) (Fig. 3).

A photocatalytic material is typically illuminated with light that balances the valence and conduction bands during the photolysis process. Exposure of light photons to irradiation is more powerful than their band gap energy. As a result of this process, electronic holes are created, which combine with organic pollutants and produce reactive oxygen species. In photocatalysis, the size of the nanoparticles plays a crucial role (Aguilar-Pérez et al. 2021). Water molecules capture the hole (hh) generated by electron–hole pairs in photocatalysis. By releasing hydroxyl radicals (OH-) from water molecules, a wide range of microbial cells and organic pollutants can be degraded. There has been a great deal of interest in the role of titanium nanoparticles and silver in environmental bioremediation, especially in wastewater treatment (Ojha 2020).

Fujishima and Honda discovered the photocatalytic thin-film mechanism while studying the electrochemical photolysis of water on TiO2 semiconductor electrodes.

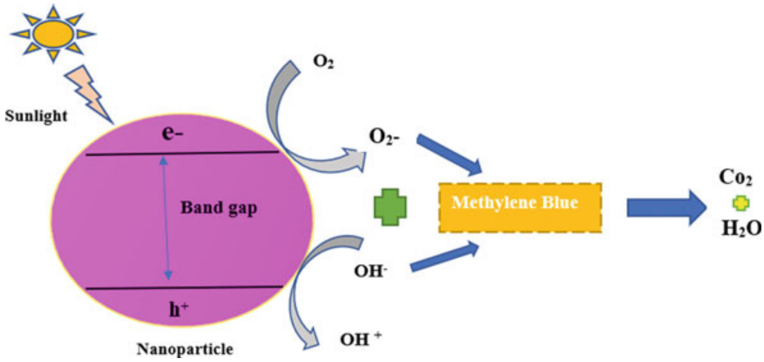

Fig. 3 Degradation of Methylene blue through photocatalysis

The titanium nano-oxide nanocomposite inactivates microorganisms by UV irradiation (Berekaa 2016). To enhance the photocatalytic activity of nanoparticles, titanium quantum dots (QDs), titanium nanotubes, titanium nanosheets, titanium nanowires, and titanium mesoporous hollow shells are used. Different morphological forms of Tio2 increase the number of photoreactive sites exposed. TiO2 nanoparticles with metal or non-metal doping should enhance charge separation or board the absorption spectrum. In addition to difficult recovery, agglomeration, and short activity, titanium oxide nanoparticles have several disadvantages (Ibrahim et al. 2016).

Copper nanoparticles are synthesized from Escherichia sp SINT7, which is copper resistant. The nanoparticles successfully degrade azo dye at a very low concentration of 25 mg g^{-1}. A blank Cango-T shows a reduction of 83.61%, while Direct Blue-1 reduces up to 88.42% (Mandeep and Shukla 2020). In addition to their function as a semiconductor, Zn NPs are also used as a photocatalyst to degrade organic dyes, phenols, and pharmaceuticals (Sherry Davis et al. 2017).

2.3 Redox Reaction

During wastewater treatment, an oxidation and reduction reaction takes place because of the redox reaction conversion of hazardous contaminants or toxic metals into non-hazardous contaminated or nontoxic metals that become more stable, less mobile, or inert. To enhance degradation and extraction capacity, redox reactions are used in remediation to change the water chemistry and microbiology by introducing specific substances into wastewater containing contaminants.

Water contaminated with organic or inorganic contaminants can be treated with this redox process technique. It is not only laboratory practice used for bioremediation, but all processes on earth use energy generated from redox reactions (Tandon and Singh 2016).

On earth, basic transformations such as the nitrogen cycle, carbon cycle, sulphur cycle, magnesium cycle, iron cycle, and nitrous cycle take place. Redox-sensitive elements such as chromium (Cr), arsenic (As), uranium (U) and copper (Cu) may be present in the environment (Tandon and Singh 2016). Redox-active CeO2 nanoparticles are used for the treatment of wastewater in a variety of industries. Redox nanoparticles react with Cr (VI) (aq) and Fe2+ species, leading to the change in surface morphology (Division 2019). As part of traditional methods, chlorine and ozone are used to disinfect; this product produces carcinogenic substances. By using nanoparticles, these problems are solved. Ferrate (VI) is used as a disinfectant for algae, bacteria, microcystins, viruses. Many natural and advanced nanomaterials exhibit disinfectant properties. Nanoparticles such as silver nanoparticles, chitosan, photocatalytic TiO2, aqueous fullerene nanoparticles and carbon nanotubes, fullerol interact with the cell membrane and cause the membrane to break down and release reactive oxygen species. Nickel, selenium, chromium, cobalt, arsenic, lead, and copper are easily controlled by redox reactions in order to determine their toxicity and mobility (Tandon and Singh 2016).

2.4 Disinfection

Water emitted from various sources contains many pathogens that may cause serious health problems in humans. Bacteria (*Escherichia coli, Pseudomonas aeruginosa, Burkholderia pseudomallei, Salmonella typhi, Plesiomonas, Yersinia enterocolitica, Vibrio cholera, Campylobacter spp., Shigella spp.*, etc.),cyanobacteria (*Anabaena, Nostoc*, Microcystis, Planktothrix), viruses (astroviruses, hepatitis A viruses, hepatitis E viruses, noroviruses enteroviruses, sapoviruses, rotavirus, adenoviruses), prions, protozoa (*Cyclospora cayetanensis, Isospora belli, Entamoeba histolytica, Giardia intestinalis, Cryptosporidium, Balantidium coli, Naegleria fowleri, Toxoplasma Gondii, Acanthamoeba spp.*), helminths (*Schistosoma spp., Dracunculus medinensis*), cysts, fungi, Rickettsia, etc., are various kind of microbes that pose a serious threat to people and ecosystems alike (Ojha 2020). Water treatment must be done properly. It is one of the simple processes in which microorganisms are killed by suitable compounds and water becomes free of microorganisms. The use of disinfectants is not limited to eliminating microbial contamination; they also help eliminate other parameters like colour, taste, oxidize iron and manganese in water bodies, and enhance filtration as well as coagulation activity (Berekaa 2016).

These disinfection practices are mostly used in the pharmaceutical industry, medical field, research labs, and diagnosis centers. In traditional disinfection methods, chlorine, ozone is used to kill compound toxics and cancerous byproducts, like halo acetonitrile, dibutyl phthalate, halo acetic acid, chlorite, and chloral hydrate. According to the report, there are about 600 byproducts of disinfection that are very harmful to living things. Nanoparticles are a safer alternative. The silver nanoparticles, zinc oxide, iron oxide, manganese oxide nanoparticles (Mn2O3, MnO2, MnO and Mn3O4) NPs, titanium oxide nanoparticles, cerium oxide nanoparticles, magnesium oxide nanoparticles, silica-based nanocomposites, and carbon-based nanomaterials play an important role as disinfectants either by generating reactive oxygen species or by disrupting cell membranes. Several metals play an important role in the disinfection of E. *coli* bacteria. A superior ability to kill bacteria has been demonstrated by silver nanoparticles (Berekaathe 2016; Ojha 2020).

A Double-layered superparamagnetic Fe3O4@SiO2@Ag@porousSiO2 nanoparticle shows good disinfectant properties against E. *coli, as well as good recycling properties.* It is a two-layer system, with Fe 3 O 4 @SiO 2 nanoparticles on the inner layers and Ag@porous SiO2 nanoparticles on the outer layers. It is believed that the inner layer of Fe 3 O 4 @SiO 2 was protected by a dense silica matrix and allowed Ag to be loaded on the outer surface. The outer mesoporous silica helps to dissolve and oxidize Ag, giving Ag+ ions. Combinational double-layered nanoparticles can meet the MCL for drinking water after treatment (Wang et al. 2019).

The use of titanium nanoparticles led to fast killing of bacteria E coli in the presence of solar irradiation followed by a first order kinetic mechanism. Iron oxide nanoparticles also show great properties, and they are easy to synthesize, cost-effective, and recyclable, which shows great disinfectant properties (Ojha 2020). Titanium nano-oxide and thin-film nanocomposite demonstrated the ability to inactivate microorganisms (Berekaa 2016).

According to a review article, iron granules used on a commercial scale successfully inactivated viruses and nanoscale zero-valent iron (nZVI) particles completely destroyed bacteria like Pseudomonas fluroscens and *B. subtilis.* Hybrid copper and ZVI nanoparticle clusters were used in a case study in Tirupur, Tamil Nadu, India to treat industrial effluents (textile, tannery, pharmaceutical) and sewage. The hybrid cluster uses more copper, ZVI, and less silver to reduce treatment costs. These clusters remove excess salts, fluorides, heavy metals, excess salt, dyes from the effluent (Kiruba Daniel et al. 2014). Nanoparticles may act as an antimicrobial agent for wastewater treatment, along with a combination of UV disinfection to improve water quality (Berekaa 2016).

2.5 Membrane

Using Membrane in wastewater treatment is one of the most effective strategies for removing water pollutants since it has a great separation capacity and does not require the addition of chemicals during operation. The efficiency of membranes is determined by the permeability of the membrane material. Membranes are generally made of cellulose acetate, polyamides, or polyacrylonitrile. In accordance with the size of membranes, there are different categories of membranes. Bacteria, suspended solids, and protozoa are removed by micro membranes. Viruses can be removed with Ultrafiltration, whereas heavy metals and organic matter can be removed with Nanofiltration (Abdelbasir and Shalan 2019).

Fouling of the membrane is a disadvantage of membrane filtration. There are two types of fouling: organic fouling and biological fouling. Organic fouling is a deposition of organic molecules inside the membrane pore, whereas biological fouling occurs when microorganisms present in the water attach to membranes and reduce flux capacity. The limitations of the membrane can be overcome by modifying the membrane with hydrophobicity parameters which are capable of enhancing organic antifouling (Ibrahim et al. 2016).

The use of nanofiltration membranes is a good practice for removing pathogens such as Cryptosporidium oocysts (Ojha 2020). Nanofiltration membranes (NF) not only remove toxic metals, but are also important for recovering nutrients from industrial wastewater. NF with 90 mm pore size is commonly used in paper and pulp industry and has the advantage of rejecting more than 70% of phosphorus. However, the disadvantage of these NF is high phosphorus, which causes fouling of the membrane. Gold nanoparticles embedded with a polymer blend of NF membrane exhibit better recovery, rejection of trivalent phosphate at 96.1%, and increased fouling resistance. Graphene oxide (GO) or attapulgite (ATP) supported on ceramic shows nearly 100% rejection of heavy metals like nickel, lead, cadmium, and copper (Mandeep and Shukla 2020).

As nanotechnology advances, new membranes are developed for water treatment. Grafting, bending, and other methods are used to modify membranes. The modifications are necessary to make the membrane highly permeable, increase catalytic properties, and degrade contaminants (Ibrahim et al. 2016).

The membranes used in bioremediation processes are produced by the electrospinning process, the purpose of using the electrospinning process is to generate polymer or composite nanofibrous membranes from different materials between 20–200 m diameter. Electrospinning provides a fine porous size with a large specific surface area and gives the membranes high water flux. A study revealed that nanomembranes can effectively remove a wide range of heavy metals and after this process, the membrane can be recovered again and reused after cleaning it (Abdelbasir and Shalan 2019).

The affinity of the membrane can be increased by adding a functional group to it. Through covalent interaction, ligands are attached to the membrane for functionalization. Cibacron blue is attached to the cellulose nanofiber membrane for functionalization during aluminium purification. Attaching ceramic nanoparticles such as alumina hydroxide or iron nanoparticles to polymer nanofiber membranes removes heavy metals from the nanofibers. As a ligand molecule, cyclodextrin can also be added to polymethyl methacrylate nanofiber membranes to improve the removal of organic waste (Ibrahim et al. 2016). Nanoparticles can be used individually or in combination with appropriate matrices to remove pollutants from industrial waste water. In a review on Microbial Nanotechnology for Bioremediation of Industrial Wastewater, it was found that porous magnesium oxide can absorb 1000 mg. g^{-1} toxic dye from the iron industry. The grafted iron oxide with hyperbranched polyglycerol had the ability to remove copper, nickel, and aluminium from wastewater in just 35 s (Mandeep and Shukla 2020).

Nanoparticles are supported on suitable supporting materials during the membrane process. For example, cellulose acetate, alginate, and polyvinyl alcohol embedded with silver nanoparticles. Different fabrication processes have been developed based on the separation process. Silver-coated Mn Zn ferrite, silver-containing thermoplastic hydrogels, silver-ion-exchanged titanium phosphate films, and silver nanoparticles within third-generation dendritic poly (amidoamine) (PAMAM) grafted onto multiwalled carbon nanotubes are used in membrane filtration. Many transport mechanisms in bacteria are inhibited by silver ions, which bind to bacterial DNA (Mandeep and Shukla 2020).

Nano-zeolites in Thin Film Nanocomposite Membranes (TFN) increase membrane permeability. 80% of the salt is rejected by these TFNs. Nano-TiO2 in TFCs increases rejection rates, reduces biofouling, reduces biological contaminants, and inactivates microorganisms using UV light (Kiruba Daniel et al. 2014).

3 Nanoparticles Used in Bioremediation

A significant increase has occurred in the biological synthesis of nanoparticles to create new materials that are cost-effective and stable with important applications in electronics, medicine and agriculture. It is possible to synthesize nanoparticles using an array of conventional methods, but biological synthesis is superior because of its ease of rapid synthesis, control, ease of processing, control of size characteristics, low cost, and eco-friendly approach. Biological contaminants (such as bacteria) and chemical contaminants including organic pollutants, are extensively removed with the help of nanoparticles. During the last decade, nanoparticles and nanomaterials have attracted considerable attention because of their unique size-dependent physical and chemical properties (Okhovat et al.2015).

Biological systems can synthesize molecules with highly selective properties and are self-organizing. Nanoparticles produced traditionally by physical and chemical methods are costly. As a way to synthesize nanoparticles at a lower cost, microorganisms and plant extracts were used. Using a bottom-up approach, nanoparticles are biosynthesized by reduction and oxidation reactions. It is usually microbial enzymes or plant phytochemicals with antioxidant or reducing properties that are responsible for reducing metal ions into nanoparticles (Sastry et al. 2003).

3.1 Nanoparticle Synthesized by Plants

The green synthesis of nanoparticles by plants has become very popular in recent years due to a single-step biosynthesis process, lack of toxicants, and presence of natural capping agents. It is advantageous to use plants for the synthesis of nanoparticles since they are readily available, safe to handle, and have a broad range of metabolites. Water-soluble phytochemicals reduce metal ions in a much shorter period than fungi and bacteria (Yadav et al. 2017).

The synthesis of nanoparticles by plants is therefore superior to that of bacteria and fungi. Compiled information indicates that the effects of nanoparticles vary from plant to plant and depend on their mode of application, size, and concentration (Yadav et al. 2017).

Silver Nanoparticles

To remove toxicity from wastewater, silver nanoparticles can be used in simulated wastewater treatment. The plants that have been reported for the synthesis of silver nanoparticle are *Elettaria cardamomum, Parthenium hysterophorus Ocimum sp, Euphorbia hirta, Nerium indicum Azadirachta indica, Brassica juncea, Pongamia pinnata, Clerodendrum inerme, Gliricidia sepium, Desmodium triflorum, Opuntia,ficus indica, Coriandrum sativum, Carica papaya,* (*fruit*) *Pelargonium graveolens, Aloe vera extract, Capsicum annuum, Avicennia marina, Rhizophora mucronata, Ceriops tagal, Rumex hymenosepalu,s Pterocarpus santalinus, Sonchus asper.* Most commonly, silver nanoparticles are used in disinfection techniques due to their toxic effect on microorganisms at certain levels.

Gold Nanoparticles

Heavy metals, fertilizers, detergents, and pesticides seriously reduce the availability of pure drinking water and potable water. Research on several fronts is advancing the idea of nano based solution using gold for cost-effective solution in water treatment, which has intriguing potential to deal with the water pollution problem. The plants responsible for synthesizing the gold nanoparticles are *Terminalia catappa, Banana peel, Mucuna pruriens, Cinnamomum zeylanicum, Medicago sativa, Magnolia kobus, Dyopiros kaki, Allium cepa L., Azadirachta indica A. Juss., Camellia sinensis L., Chenopodium album L., Justicia gendarussa L., Macrotyloma uniflorum, Mentha piperita L. Mirabilis jalapa L., Syzygium aromaticum* (*L*)*, Terminalia catappa L., Amaranthus spinosus* (Li et al. 2011).

The degradation of methylene blue is achieved by using more than 20 plant-mediated gold nanoparticles. Oil of leaves of *Myristica fragrans, Fagonia indica, Persea americana* seed extract*, Nigella arvensis leaf* extract*, Lagerstroemia speciosa, Sterculia acuminate, Mimosa tenuifolia, Salmalia malabarica, Sesbania grandiflora L.,* Edible coconut oil, *Parkia roxburghii,* leaf extract, *Costus Pictus* leaf extract, *Actinidia deliciosa* fruit*, Costus speciosus* rhizome, *Citrifolia* fruit extract*,* *Hydrocotyle asiatica* leaf extract can produce gold nanoparticles and degrade methylene blue under different conditions (Dabhane et al. 2021). Although Gold nanoparticles have been used to degrade dyes in several reports, this process is not commercially viable because the gold salt is expensive and the nanoparticle cannot be recovered after degradation. A commercially viable process could be created by using nanoparticles supported by matrices.

The catalytic activities of gold and silver nanoparticles were determined by catalytic degradation of pollutants with excess amounts of NaBH4 at room temperature. A photocatalytic mechanism can be used to bioremediate industrial water using such nanoparticles (Vo et al. 2019).

CuO Nanoparticle

Plant-mediated copper oxide nanoparticles are the easiest, simple and most efficient way for the production of nanoparticles. *Madhuca longifolia* extract mediated CuO nanoparticles exhibit great characterization aspects like great crystalline nature on XRD and on change in different sizes it shows an increase in photoluminescence property. This nanoparticle shows photocatalytic degradation of methylene blue (Das et al. 2018).

3.2 Nanoparticles Produced by Bacteria

Bacteria produce enzymes that catalyze specific reactions which are responsible for the synthesis of nanoparticles. This provides a new rational biosynthetic strategy that involves the use of enzymes, microbial enzymes, vitamins, polysaccharides and biodegradable polymers. The extracellular secretion of enzymes has the advantage of producing highly pure nanoparticles of size 100–200 nm that are free of other cellular proteins. The properties of nanoparticles are determined by optimizing the parameters controlling organism growth, cellular activities, and enzymatic processes. A large-scale synthesis of nanoparticles using bacteria is attractive because it does not require hazardous, toxic, and expensive chemical materials (Iravani 2014). Bacteria are considered potential sources of nanoparticle synthesis, like gold, silver, platinum, palladium, titanium, titanium dioxide, magnetite, cadmium sulphide, and so on.

Silver Nanoparticles

Silver nanoparticles (Ag NPs) are highly toxic to microorganisms and thus have strong antibacterial effects against a wide range of microorganisms, including viruses, bacteria, and fungi. Silver nanoparticles attached to filter materials have been considered promising for water disinfection due to their high antibacterial activity and cost-effectiveness. The bacteria responsible for the synthesis of silver nanoparticles are

Bacillus cereus, Oscillatory willie, Escherichia coli, Pseudomonas stuzeri, Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Lactobacillus strains, Corynebacterium, Staphylococcus aureus, Ureibacillus thermosphaericus (Li et al. 2011). *Pseudomonas aeruginosa* JP-11 was extracted from marine water produced 40 nm, spherical shape cadmium sulphide nanoparticle removes cadmium pollutant from aqueous solution. Other bacteria like *Klebsiella pneumoniae, Escherichia coli, Pseudomonas jessinii isolated from* tiger nuts, carrot juice and faces are responsible for silver nanoparticles. *Shewanella loihica* PV-4, produced spherical shape silver nanoparticle responsible for degradation of methyl orange dye (Gahlawat and Choudhury 2019).

3.3 Nanoparticles Produced by Yeast and Fungi

The fungi are an excellent source of extracellular enzymes that influence nanoparticle synthesis. Many of them have been used for the biosynthesis of nanoparticles. As compared to bacteria, fungi can produce larger amounts of nanoparticles (Munisamy).

It is equally important to understand the nature of the biogenic nanoparticles. As a result of microbiological methods, nanoparticles are formed more slowly than those derived from plant extracts. Enzymes produced by fungi are responsible for synthesis of nanoparticles. Due to the wide diversity, easy culture methods, reduced time, and cost-effectiveness of fungi, they offer an advantage over other methods. Thus, nanoparticles can be synthesized in an eco-friendly manner (Ali et al. 2011).

CuO Nanoparticles

CuO nanoparticles can be produced using fungus-like *Penicillium chrysogenum.* In this study, copper sulfate with fungal extract was exposed to gamma dose radiation. The nanoparticles form were found to be effective against six different kinds of fungi *Ralstonia solanacearum, Fusarium oxysporum, Ralstonia solanacearum, Aspergillus niger, Penicillium citrinum, Erysiphe cichoracearum, Erwinia amylovora, and Alternaria solani.*

Maximum antifungal activity of penicillium-mediated copper oxide nanoparticles was observed in *Fusarium oxysporum* and it was least effective in *Erwinia amylovora.* This nanoparticle is best for disinfection of agricultural packaging tools because these six fungi infects agricultural crops and when it comes to the packaging industry there might be a chance of contamination of water. It is necessary to take action against these fungi for future contamination (El-Batal et al. 2020).

Gold nanoparticles synthesized using fungus *Streptomyces griseoruber* extracted from soil samples of Mercara region are responsible for degradation of methylene blue (Gahlawat and Choudhury 2019).

4 Target Specific Removal of Pollutants Using Nano Bioremediation

Studies are being conducted on removing contaminants from industrial wastewater, which contains various types of contamination. A few examples of nanoparticles that have been used for metal or nonmetal removal from industrial wastewater are shown in Table 1. Industrial wastewater contains major metals and nonmetals such as Cr (VI), Pb (II), Mercury, Cu II, Cd (II) / Pb (II), Ar (III), Nickel, Anionic chromat (Ibrahim et al. 2016) (Puthukkara et al. 2021a).

4.1 Metal or Non-Metal

Chromium VI

1 g/L of guar gum zinc oxide nanoparticles can remove almost 98.63% Cr (VI) within 50 min at pH 7. Green tea leaf extract, *Syzygium jambos* (*l.*)*, Eucalyptus globulus* leaf*, Citrus limetta* fruit peel mediated iron nanoparticles also remove chromium VI from industrial wastewater (Ibrahim et al. 2016).

Pb (II)

A graphene oxide/zinc oxide nanocomposite (GO-ZnO), iron phosphate-mediated magnetite nanoparticle (Fe3O4), and a Syzygium aromaticum flower extract-mediated zerovalent iron nanoparticle (ZIF) have been shown to remove Pb II. The adsorption capacities of CeO2, Fe3O4, and TiO2 are 189 mg Pb/g, 83 mg Pb/g, and 159 mg Pb/g, respectively. The TiO2, Fe3O4 and CeO2 nanoparticles do not show any toxicity, but CeO2 exhibits high phytotoxicity.

Mercury (Hg2+)

Mercury ions are removed by iron sulfide (FeS) nanoparticles synthesised using carboxymethylcellulose (CMC) as a stabilizing agent.

Cadmium (II) / Lead (II)

Cd (II) and Pb (II) were removed from wastewater using NiO nanoparticles. Both heavy metals can be absorbed by the NiO nanoparticles. NiO nanoparticles had maximum adsorption capacities for Pb (II) and Cd (II) ions of 625 mg/g and 909 mg/g, respectively. Contaminants are removed spontaneously and endothermically during this process.

Arsenic (III)

Fe3O4 nanoparticles with ascorbic acid show 46.06 mg/g absorption of arsenic (III) and arsenic (V) from contaminated sites. The advantage of these nanoparticles is that they prevent metals from leaching from water.

Copper (II)

The copper (II) adsorption capacities of ZnO and MgO are 593 and 226 mg/g, respectively, at 3 to 4 pH. Iron phosphate nanoparticles coated with sodium carboxymethyl cellulose can absorb copper (II) from soil and water (Table 1).

4.2 Organic or Inorganic Molecules

Various types of nanoparticles are used for removing organic and inorganic pollutants. They are mostly carbon-based nanoparticles that are used to remove toxic compounds from the environment. Dye, pesticides, benzene/phenol derivatives and pharmaceuticals are among the organic and inorganic pollutants released into industrial water. Table 2 provides data regarding different types of organic/inorganic pollutants, specific contaminants released by different sectors and nanoparticle-based solutions for removing contaminants.

Organic Dyes

Different dyes are eluted in the textile industry. Methylene blue, methylene orange, bromophenol blue, malachite green, Reactive Blue 4 (RB4), Alizarin red S (ARS) and Morin are some of the most common dyes. Due to electrostatic interaction, methylene blue can interact with magnetite carbon nanotubes and exhibit high absorption capacity. Carbon nanotubes (CNT) prepared by acid washing and oxidation of nanoparticles in purified air are used to degrade methyl orange. A high CNT dosage is responsible for inhibiting the surface methyl orange dye molecules. Dye degradation is affected by temperature and pH. Dye-like reactive blue degrades at high temperatures, due to the high temperature, particle diffusion between dye molecules increases, leading to the degradation of the dye. The adsorption capacity of Alizarin red S (ARS) and Morin is increased with low pH.

Additionally, plant extract synthesized nanoparticles are capable of degrading dye molecules. Mango peel, *Cupressus sempervirens, Camellia sinensis,* Green and black

Table 1 Types of contaminant and nanoparticles used in removal of contaminantes

Types of Contaminants	Target specific contaminant	Nanoparticles used in bioremediation	References
Metals /Non-metal	Heavy metals	Thiol-functionalized superparamagnetic nanoparticles, ZnO, FeO3	Ibrahim et al. (2016)
	Cr (VI)	Guar gum–nano zinc oxide (GG/nZnO)	
		Green tea leaf mediated zerovalent iron nanoparticle	
		Syzygium jambos (*l.*) Alston leaf extract-based ZVI nanoparticle	Puthukkara et al. (2021a)
		Eucalyptus globulus leaf	
		Citrus limeta fruit peel	
	Pb (II)	Nanocomposite GO–ZnO(OH)2, Magnetite (Fe3O4)	Ibrahim et al. (2016)
		Iron phosphate mediated nanoparticle	
		Nano ZnO nanoparticle	
		CeO2, TiO2 nanoparticles	
		Syzygium aromaticum flower mediated zero-valent iron nanoparticles	Puthukkara et al. (2021a)
	Mercury	Iron sulphide (FeS) nanoparticle	Ibrahim et al. (2016)
	Copper II	Iron phosphate nanoparticle	
		Magnesium and zinc oxide (MgO and ZnO)	
		carbon nanotube modified with silver nanoparticle	
	Cd (II) / Pb (II)	Nanocrystalline hydroxyapatite (nHA)	
		NiO nanoparticles	
	Ar (III)	Fe3O4 nanoparticles Coated with ascorbic acid	
	Nickel	Carbon nanotube modified with sodium hypochlorite	
	Anionic chromate	Oxidation on carbon nanotube	

Table 2 Nanoparticle used in removal of organic/inorganic pollutant

Organic /inorganic Contaminants	Target specific contaminant	Nanoparticles used in bioremediation	References
Organic dyes	Methylene blue (MB)	Carbon nanotubes loaded with magnetite	Ibrahim et al. (2016)
		Psidium guajava leaf extract-based ZVI nanoparticle	Puthukkara et al. (2021a)
	Methyl orange (MO)	Carbon nanotube	Ibrahim et al. (2016)
		Mango Peel mediated ZVI nanoparticle	Puthukkara et al. (2021a)
		Cupressus sempervirens leaf mediated ZVI nanoparticle	
	Bromophenol blue	*Camellia sinensis* leaf extract mediated zero-valent valent iron nanoparticle synthesis	
	Malachite green	Green, oolong and black tea leaf extract mediated ZVI nanoparticles	Ibrahim et al. (2016)
	Reactive blue 4 (RB4)	Carbon nanotube	
	Alizarin red S (ARS) and Morin		
Pesticides	Dubinin	Carbon nanotube	
	Diuron and dichlobenil		
	Atrazine		
Benzene Derivatives Phenolic Compounds	2,4,6-Trichlorophenol (TCP)	Carbon nanotubes with treatment of HNO3	
	Benzene, toluene, ethylbenzene, and p-xylene	Carbon nanotube with treatment of sodium hypochlorite (NaOCl)	
	1,2-Dichlorobenzene (1,2 DCP)	Graphitized carbon nanotube	
	Chlorophenol	Graphitized carbon incorporated by Ni/Fe Nanoparticles	Zhuang et al. (2021)
Pharmaceuticals	Ciprofloxacin (CPI)	Graphitized (MG), hydroxylated (MH) and carboxylized (MC)	Ibrahim et al. (2016)
	Ibuprofen (IBU)	HNO_3 on carbon nanotube (OMWCNTs)	

(continued)

Table 2 (continued)

Organic /inorganic Contaminants	Target specific contaminant	Nanoparticles used in bioremediation	References
	Tetracycline (TC)	sodium hypochlorite on carbon nanotube	
	Epirubicin (EPI)	Modification of carbon nanotube with HNO3	
Natural organic maters (NOMs)	Humic acid (HA)	Carbon nanotube with modification of HNO3 and H2SO4	
	Fulvic acid (FA)		

tea extracts assisted synthesis of zero-valent iron nanoparticles are responsible for degradation of methylene blue, methylene orange, bromophenol blue and malachite green dyes (Ibrahim et al. 2016; Puthukkara et al. 2021b).

Pesticides

Carbon nanotubes are responsible for degradation of diuron, dichlobenil, atrazine and dubinin. Diuron degradation is influenced by hydrogen bonding. Addition of an oxygen-containing group increases the adsorption of pesticides on carbon nanotubes (Ibrahim et al. 2016).

Benzene Derivatives, Phenolic Compounds

Benzene-containing compounds like 1,2-Dichlorobenzene (1,2 DCP) are degraded at high pH with the help of graphitized carbon nanotubes. Dye degradation requires a low pH, but compounds like benzene require a high pH for degradation. A decrease in adsorption capacity results from more water molecules being formed due to photocatalytic degradation. With the help of HNO3, carbon nanotubes can also be used for the degradation of 2,4,6-Trichlorophenol (TCP). Carbon nanotubes oxidized with sodium hypochlorite (NaOCl) generally degrade compounds such as p-xylene, ethylbenzene, toluene and benzene (Ibrahim et al. 2016).

Pharmaceuticals

The wastewater released by pharmaceutical industries contains several traces of drugs which adversely affect both humans and the environment. A number of drugs in pharmaceutical industry wastewater are epirubicin (EPI), ciprofloxacin (CFX), tetracycline (TC), ibuprofen (IBU). Carbon nanotubes are used to degrade pharmaceutical drugs (Ibrahim et al. 2016) (Table 2).

Table 3 Bioremediation of microbial contamination through nanoparticles

Microbial Contaminants	Target specific contaminant	Nanoparticles used in bioremediation	References
	E. coli	Silver nanoparticles, Ag Nanowire	Yadav and Kumar (2017)
		AgNPs/PU, Ag/CNT/PU sponges, AgNPs polypropylene filters, Polysulfone/AgNPs, AgNW/CNT	Ojha (2020)
		MgO nanoparticles	Ojha (2020)
	Bacillus subtillus	Magnesium nanoparticles	Yadav and Kumar (2017)
	P. aeruginosa,	Magnetic Fe3O4/SiO2,	Dimapilis et al. (2018), Al-Issai et al. (2019), Wang et al. (2019), Ojha (2020)
	Staphylococcus aureus	Silver nanoparticles, ZnO, CuO, Tio2	
	Salmonella typhi	Silver nanoparticle, ZnO, CuO, Tio2	

Natural Organic Matter

For absorption of natural organic matter like Fulvic acid (FA), Humic acid (HA) carbon nanotubes are used with a combination of H_2SO_4 and HNO_3.

4.3 Microorganism

Microorganisms play a great role in toxic metal reduction but sometimes microorganisms may lead to contamination of water. Nanoparticles from various syntheses can remove microorganisms' contamination. In most cases, silver nanoparticles are used to kill microorganisms. The fungi mediated silver nanoparticle is found to be effective against bacteria like *S. aureus* and *C. violaceum* (Durán et al. 2007), Table 3 shows Microbial contamination and nanoparticles for removal of it.

5 Recent Research Trends and Advances in Bioremediation

Various industries emit wastewater that contains Microplastics (MPs), which are plastic particles with a size of about 5 mm, and nano plastics, which are plastic particles with a size less than 100 nm. Plastics of this type are either formed by the degradation of larger plastic materials or directly from products in which they are used. In both cases, such plastics have serious health risks. Studies on drinking water treatment plants show more than 400 mg MPs per litre, which is dangerous to human health. Currently, no treatment is available for the complete removal of MPs. Due to its hydrophobic surface and ability to absorb pollutants, MPs may cause problems in other wastewater treatment systems. As a result, it is difficult to evaluate the effectiveness of particular wastewater treatment methods. MPs can be removed at a certain level using activated carbon (AC) infiltration (Jjagwe et al. 2021).

Recently, one interesting strategy for wastewater treatment has been the use of hydrogel-mediated composites for the removal of organic and inorganic pollutants. Hydrogel based solutions have a good adsorbent capacity as compared to conventional adsorbents.

The polymer networks in hydrogel have a large absorption capacity, and they have several better properties like durability, porosity, photostability, and odourlessness. Yi et al. demonstrated the five-cycle reusability of hydrogels formed from graphene oxide, polyvinyl alcohol, and alginate on the methyl orange dye in 20 min. Using two different kinds of polymer of alginate, i.e., alloy site nano-composite beads and calcium alginate show reusability after ten cycles of the cycle (Thakur et al. 2018).

A novel kind of titanate nanocomposite sheet immobilized from silk fibroin has been reported by Magri et al., approximately 75% of Pb2+ can be absorbed on this sheet. During the whole process, even after several rounds of treatment, it does not lose its capacity or show any degradation of the composite material. The selectivity of these immobilized titanate sheets on solid silk fibroin nanocomposite was enhanced by the addition of sodium ions (Magrì et al. 2018).

Today, researchers are making use of surface functionalized silica nanoparticles for water purification. Silanol group on silica increases surface activity of nanoparticles and protects them from leaching. Wang et al. describe the development of silica-based adsorbent models for heavy metals. Study by Di Natalea et al. showed that carbon nanoparticles supported by silica are capable of absorbing heavy metals from wastewater, such as Pb2+, Ni2+ and Cd2+. In certain conditions, such as when the temperature is around 100 0C and the pH is neutral, Ni2+ is removed faster than Cd2+. Other investigations of heavy metal removal by Sheeta et al. using different nanostructures such as silica/graphite oxide composites, graphite oxide, and silica nanoparticles. Composites of silica/graphite oxide in a ratio of 2:3 exhibit the highest efficiency of heavy metals among these three materials. In a recent study, Li et al. developed a new silica nano adsorbent whose surface has a functionalizing component designed specifically to eliminate heavy metals from wastewater. The novel kind of surface modification is made with five kinds of functional groups; these modifications are unique and were initially used for the removal of heavy metals. The silica modified with EDTA as a functionalizing group was able to absorb more Pb than the other four groups. As a method of removing trihalomethanes (THMs) from water by using the sintered process, Ulucan et al. reported that Fe2O3 nanoparticles sintered in zeolite showed a superior level of absorption (Janani et al. 2022).

For removal of Pb^{2+} from aqueous medium two kinds of nano-zero-valent iron were used. One type is iron produced by a reduction method and the other is commercially available iron. This comparative study shows zero valent iron showed more absorption of Pb2 + and in less than 15 min Pb^{2+} was removed from the aqueous system. Elliott et al. synthesized nano-zero-valent iron nanoparticles using sodium borohydride and ferrous sulphate and successfully removed Hexachlorocyclohexane from contaminated water. This nanoparticle formulation can remove almost 95% contaminated Hexachlorocyclohexane with help of a little amount of zero-valent iron nanoparticle (2.2 to 27 g.L^{-1}) within 2-day (Puthukkara et al. 2021a).

There are many applications of nanotechnology in bioremediation. It is known that enzyme-based nanoparticles can be used for bioremediation since enzymes act as biocatalysts. The main disadvantage of enzymes is that they are less stable than synthetic catalysts. Since enzymes have a short lifetime, they are not applicable commercially. The stability of enzymes can be increased in many ways. Enzymes play a significant role when attached to the nanoparticle (Rizwan and Ahmed 2018). The advantage of nanoparticles is that they extend the half-life of enzymes by providing extra stability, protecting the enzymes from biotic and mechanical degradation. Enzymes encapsulated within nanoparticles are more stable and can be reused several times, the nanoparticles provide support for the enzymes and protect them from protease attack and therefore biodegradation of enzymes is prevented, ensuring that enzymes last longer. The properties of nanoparticles were tested using complex nanofiber esterase enzymes over a 100-day period. Yadav and Kumar (2017) proved that enzymes continue to function after 100 days.

An enzyme can be separated easily from a product or reactant by combining with iron nanoparticles, which have great magnetic properties. An enzyme can also play an important role in bioremediation, but a combination of enzymes with nanoparticles can also be helpful. A study of this type was conducted by Qiang et al. These experiments used two different enzymes, peroxides and trypsin. As a result of combining these enzymes in core–shell nanoparticles, the activity efficiency, stability, and lifetime of the enzymes was enhanced from an hour to a week. It helps to protect enzymes from oxidation (Rizwan and Ahmed 2018).

It is necessary to recycle waste materials produced by industry into useful products in order to reduce pollution. Due to advances in technology, it is now possible to turn this waste into useful products thanks to excellent technology. In the industry, this concept is more easily applied to the production of biomolecules, adsorbents, biogas, clinker, and biohydrogen from waste. In 2019, Kumar described fermentative bacteria producing biohydrogen in a dark reaction in the presence of nanoparticles (Mandeep and Shukla 2020).

Several researchers are interested in converting industrial wastewater into biohydrogen. There is evidence that Elreedy et al. used mixed kinds of bacteria cultures to produce monometallic, bimetallic and multimetallic nanoparticles for biohydrogen production. The study found that biohydrogen is produced by nanoparticles with multiple combinations of metal ions. As a result, various types of nanoparticles, hydrogenase and dehydrogenase activities increased (Preethi et al. 2019). Gadhe et al., found that biohydrogen production increased in addition to nickel oxide and hematite nanoparticles. The production of biohydrogen is lower in monometallic nanoparticles than in combinations of different metals. Ferredoxin oxidoreductase and hydrogenase enzymes, a mixed metal nanoparticle exhibits 8.83 mmol/g COD (Mandeep and Shuklathe 2020).

In the environment, hydrophobic chemicals such as polycyclic aromatic hydrocarbons (PAHs) tend to persist for long periods of time and affect environmental conditions. Tungittiplakorn et al. develop nanoparticles with modified polyethylene glycol urethane acrylate to deal with this problem. Since polycyclic aromatic hydrocarbons are not soluble in water, they cannot be removed easily by the normal biosorption process; therefore, modified nanoparticles are used. Polymer embedded nanoparticles increase the solubility of hydrophobic chemicals or contaminants (Rizwan and Ahmed 2018).

ZVI can degrade a variety of chlorinated aromatics and polychlorinated biphenyls, as well as aliphatic compounds. During these degradation processes, chlorinated by-products are formed, and these by-products affect the reactivity of iron nanoparticles. This problem will also be solved with advances in nanotechnology. Combination metal therapy plays an important role in chemical degradation. When zero-valent iron nanoparticles combine with metals like Ni, Cu, Zn, and Pd, they show a greater catalytic response than iron nanoparticles alone.

Most commonly, palladium is used as a catalyst for dehalogenation in bioremediation processes using nano-zero-valent iron. By using bimetallic nanoparticles such as Ni/Fe, Xie et al. report on the degradation of polybrominated diphenyl ethers (PBDEs) in the environment. Different types of organic coatings are applied in order to increase the productivity of nanoparticles. These coatings play a variety of roles in activating ZVI molecules. It plays an important role in the modification of the ZVI surface resulting in an increase in the adsorption rate of contaminants on the surface of nanoparticles. Due to redox reactions, electrons get shut down and are responsible for increasing the speed of reaction, so these coatings are also essential to redox reactions. Hydrophobic compounds become more mobile when they are coated with electrolyte organic compounds. Hydrophobic compounds become more mobile when they are coated with electrolyte organic compounds. In the Fe-Pb bimetallic system, contaminants are adsorbent to a greater extent and there are no harmful by-products formed at the end. As a result of the reaction, reactive oxygen species are produced, which are again helpful to dechlorinate the reaction (Koul and Taak 2018b).

The Texas Rice University developed a new kind of nanomaterial called a nano mat, which resembles paper and is used to clean oil spills from ground surfaces as well as water bodies. Numerous petroleum hydrocarbon releases from the oil industry have toxic effects on aquatic environments. The researchers found that thin metal and carbon can trap oil droplets. Multifunctional nanowire structures with a coating of zirconium particles, which have multiple faces, can be used to separate oil from water. A new kind of structure can absorb particles with molecular weights 10 times greater than their original weight (Mohammadi et al. 2020).

6 The Drawback of Nano Bioremediation

In addition to being beneficial, nanoparticle-based solutions are also toxic. A limited amount of literature exists on nanotechnology's drawbacks in bioremediation. Below are some disadvantages of nanotechnology in wastewater treatment. In terms of wastewater treatment, hydrogel has many advantages, but one major disadvantage is the difficulty in recovering it after treatment. After treatment, the substance tends to dissolve and form a solution, so it is difficult to recover it (Thakur et al. 2018).

Advanced nanoparticles such as metallic nanoparticles MNPs accumulate in the environment and are toxic to various ecosystems, including aquatic, terrestrial, and air. Nanoparticles may be accumulated in the environment in different ways; they may enter the environment through the site where they are produced or during their transportation process at the time of their actual application or final disposal. As Nano-engineered materials pass through the aqueous medium, they affect natural processes such as chemical, physical, and also biological processes such as reactions, aggregation, transformation, sedimentation, dissolution, transformation, and sorption (Aguilar-Pérez et al. 2021).

Sharifabadi et al. studied the disinfection of bacteria using modified sodium dodecyl sulphate when it was combined with an ag/cation resin filter system. They found that after treatment there were no bacteria present, but when using a different filter like Ag/ fibre, Ag/sand, Ag/zeolite, and Ag/anion resin show low bacteria removal. This is one of the drawbacks of nanotechnology (Manikandan et al. 2021).

Nanoparticles are rapidly incorporated into animal cells as compared to plant cells because plant cells have a maximal level of external barrier. A nanoparticle present in the soil accumulates in plants through their roots, and in an aqueous medium, heteroaggregation and settlement of the particles may contribute to toxicity. The interaction of nanoparticles with natural organic matter affects the aquatic environment. Various processes are affected by these interactions, including surface transformation, adsorption, dissolution (oxidation and sulfidation), and stabilization/aggregation. According to a study carried out by Cáceres-Vélez et al. on the toxic effect of AgNPs on zebrafish in the presence of humic acid (HA). The result from the experiment shows that if there is more amount of humic acid then AgNPs uptake by zebrafish will reduce. But it doesn't change the effect of AgNPs on zebrafish. The presence of AgNPs in zebrafish bodies leads to toxicity in their body. The main advantage of this mechanism is that it will lower the toxic effects by reducing the uptake of AgNPs in the presence of humic acid. Another experiment was conducted by Selmani et al. to determine the effect of coated selenium nanoparticles on aquatic organisms *Vibrio fischeri* and *Daphnia magna.* The stability of nanoparticles in an aqueous environment depends on the type of medium and surface coating. A comparative study between selenium and selenium coated polyacrylic acid (PAA) shows a toxic effect on 24 and 48 h of exposure. There is another kind of nanoparticle that is most commonly used in bioremediation i.e., iron oxide nanoparticles. Besides their use in bioremediation, it causes toxic effects on the nitrogen cycle (Aguilar-Pérez et al. 2021).

7 Conclusion

We conclude from these chapters that nanotechnology-based solutions for industrial waste are a promising technology for removing pollutants from water bodies. Throughout the last few decades, due to rapid industrialization, new industries have developed day by day. These industries offer immense comfort products for the benefit of humans. Various industries release toxic chemicals that have many side effects. The conventional methods remove toxic chemicals from the environment and provide a toxin-free environment, however if nanotechnology-based solutions are added to conventional methods, speed and effectiveness of the process will be enhanced. In today's world, nanotechnology-based bioremediation solutions can remove various contaminants including organic, inorganic, and biological contaminants. Nanoparticles make this possible due to their unique properties. Because of their easy synthesis, cost-effectiveness, and great functionality, they are widely used. Environmental toxic pollutants can be removed using methods such as adsorption, membrane-based, photocatalysis, disinfection, and redox reaction. Each of these methods has advantages and disadvantages.

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Plant Mediated Nanomaterials: An Overview on Preparation Strategies, Characterisation, and Their Potential Application in Remediation of Wastewater

Neha Kumari, Lakhan Kumar, and Navneeta Bharadvaja

Abstract Waste water containing inorganic/organic pollutants and heavy metals has become a major problem nowadays. Some heavy metals are toxic even if they are present in trace amount. So, removal through adsorption of these heavy metals is necessary as they are non-biodegradable and can easily enter in the soil and consequently harm plants and other life-forms. Nanotechnology provides a potential approach for environmental remediation as it helps in photocatalytic degradation of dyes and heavy metal adsorption from aqueous environment. Nanoparticles can be synthesized from various techniques including chemical, physical and biological. Synthesis of nanoparticles from plants is an eco-friendly, inexpensive and simple way to remediate environmental pollutants. Here, we reviewed the synthesis of nanoparticles using a wide range of plants. Plant extracts, contains a number of bioactive compounds, which serve as reducing and capping agent for the metal precursor. Plant based nanomaterials have shown ability to selectively sense the toxic hazardous heavy metals like Pb, As, Ni, Hg, Cr, Zi, Co, Fe, Mn, Pd and Se in various environmental niche. The chapter commences with the introduction to environmental degradation and discusses the phytonanoparticles synthesis approaches and their characterization. Besides this, it illustrates on role of various plant bioactive compounds in synthesis of nanomaterials. Further, it discusses role of various plant mediated nanomaterials in remediation of dyes and heavy metals. The article concludes by highlighting challenges and prospects of this emerging green technology for environmental pollution remediation.

Keywords Wastewater · Inorganic/organic pollutants · Adsorption · Photocatalytic degradation · Nanotechnology · Nanoparticles

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1 Introduction

Phyto-nanotechnology is the emerging branch of nanomaterials synthesis from plant biomolecules that are used for a number of purposes including biomedical, microfabrication, energy storage devices, agriculture, health care, and remediation purpose as it has large surface area and high reactivity. Phyto-nanotechnology is known to be successful to reduce the environmental pollution which involves water treatment and purification of gases. Treatment of ground water is also somewhere related to inorganic and organic pollutants present in the soil. According to United Nations report, globally 0.5% of fresh water is present on earth and 80% of freshwater goes back to the ecosystem without being treated. Contaminated water and soil have become a great issue for us and the environment as well. Water can be contaminated by the presence of different chemicals and heavy metals like lead, arsenic, nickel, mercury, chromium, zinc, cobalt, and selenium. They are present in trace amount but still are highly toxic. Metallic elements are considered to be toxic due to its high density, specific gravity and atomic weight. There are 50 heavy metal presents out of which 17 are highly toxic and. normally, most of the heavy metals are found on the earth crust but due to excessive industrialization, deforestation and various other human activities these metals transfer to ground water (Chowdhury et al. 2016). Not all the heavy metals are hazardous, some are essential to humans like cobalt, copper, zinc and mercury but they can be harmful if present in excessive amount. Toxicity of a metal depends upon its dosage, chemical reaction and means of exposure. Sources of heavy metals like mercury, cadmium, arsenic, lead include coal mining, contact with acid rain, TDS refining procedure of other metals, manufacturing of chemicals and corrosion of pipes (Rosenberg 2015). Exposure of contaminated water for drinking purpose can affect human health in many ways. It can lead to several types of cancer, organ damage, neurotoxicity, nephrotoxicity, malfunction of kidney, lungs, liver, circulatory system, learning difficulty, rheumatoid arthritis and even death in extreme cases (Lellis et al. 2019). Also, in agriculture, the toxic heavy metal present in the soil can be taken up by plants leading to ROS strengthening, crops damage and adverse effect on human and animal health.

On the other hand, dyes are known to be carcinogenic and pollute water bodies. Exposure of dye in water bodies result in reduction in availability of light to the life forms living under water which leads to the reduction in the rate of photosynthesis, affecting the growth of the plant. 15–50% of azo dye is found in water bodies because it does not bind to fabric. This wastewater incorporated with dye when used in irrigation affects the soil microbial communities, enzyme activity and growth of plant. Azo dyes are chemically stable, non-biodegradable, durable coloured compounds that are carcinogenic and mutagenic in nature (Ismail et al. 2019) Congo red dye, also known as carcinogen. Benzedrine exhibit optimal, thermal and physiochemical stability which contributes to their non-biodegradable nature (Rai et al. 2014). Triphenylmethane dye used in manufacturing process such as textile dyeing, food, and cosmetics etc., causes reproductive diseases in rabbit and marine animals. O-phenylenediamine used a substrate in dyeing composition acts as a xenobiotic and recalcitrant. It causes breathing problem, ingestion and eye irritation in humans. Brilliant Cresyl Blue is used in scientific labs and industries. Rhodamine 6 G is a fluorescent dye widely used for staining purpose but has toxic effects on microorganisms and humans. Rhodamine B dye is a water soluble red dye that causes eye and skin diseases (Glossman-Mitnik 2013). Methyl blue is a type of cationic dye extensively used in dyeing paper, clothes leather etc. Jaundice, cyanosis, high blood pressure, vomiting are some of the common diseases associated with soluble methyl blue dye (Kushwaha et al. 2014). There are many other water soluble dyes like malachite green dye, crystal violet dye, and phenol red dye that reduce the transparency of water, affect photosynthesis of aquatic ecosystem and are hazardous to humans.

Recent studies show that nanoparticles successfully remediate organic, inorganic pollutants, and heavy metals from the aqueous solution. There are several methods to synthesize nanoparticles, but biogenic synthesis of nanoparticles is chosen over other chemical and physical methods since they are simple, eco-friendly, cost effective and clean. Biological synthesis can be done from various sources like bacteria, fungi, algae and plants. In this chapter, we are focusing on phytonanotechnology which utilise plant extracts which act as reducing and capping agent in the synthesis of nanoparticles and provide stability. Generally, the plant extract is prepared as a stock and certain amount of metal precursors are allowed to react with plant extract under optimized reaction conditions. After a particular time, visual observation like colour change indicates the formation of nanoparticles without the use of any toxic, expensive chemical or the formation of any harmful by product. Metals and oxides of metals act as adsorbents following the adsorption isotherm, adsorption thermodynamics and adsorption kinetic modelling (Guerra et al. 2018). The Nano Zero Valent Iron particle is best for the reduction process as it makes use of phyto-compounds present in the plant extract. Zero valent form of iron is efficient in adsorption of hexavalent chromium (Madhavi et al. 2013a). Similarly zero valent form of silver nanoparticles removes cadmium from aqueous solution (Al-Qahtani 2017). To keep the particle stable and prevent its aggregation certain stabilizing agents are used like carboxyl methyl cellulose, sodium borohydride etc. Maghemite nanoparticles is effective against lead and cadmium from water instead of electro exploding wire techniques (Yadav and Fulekar 2018a). The chapter aims to cover several aspects of nanomaterials synthesis, their characterization and potential application in environmental remediation.

2 Synthesis of Nanoparticles

Nanoparticles are generally produced by top down and bottom-up approaches that require heavy machines, chemicals and a very high maintenance cost. So, instead of using these physical and chemical methods, biological method which is less time consuming, non-toxic and economically suitable can be preferred. The biological method involves microorganism assisted biogenesis using yeast, fungi, bacteria and algae, bio template and plant extract assisted biogenesis. In this chapter, we focused on studying various approaches of synthesis of nanoparticles using plant extracts (Fig. 1). The biomolecules present in the plant extracts act as reducing and capping agents which help in bio-reduction of metal ions under optimized conditions. Silver nanoparticles formed from the *Gardenia jasminoides* extract help in reduction of silver ions to zero oxidation state without using any toxic chemical to form silver nanoparticles (Lü et al. 2014). Hence, bio-compounds like polyphenol, flavonoid, alkaloids, proteins, enzymes and co-enzymes help in reduction to form nanoparticles whose size depends upon the phytochemicals compounds present in the plant extract. Synthesis of metal nanoparticles with different plant extract is given in Table 1.

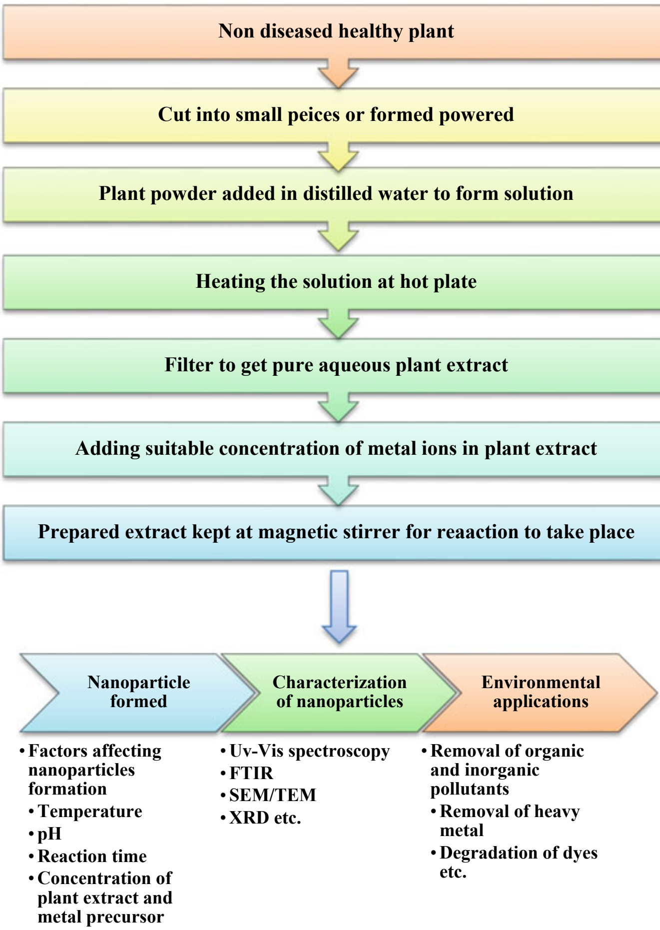

Fig. 1 Schematic representation of plant mediated synthesis of nanoparticles, their characterisation, and applications

Table 1 Nanoparticle synthesis from various plants, their characterization, and potential applications

Metal precursor	Plant	Characterization techniques					Observation		Applications	References
		UV–vis	FT-IR	SEM	TEM	EDX	Initial	Final		
Copper nanoparticle Copper sulphate pentahydrate	*Piper Retrofractum Vahl*	234–255 nm	550–570 cm	Spherical shape high Cu content-70.3%	2–10 nm	Crystallinity phase −26.4%	Yellow	Greenish black	Inhibit *E.coli* and *Staphylococcus aureus*	Amaliyah et al. 2020)
Copper nanoparticle Copper sulphate pentahydrate	*Cedrus deodara*	205 nm	607 cm	Agglomerated and form large particle	Spherical	No impurities	Greenish	Dark brown	Inhibit against pathogenic strains	Ramzan et al. (2021)
Copper nanoparticle Copper acetate monohydrate	*Punica granatum peel* extrac	–	Broad spectrum at 3400 for OH present at surfaces of CuO	Spherical shape, diameter = 12.5 mm	–	**XRD**-crystalline size = 35.80 nm monoclinic phase	Blue	Brown	Antibacterial activity against *E.coli*	Siddiqui et al. (2020)
Copper nanoparticle Copper sulphate pentahydrate (5 mM)	*Celastrus paniculatus* leaf extract	269 nm		Spherical diameter = 5 nm	2–10 nm	**DLS-** zeta potential = −22.2 mV Zeta deviation = 3.61 mV EDS-purity (79.87%)	Yellow	Green	Antifungal property Degradation efficiency on organic dye	Mali et al. (2020)
Copper nanoparticle Copper sulphate pentahydrate	*Neem* flower extract	550–560 nm		Tightly packed nanocrystal	Spherical (5 nm)	–	Light blue = light green	Dark yellow = brown ppt	**Antibacterial study** Max. efficiency against *Proteus mirabilis* = 40 mg/ml	Gopalakrishnan and Muniraj (2021)
Copper nanoparticle Copper sulphate pentahydrate	Red extract cabbage (*Brassica oleracea var. capitata f. rubra* extract)	255 nm	3100–3600 cm Band energy gap = 2.75 eV	Spherical	Particle size = 77.5 nm	**XRD-**crystalline nature, Fcc structure, crystalline size = 78 nm	–	White ppt	Antibacterial agent against *E.coli* and *S. aureus*	Fernandez and Rajagopal (2020)
Copper nanoparticle Copper sulphate pentahydrate	Seedless dates	576 nm	624.57 cm	–	Spherical	**XRD** **DLS** **Particle size distribution** = mean diameter 78 nm **Zeta potential** = +41 mV	Pale yellow	Red brown color	More feasible than chemical method	Mohamed (2020)
Copper nanoparticle Copper chloride	*Tinospora cardifolia*	248 nm	3329 cm − OH stretching 1620-C=O stretching 1395-OH bending 1319-C-O bending 1049- C-O–H stretching	Spherical	**XRD-** crystalline phase -metallic copper and copper oxide	**PSD** = 63.5 nm **Polydispersity index** = 0.26 **Zeta potential** = −33.98 mV **AFM** = 178 nm height	Sky blue	Dark green	Antimicrobial activity Efficacy of coated cotton against gram-positive is 65% & gram-negative is 50.5%	Sharma et al. (2019)

(continued)

Table 1 (continued)

Metal precursor	Plant	Characterization techniques					Observation		Applications	References
		UV–vis	FT-IR	SEM	TEM	EDX	Initial	Final		
Zinc oxide nanoparticle Zinc nitrate hexahydrate	*Cassia fistula* plant extract	370 nm	Photolytic degradation- (batch reactor) = 5 ppm dye conc. Gives 90% degradation; ph-2 & 4 96.26% & 98.71% Photodegradation efficiency Antioxidant (DPPH) Ic50 = 54 Mg/ml	Hexagonal wurtzite structure	5–15 nm	XRD- 100,002,101 plane—increases purity of particles	–	–	Exhibit Antioxidant, Bactericidal property and catalytic property against methyl blue	Suresh et al. (2015)
Silver nanoparticle Silver nitrate	*Morinda tinctoria* Leaf extract	420 nm	3296 cm—carboxylic group 3436–3220 cm—O–H stretching, H bonded phenol and alcohol group 1634 cm—N–H bending primary amines 1672 cm—C = O stretching	Spherical rod shaped	79–96 nm	4 peaks of 2 theta 38.26 degree = (111) 44.44 = (220) 64.58 = (220) 77.67 = (311) Fcc and crystalline in nature	Pale yellow	Dark brown	Degradation of methyl blue dye	Vanaja et al. (2014)
Silver nanoparticle Silver nitrate	Neem leaf Neem bark Mango leaf Green tea Pepper seeds	420	-	Spherical	–	–	Pale yellow	Brownish yellow	Calorimetric sensing of toxic metal (Hg2+, Pb2+, Zn2+, Cr 3+, Cd2+, Ca2+. Cu2+, Mg2+, Ni2+, Fe2+)	Karthiga and Anthony (2013)
Silver nanoparticle Silver nitrate	*Amaranthus gangeticus Linn leaf extract*	416 nm	1635 cm—C = O stretching 3441 cm—OH & NH2	Globular shaped	11–15 nm	–	–	Brown	Antifungal and antibacterial activity inhibitory activity towards gram +ve & −ve Degrade congo red dye	Kolya et al. (2015)
Silver nanoparticle Silver nitrate	*Nigella Sativa* seed extract	426 nm	3423- 2880 cm Indicates CH, −OH, −NH, C=C acting as reducing & stabilizing/capping agent	Spherical	10.88 nm	XRD- 2 theta = 38.14 degree- (111); 44.36 degree (200); 64.71 (220) 77.40 (311)	White	Light brown	Congo Dye degradation	Chand et al. (2021)

(continued)

Table 1 (continued)

Metal precursor	Plant	Characterization techniques					Observation		Applications	References
		UV–vis	FT-IR	SEM	TEM	EDX	Initial	Final		
Silver nanoparticle Silver nitrate	*Zanthoxylum armatum* leaves	419 nm	3431 cm- N–H stretching OH hydroxyl grp 2922 cm- C-H stretching 1744 cm—carbonyl stretching 1630 cm- N–H bond 1375 & 1238 cm—N = O symmetry 1045 cm- C-N amines	Spherical shape	Diameter = 15–20 nm	**Zeta potential** = −21.2 mV Average size −36 nm **XRD** Fcc 2 theta = 38.23 degree (111); 46.45 (200); 66.65 (220); 77.55 (311) Avg crystallize size—22 nm	Colourless	Brownish	Dye degradation—safranin O Methyl red Orange and methyl blue	Jyoti and Singh (2016)
Silver nanoparticle Silver nitrate	*Viburnum opulus* fruit extract	513 nm; 415 nm (350–450 nm)	3398 cm- stretching of O–H 1733 cm- C=O stretching 2931 cm—C-H stretching 1380 cm-C-O stretching 1030 cm- C-O bending	Spherical	7–26 nm Avg size- 16 nm	**XRD-** 2 theta 38.33 degree (111); 44.56 (200); 64.62 (220); 77.44(311);82.41 (222); 32.2-organic compound in sample (Fcc) **TGA-** 35.14% bioactive compounds present at AgNP surface	Faint red	Yellowish brown	Dye degradation Brilliant blue Tartrazine Carmoisine	David and Moldovan (2020)
Silver nanoparticle Silver nitrate	*Calendula officinalis*	436 nm	2 theta – 665 cm; 3438 cm; 1635 cm-stretching vibration of carbonyl grp; 3338 cm-O–H stretching	Uniform and spherical	50–60 nm 140–150 nm	**XRD-** Fcc, crystal size = 14.37 nm	Yellow	Brown	Methyl blue and methyl orange degradation	Chandra Paul et al. (2020)
Silver nanoparticle Silver nitrate	*Dahlia pinnata* leaf extract	460 nm	1064 cm- C-N bond 3265 cm- O–H stretching 2916 cm- C-H stretching 1423 cm- C-H bending 673- stretching vibration of halo alkene 1595 cm- C-H present	Almost spherical	Diameter- 15 nm	**XRD-** Face centre cubic structure, 27.5- (220); 32.75- (122); 46.25(111); 54.65(331); 57.25(241); 76.68(311)	Colourless	Dark yellow	Rapid colorimetric detection of Hg2 +	Roy et al. (2015)
Silver nanoparticle Silver nitrate	*Panax Ginseng* root extract	404 nm	3640 cm- O–H stretching 1740- carbonyl stretching vibration of aldehyde 1640- symmetry-COO stretching 1407- asymmetry-COO stretching 1065- bending vibration of C–C-O & C–C-OH	spherical	4–20 nm	**–**	–	Dark brown	Detection of Hg2+	Tagad et al. (2017)

(continued)

Table 1 (continued)

Metal precursor	Plant	Characterization techniques					Observation		Applications	References
		UV–vis	FT-IR	SEM	TEM	EDX	Initial	Final		
Gold nanoparticle Gold(lll)chloride trihydrate	*Capsicum annum*	335.26 nm	3324- 3329 cm- = –OH grp 2100 cm- -CN grp 1636 cm- C = O grp	Triangular	–	**XRD-** 111, 200, 220, 311 degree Crystal size = 13.71 nm	Yellow	Dark red	Water pollution removal and Show strong antimicrobial activity at low concentration	Baran et al. (2020a)
SnO2 nanoparticle (SnCl2)	*Vitex agnus-castus* fruit extract	373.45 nm	3434.27 1633.02 1027.50 647.45 Anti-symmetric Sn–O-Sn; Sn–O symmetric	Spherical	4–13 nm Avg size = 8 nm	**XRD** 26.9 degree- (110);34- (101); 38-(200); 52-(211); 58-(002); 62.1-(310); 65.2-(301); 71.4(202)	–	Light grey	Photocatalytic degradation of organic dye RhB, under and removal of heavy metal Co+2	Ebrahimian et al. (2020)
Iron nanoparticle (FeSO4.7H2O)	*Eucalyptus globules* leaf	'graph given = above 420 nm	3346.25 cm; 1635.58; 524.68 cm (polyphenolic content present	Spherical	50–80 nm	**XRD** Peak at 2 theta 46.40 indicates zero valent iron	Brown	Black	Adsorption of Cr(VI)	Madhavi et al. (2013a)
Maghemite nanoparticle 2 M FeCl3+1 M FeSO4.7H2O	*Tridax* plant	210 nm	454 cm, 569 cm, 632 cm, attributed to Fe–O; 563 cm—gamma attributed to FeO 1443–1600 attributed to C=C/C=N bond 326, 402, 500, 706 are peaks of Maghemite	Cubic spinal structure (spherical and cuboidal shape)	9.59–15.42 nm	**XRD** 18.22, 29.3,30.02,32.08,43.14,57.48 degree with miller indices 011,210,220,310,330	–	Blackish brown ppt	Removal of heavy metal Pb and Cd	Yadav and Fulekar (2018a)
Zinc Nanoparticle Zinc acetate (0.1 M)	*Sphagneticola trilobata* (leaf, stem and root extract)	-	3144 cm-C=O stretching 1665 cm –N–H stretch 1640 cm-N–H bending in amine & amide grp	Irregular and complex	65–80 nm	**EDS-** zinc 41.4% synthesized in Nanomaterial **XRD-** 200,111,221 planes correspond to 2 theta angle Face center cubic structure	–	Yellow colour	Removal of Chromium by DPC method	Shaik et al. (2020a)
Cupric Oxide nanoparticle	*Calotropis procera*		3444 cm –OH grp; 1636 cm; 1231 cm;1084– protein latex 519; 598 cm –Cu–O band	Spherical	10–15 nm; diameter = 15.06 nm standard deviation- 5.17	**XRD** Peak on range 20 < 2 theta <80 degree Monoclinic symmetry	Green ppt	Black ppt	Removal of Cr(VI) from aqueous solution	Dubey and Sharma (2017)

(continued)

Table 1 (continued)

Metal precursor	Plant	Characterization techniques					Observation		Applications	References
		UV–vis	FT-IR	SEM	TEM	EDX	Initial	Final		
Iron based NP FeSO4.7H2O	*Eucalyptus leaf*	–	3388 cm −O–H stretching; 2932 &2840 −C-H stretching;1720—carbonyl grp; 1605& 1515 −C=C aromatic skeletal vib.;1330 −CH3 asymmetric vib.;1048- C-N stretching NP rxn with Cr(VI) & Cu(II) show band at 460 & 546 cm -Fe–O stretch	Polydispersed	20–80 nm	**EDS-** Element % of Fe = 16.3; C = 47.4 O = 36.3 **XRD** 2 theta at 44.8; 24 degree show biomolecule capped with Fe NP	–	–	Removal efficiency of Cr (VI) and Cu (II) when existed together or separated	Weng et al. (2016a)
Zero valent silver NP Silver nitrate	*Ficus Benjamina*	420 nm	3461- O–H stretching; 1632 cm—amide grp	Dendritic structure	60–105 nm	**–**	–	Brown	Cadmium removal from aqueous solution	Al-Qahtani (2017)
Zero valent Iron NP	*Rose damascene, Thymus vulgaris, Utrica dioica* leaf extract	–	3400 & 3430 cm for polyphenols; 1126–1190 cm for carbonyl grp; 1628 &1640 cm corresponds to C=C in alkene grp; 615–617 indicating aromatic compounds of alkanes	Non uniform exhibit differed space & void space	100 nm **BET** Surface area = 1.63(TV); 2.42(UD); 1.42 (RD)m3/g total pore vol = 4.52*10–2(TV) 2.97*10^{-2} (UD); 2.08*10^{-2} (RD)	**XRD** Perfectly index crystalline Fe	–	–	Removal of Cr(VI) from aqueous solution	Fazlzadeh et al. (2017)
Silver nanoparticle Silver nitrate	*Perilla frutescens*	469 nm	615 & 627- C-H group; 1048 & 1124—stretching vibration; 1381 &1374 -C-N stretching vibrations; 1608 & 1601- secondary amide groups	Spherical, rhombic, triangular and rod shaped	25.71 nm	**Zeta potential-** −23.33 mV FCC cubic	Yellow	Brown	Exhibits antioxidant, anticancer and antibacterial properties	Reddy et al. (2021)
Copper nanoparticle $Cu(OAc)_2$	*Stachys lavanaulifolia*	400 nm	3100–3385-hydroxyl group; 2922, 1618, 1398 represent saturated hydrocarbon; C=C and C=O—aromatic stretching frequency; 3400- O–H stretching; 1473-bonding vibration of sp^2 bond 1627- carbonyl stretching	Monodispersed	20–35 nm	**XRD** 2 theta = 32.59, 35.61, 38.78, 48.82, 53.24, 58.37, 61.60, 66.31, 68.15, 72.46, 75.30 assigned to (110), (−111), (111), (−202), (020), (202), (−113), (220), (311), and (-222) planes	Pale yellow	Dark brown	Represents catalysed C-heteroatom coupling reaction	Veisi et al. (2021)

(continued)

Table 1 (continued)

Metal precursor	Plant	Characterization techniques					Observation		Applications	References
		UV–vis	FT-IR	SEM	TEM	EDX	Initial	Final		
Iron based nanoparticle $FeSO_4.7H_2O$	Green tea extract	–	1611- C = C stretching vibrations; 1362- C-N bond; 1039- C–O–C bonding	–	50–80 nm	**GC–MS** Phenol, 1,1-biphenyl, 2-ethyl, 1,2,3- benzenetriol, caffeine and bis (2-ethylnexyl) phthalate involve in FeNP synthesis as reducing and capping agent			Removal of hexavalent chromium	Hao et al. (2021)
Nickel oxide nanoparticle $Ni(NO_3)_2.6H_2O$	*Abutilon indicum* leaf extract	200–385 nm	3410 (O–H); 2990 (C-H); 1702 (C = O) 1650 (amine I and amide II); 1259 (O–H) 1140 (C-O)	Agglomerated form	452.49 nm	**XRD** Highly crystalline attributed to (111), (200), (220), (311), (222) plane with diffraction angle 37.5, 43.26, 63.11, 75.46, 49.46 degree respectively	Yellow	Green	Exhibit anticancer, antioxidant, and antibacterial property	Khan et al. (2021)
Zinc oxide- based nanoparticle $Zn(NO_3)_2.7H_2O$	*Saponaria officinalis* extract	380 nm	-	Agglomerated form	100–200 nm diameter	**XRD** Hexagonal wurtzite phase attributed to (100), (002), (101), (102), (210), (103), (200), (212), (201) planes		Dark brown	Dye degradation efficiency of methyl blue and show antibacterial activity	Tănase et al. (2021)
Silver nanoparticle Silver nitrate	Plant gum of- *Araucaria heterophylla* *Azadirachta indica* *Prosopis chilensis* (bark of tree)	–	–	Spherical	30 nm DLS = 50 nm 35 nm; DLS = 50 nm 50 nm DLS Diameter = 75 nm	**Zeta potential (EDX)** −20.87 mV −23.65 mV 16.41 mV			(1) Effective against gram positive and gram-negative bacteria (2) not much effective against cell lines, but still exhibit anticancer anti-cancer activity against human breast cancer cell line MCF 7 at higher concentration (3) Chromium removal efficacy was not efficient	Samrot et al. (2019)

3 Role of Phytocompounds in Nanoparticles Synthesis

Phytocompounds play a major role in the formation of nanoparticles. Due to extensive study on phytochemicals analysis, our understanding has increased about them. It has facilitated in identification of particular biochemical compound present in the plant which acts as a reducing, capping and stabilising agent in nanoparticles synthesis. The phytochemicals analysis, if carried out by performing phytochemicals tests can give the exact phyto component participating in reduction, which can be further determined by FTIR (Fourier Transform Infrared Spectroscopy) analysis. FTIR studies reveal that amino acids help in reduction of metal ions. For example, alpha amino acids reduces silver ion (Tan et al. 2010). Flavonoids are secondary metabolites produced by plants that have the ability to transform enol-form to keto form by releasing one hydrogen atom. Functional group of phenolic acids comprises the phenolic ring which is responsible for chelating metal and carboxylic acid. In synthesis of silver nanoparticle, using plant extracts of three plants namely *Schinus molle, Equisetum giganteum*, and *Ilex paraguariensis*, removal of electrons occur so that Gallic acid is oxidised to quinine (Barberia-Roque et al. 2019). Phytocompounds polyols and polysaccharides actively present in *Cinnamomum verum* help in reducing Ag + ion in silver nanoparticle synthesis (Sathishkumar et al. 2009). Its functional group containing carbohydrate and hydroxyl group is known to be soluble in water while methyl and isopentyl are lipophilic in nature. It also has potential to donate hydrogen atom, thus helping in reduction process. Flavonoids are divided into major subclasses which included chalcones, flavones, flavanols, isoflavones and anthocyanidin. Quercetin, which comes under the class of flavanol, chelates metal ion at three different positions namely the catechol group, carbonyl group and hydroxyl group. Quercetin and plant pigmentation helps in bioreduction of silver nanoparticle synthesized from *Acalypha indica* leaf extract (Krishnaraj et al. 2010). Flavonoids have potential to tolerate heavy metals like cadmium and zinc in *Arabidopsis thaliana*. Terpenoids are the derivative of essential oil and have diverse structure containing 5 carbon isoprene units. They show strong antioxidant activity. They also help in bio-reduction by deprotonating the OH^- group to form conjugate base structure and preventing it from further oxidation. Terpenes, which are converted into terpenoids upon oxidation, help in metal reduction. This active redox reaction leads to the formation of nanoparticles. *Lantana camara* leaf extract contains terpenoids as their main reducing and capping agent in the synthesis of silver nanoparticles (Ajitha et al. 2015). Excessive thermal heating can inactivate essential phytocompounds. Monosaccharide sugar like glucose and fructose also take part in formation of metallic nanoparticles through the conversion of ketone to aldehydes. Disaccharides and polysaccharides form an open chain with the help of monosaccharides up to 7–8 units and provide the metal ion to aldehydes group and facilitate reduction process.

4 Characterization of Nanoparticles

To detect the reduction of metal precursor, the optical absorbance of synthesised nanoparticles is performed by a UV–Vis spectrophotometer. The spectra are recorded within the range of 200–700 nm. UV–Vis spectrophotometer is used to determine the concentration of different molecules present in a solution utilising the characteristics wavelength of the molecule at which it maximally absorbs the light. After the plant extract and metal precursor are mixed, a change in colour of the solution is observed as the time progress. One concern is there that with time aggregation of nanoparticles proceeds, which ultimately alter the peak of absorbance. At particular wavelength of localized surface plasmon resonance, we get different maximum absorbance of nanoparticles as compared to literature values of those particular metal precursors. A study reported that the gold nanoparticles synthesized from *Garcinia mangostana* fruit peels turned purple instead of traditional yellow (Xin Lee et al. 2016). Maximum absorbance of silver and gold nanoparticles synthesized from *Rumex roseus* leaf extract was recorded at 429 nm and 549 nm respectively by UV–Vis spectrophotometer (Chelly et al. 2021). SEM/TEM is used to determine the morphology of the synthesized nanoparticles. In TEM analysis, once electron hit the sample it gets absorbed, and gives much higher resolution than light microscopy whereas in SEM analysis of nanoparticles, the electron scans for different region of sample. In some regions, more scattered electrons are present due to which the absorbed atom is less and in other region less scattered electron found give a clear indication of more absorbed atom. This scattering of electron in different region results in more contrasting three- dimensional image as obtained from SEM micrograph. SEM/TEM studies are done to observe the shape and size of the synthesised nanomaterials. *Garcinia magostana* mediated magnetite nanoparticles are of 13.42 nm and displayed diffraction rings of Fe_3O_4 phase (Yusefi et al. 2021). FTIR spectra determine the functional group present in the plant extract which are held responsible for the synthesis of nanoparticles. The principle behind FTIR tell us that the bond between two atoms or molecules are not fixed and are involved in different types of motions called bond vibrations. Every compound has its signature vibration and stretching between the bonds by which the functional group can be determined. For example, formaldehyde has carbonyl carbon attached with oxygen where stretching and wagging goes on simultaneously. Therefore, absorbance determines the type of functional group present in the sample. The peaks in the spectrum show the bending and stretching vibrations of the biomolecules. Zinc oxide nanoparticles synthesized from *Cayratia pedata* leaf extract exhibit zinc bonding at 400 cm^{-1} and oxygen bonding at 600 cm^{-1} (Jayachandran and Nair 2021). Crystal structure of nanomaterials are analysed by XRD. We use X-ray crystallography because X-ray has shorter wavelength. Its working involves projecting a high energy electron beam falls on a rotating target that throws out the X-ray generated, which are then measured by a detector containing photomultiplier tube of X-ray diffractometer. The position of the peaks is determined by the planes which diffract coherently at an angle where Bragg's law holds good.

5 Different Types of Plant Mediated Nanoparticles Helping in Dye Degradation and Heavy Metal Removal from Aqueous Solution

Chemical compounds which are released from industries, households, oil pollution, acid rain, sewage and agriculture waste makes remediation process of water more complicated and difficult. There are many techniques available for purification of water but the nanotechnological approach has been termed as potential method due to the small size and high reactivity of nanoparticles. Several types of plant-based nanomaterials have been explored for remediation of environmental pollutants as illustrated in Fig. 2.

5.1 Nanoscale Zero Valent Iron (nZVI) Nanoparticle

Nanoscale zero valent iron (nZVI) is preferred because it can be separated easily under the influence of external magnetic field. nZVI cannot remove contaminants on its own because of high probability of aggregation which can easily alter the surface chemistry to make it unreactive (Pasinszki and Krebsz 2020). To prevent these problems, nZVI nanoparticles come into use without damaging the active catalytic sites of nZVI. Sodium borohydride is used for stabilizing these nanoparticles as it increases surface reduction and prevents oxidation. Polyvinyl-pyrrolidone (PVP), polyethylene glycol (PEG), and carboxymethyl cellulose (CMC) used in coating of nZVI increase the stability by catalytic reduction of ketones to alcohol (Parimala

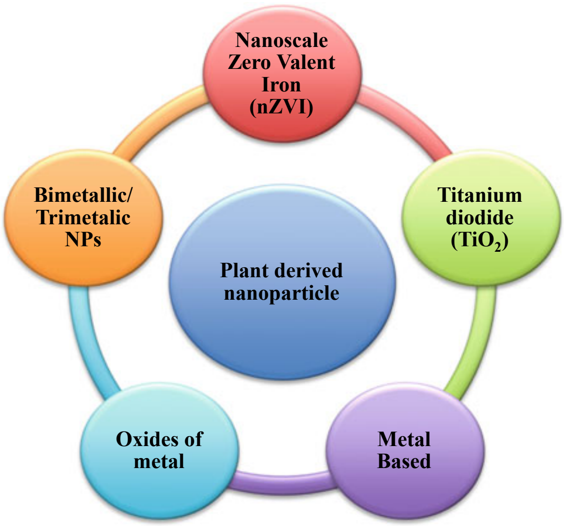

Fig. 2 Types of plant derived nanoparticles

and Santhanalakshmi 2014). Fenton's reagent is also used in remediating wastewater along with nZVI nanoparticle as it produces hydroxyl radicle at low pH with higher concentration of H_2O_2 and Fe^{2+}. Reduction of oxygen by nZVI and subsequent formation of hydrogen peroxide leads to hydroxyl radical formation and an oxidized organic compound (Babuponnusami and Muthukumar 2014). Nanoscale zero valent iron nanoparticle synthesis from green tea extract using bentonite for stabilization successfully degraded 96.2% RB 238 dye within 60 min followed by Fenton like oxidation (Hassan et al. 2020). Catalysis of dye resulted in the formation of hydrogen peroxide in which the OH radicle and dye degraded to form carbon dioxide and water molecule. Nanoscale zero valent iron used along with magnetite nanoparticle followed Fenton's reaction to remediate wastewater by degrading chlorinated compound like 2, 4-Dichlorophenoxyacetic Acid at pH 3–6.5 within 90 min (Nanoparticles 2020).

5.2 Titanium Dioxide (TiO_2) Nanoparticle

Titanium dioxide obtained from the three different minerals namely anatase, brookite and rutile. Anatase grade of TiO_2 exhibits similar properties as of rutile but brookite form is very rarely formed and is highly unstable. TiO_2 nanoparticle synthesized from Cinnamon powder are of spherical anatase phase and shows enhanced photocatalytic property for solar cells with band gap of 3.2 eV which is determined by UV–Vis spectroscopy (Nabi et al. 2020a). Titanium dioxide is a potent photocatalyst which gets activated by UV light such as sunlight for photocatalytic reaction to take place. Titania exhibits unique physical properties due to which TiO_2 is insoluble in water and show white colouration or precipitate, that's why it is extensively used as food additive. Chemical and physical methods have several limitations like small scale production, not environment friendly, maintenance of temperature and pressure leads to high cost, use of surfactants result in toxicity and complexity. TiO_2 nanoparticles can be successfully synthesized from biological methods like *Aloe vera* extract, *Annona squamosa* peel extract, and *Jatropha curcas* leaf extract, *Nycetanthes Arbor-Tristis* leaf extract, *Psidium guajava* and various other plant extracts given in Table 2.

TiO_2 is another way to generate hydroxyl radical which oxidizes contaminants present in water under the influence of light. Under the exposure of UV light, the band energy gap of TiO_2 is 3–3.5 eV. The transfer of negative charged electron from valence band to conduction band exhibited by the forming positive charged holes. The photogenerated positive charge carriers (holes) from valence band diffuse to photocatalyst surface and react with water molecule to form free radical which is further oxidizes (Nakata and Fujishima 2012). The negatively charged electron from conduction band helps to promote reduction followed by reacting with atmospheric air to produce non-hazardous compound like water molecule, carbon dioxide etc. To enhance the photocatalytic efficiency, TiO_2 is trapped within nanoparticles without damaging the active sites followed by surface modification via doping with carbon, nitrogen and metal (Zahra et al. 2020).

Table 2 Types of phyto-nanoparticles and their application in treatment of wastewater

Type of nanoparticles	Plant source	Absorption capacity/percentage degradation	Applications	References
nZVI	*Rosa damascene (RD)* *Thymus vulgaris (TV)* *Utrica dioica (UT)* leaf extract	94.87% 83.45% 86.8%	Removal of Cr(VI)from aqueous solution	Fazlzadeh et al. (2017)
Kaolin-supported nZVI	*Ruellia tuberosa*	99.8%	Degradation of reactive black 5 azo dye	Khunjan and Kasikamphaiboon (2021)
nZVI	*Spinacia oleracea*	COD- 73.82% BOD- 60.31%	Removal of chemical and biological oxygen demand from municipal waste water	Turakhia et al. 2018)
nZVI	*Cupressus sempervirens*	95.4%	Decolourization efficiency of methyl orange	Ebrahiminezhad et al. 2018)
nZVI	Oak Mulberry Cherry Leaf extract	–	Removal of As(III) and Cr(VI) from aqueous solution	Poguberović et al. (2016)
nZVI	*Mentha piperita*	$PO_4{}^3$ –85.01% NH_3 –99.51% NO_3 –86.33% Pb^{2+} –83.4% Cl –79.33%	Removal of agricultural contaminants like phosphate, ammonia, nitrate, lead, and chloride from aqueous solution	Shad et al. (2020)
nZVI	*Shorea robusta* leaf extract	96%	Degradation of congo red dye from aqueous solution	Jha and Chakraborty (2020)
nZVI	Grape seed extract + Fe^{3+}	82.8–96.1%	Removal of Cr(VI) from aqueous solution	Guo et al. (2020)

(continued)

Table 2 (continued)

Type of nanoparticles	Plant source	Absorption capacity/percentage degradation	Applications	References
nZVI	*Ficus Benjamina* leaf extract	75.5–85%	Cadmium removal from aqueous solution	Al-Qahtani (2017)
TiO_2 Au-TiO_2	*Azadiracta indica*	–	Degrade methyl red dye	Sankar et al. (2015)
	Cinnamomum tamala leaves	64%	Degradation of methyl orange	Naik et al. (2013)
	Jatropha curcas leaf	82.26% 76.48%	Removal of chemical oxygen demand(COD) Removal of Cr from tannery wastewater	Goutam et al. (2018)
	Piper betel (PS) *Ocimum tenuiflorum* (OT) *Moringa oleifera* (MO) *Cariandrum sativum* (CS)	MO degrades faster among all within 30 min	Degradation of malachite green dye	Pushpamalini et al. (2020)
	Lemon peel extract	70%	Degradation of rhodamine B dye	Nabi et al. (2020b)
	Syzygium cumini leaf extract	75.5% 82.53%	Photocatalytic removal of chemical oxygen demand (COD) Removal of lead (Pb)	Sethy et al. (2020)
TiO_2	*Caltropis gigantea* leaf extract	96.7%	Catalytic degradation of metformin by solar photocatalysis	Prashanth et al. 2021)
Metal -FeNP	Green tea extract	81% 31%	Removal of methyl chlorobenzene (MCB) from wastewater Removal of chemical oxygen demand(COD)	Kuang et al. (2013)

(continued)

Table 2 (continued)

Type of nanoparticles	Plant source	Absorption capacity/percentage degradation	Applications	References
FeNP	*Eucalyptus leaf* extract	N-71.7% COD-84.5%	Removal of total nitrogen and COD from wastewater	Wang et al. (2014)
Nickel NP	*Camellia sinensis* leaf extract	94.5%	Degradation of CV dye	Bibi et al. (2017)
AgNP	*Morinda tinctoria* leaf extract	95.3%	Degradation of methyl blue dye	Vanaja et al. (2014)
FeNP	*Camellia sinensis leaf extract* *Vitis vinifera extract*	RR-52% RY-64% RB-78% RRB-76% RR-49% RY-61% RB-71% RRB-68%	Degradation of Reactive Red 195 (RR), Reactive Yellow 145 (RY), Reactive Blue (RB) and Reactive Black 5 dyes	Raman et al. (2021)
AgNP	*Nigella sativa* seed extract	98.5%	Decolouration of Congo red dye	Chand et al. (2021)
AgNP	*Zanthoxylum armatum* leaves	SO-1.02 × 10^{-3} MR-1.03 × 10^{-3} MO-1.86 × 10^{-3} MB-1.44 × 10^{-3}	Dye Degradation of safranin O (SO), Methyl red (MR), Methyl orange (MO), Methyl blue(MB)	Jyoti and Singh (2016)
AgNP	*Calendula officinalis*	MB- 27.12% MO-69.79%	Degrade methyl blue (MB) and methyl orange(MO) dye in aqueous solution	Chandra Paul et al. (2020)
AuNP	*Capsicum annum*	Pb-63.46% Cd-60.20% Cu-51.50% Fe-68.20% Ni- 42.18% Co- 23.47% Mn-21.62% Zn-35.37% Pb-75.75%	Removal of contaminant present in water including Pb, Cd, Cu, Fe, Ni, Co, Mn and Zn	Baran et al. (2020a)

(continued)

Table 2 (continued)

Type of nanoparticles	Plant source	Absorption capacity/percentage degradation	Applications	References
SnO_2 NP	*Vitex agnus castus* fruit extract	RhB- 91.7% Co^{+2}–93.6%	Photocatalytic degradation of Rhodamine B dye and removal of heavy metal Co^{+2}	Ebrahimian et al. (2020)
FeNP	Green tea extract	19.9 mg/g	Removal of As (V) at 298 K	Wu et al. (2021)
Oxide of metal—iron	Pomegranate seed extract	95.08%	Degradation of reactive blue 4 dye from aqueous solution	Bibi et al. (2019)
iron	*Cynometra ramiflora* fruit extract	94%-91%	Degradation of methylene blue dye under sunlight irradiation	Bishnoi et al. (2018)
iron	*Piper betle* leaves	MG-93% MO-73.29%	Degradation of malachite green (MG) and methyl orange (MO) dyes	Badmapriya and Asharani (2016)
Copper	*Carica papaya* leaf extract	–	Degradation of Coomassie brilliant bye R-250 dye	Sankar et al. (2014)
Copper	*Pridium guajava* leaf extract	NB- 93% RY160-81%	Degradation of Nile blue (NB) dye and reactive yellow (RY160) dye	Singh et al. (2019)
Alpha- manganese oxide	*Ficus Retusa*	MO- 116.1 mg/g MR- 74.02 mg/g	Determining adsorption efficiency of azo dye like methyl red and methyl orange	Srivastava and Choubey (2021)
Zinc	*Carissa edulis* extract	97%	Degradation of Congo red dye	Fowsiya et al. (2016)
Zinc	*Trianthema Portulaca* strum's extract	91%	Show antimicrobial activity against Staphylococcus aureus and E.coli, Antifungal activity against *Aspergillus niger* and *Aspergillus flavus* and degradation of Synozol Navy blue-KBF textile dye under solar irradiation	Khan et al. (2019)

(continued)

Table 2 (continued)

Type of nanoparticles	Plant source	Absorption capacity/percentage degradation	Applications	References
Zinc	*Calliandra haematocephala* leaf extract	88%	Catalytic degradation of methylene blue dye under solar radiation	Vinayagam et al. (2020)
Zinc	*Saponaria officinalis*	42%	Degradation of methyl blue and exhibit antibacterial property	Sharma et al. (2019)
Bimetallic—Fe/Pd NP	Grape leaf aqueous extract	98%	Removal of orange (II) dye	Luo et al. (2016b)
nZVI/Ni	Green tea extract	100%	Removal of Cr(VI) for groundwater	Zhu et al. (2018)
Ni/Fe NP	*Punica granatum* peel extract	76–78%- groundwater 68–72%- lake water 92–94%- DW	Removal of tetracycline from ground water, lake water and deionized distilled water	Ravikumar et al. (2019)
nZVI-Cu NP bentonite supported nZVI-Cu NP	Pomegranate rind extract	72% 95%	Removal of antibiotic tetracycline from natural water	Gopal et al. (2020)
Ag/AgCl NP	*Azadirachta indica*	92.5%	Degradation of methyl blue dye from aqueous solution and exhibit antibacterial property	Panchal et al. (2021)
Mn:CeO_2	*Cassia angustifolia* Seed extract	–	Photo degradation of malachite green dye	Antony and Yadav (2021)
$NiFe_2O_4$ NP	*Juglans regia*	85%	Photocatalytic degradation of congo red dye and ciprofloxacin from water	Taj et al. (2021)

5.3 Metal and Metal Oxide Nanoparticle

Metals and oxides of metal nanoparticles are governed by physical and chemical properties of metals which play a major role in providing stability. Also, catalytic property enhances the degradation of contaminants like toxic metals, organochlorinated pesticides, polychlorinated biphenyl (PCB) (Nguyen et al. 2018). Metal oxide nanoparticles are very specific to size, shape as well as nanostructure. They have high density which results in small size of nanoparticles. So much of concern with the size is related to reactivity, magnetic and electric property of nanomaterials. Rather than remediating wastewater, iron oxide and magnetite are also considered as a potential approach in MRI and MSD. Similarly, silicon dioxide, manganese oxide, copper oxide, zirconium oxide, acts as catalysts in oxidation process and also possess electrolytic property. CeO_2 nanoparticle synthesised from *Oleo Europaea*, *Rubia cordifolia* leaf show various catalytic properties, used in medical sciences and optical sensor technology (Nadeem et al. 2020). Iron oxide nanoparticle conflicts our interest, used in removal of contaminants from water. iron oxide nanoparticle potentially removes lead, cadmium and chromium from aqueous solution (Ehrampoush et al. 2015). Bio-compounds such as coumarin and olefins facilitates reduction of metal ion by donating electron followed by hydroxyl and methyl group containing compound. polyphenolic compounds chelate the metal ion therefore nanoparticle can be reused up to five cycles without losing stability (Groiss et al. 2017). Several examples of metallic nanoparticles and metal oxide nanoparticles are given in Table 2.

5.4 Bimetallic/Trimetallic Nanoparticle

Combination of metals by optimizing their energies and reaction conditions refers to bimetallic or trimetallic nanoparticles based upon the number of metals participating in the formation and enhance their remediation strategy. They can be used as multipurpose tool. Bimetallic increases the efficacy of reduction of metal by altering the individual component, or geometrical structure to achieve better functionality and application. *Phoenix dactylifera* synthesised bimetallic copper-silver nanoparticle exhibit catalytic property to degrade methyl blue from aqueous solution and antibacterial activity against *Bacillus subtilis* and *Escherichia coli* (Al-Haddad et al. 2020). Some monometallic nanoparticle aggregates easily and loss their reactivity therefore addition of catalytic metal is preferred which result in formation of bimetallic nanoparticle in order to increases the reactivity, catalytic selectivity and great efficiency useful in multiple applications like exhibiting antimicrobial property, anticancer property and potent nano catalyst. Green synthesis of Au–Ag bimetallic nanoparticle by using *Pulicaria undulata* extract shows catalytic activity in reduction of 4-nitrophenol to 4-aminophenol under the influence of sodium borohydride (Khan et al. 2020). Silver nanoparticle which exhibits excellent antibacterial property used in pharmaceutical sector and antimicrobial property used in cosmetics, biosensor, food processing etc,. On the other hand, gold nanoparticle used in medical field like cancer therapy. The idea is to combine the dual nature of silver and gold nanoparticle to improve the application and efficiency in bimetallic nanoparticle. Fe/Pd nanoparticle synthesis from aqueous extract of grape leaf in order to determine the reactivity of bimetallic nanoparticle in comparison with monometallic iron nanoparticle (Luo et al. 2016a).

Green synthesis of Au/Pt/Ag trimetallic nanoparticle using *Lamis albi flos* extract for determination of antimicrobial activity (Dlugaszewska and Dobrucka 2019). *Vitex angus-castus* synthesized Au-ZnO-Ag trimetallic nanoparticle has ability to degrade methylene blue dye within 36 min as well as 97% degradation of crystal violet dye (Dobrucka 2019). Nanomaterial along with nanoparticles is more specific in treatment of water as it increases the degradation speed and reactivity, preventing any kind of by product formation. Nanomaterials when reacts with pollutants (inorganic/organic) or heavy metals, result in photocatalytic reaction, chemical reaction, absorption and adsorption. Au–Ag-Sr nanoparticle synthesize from root extract of three different plants namely *Coriandrum sativum, Aloe indica* and *Plectranthus amboinics* by using gold chloride, silver nitrate and strontium chloride as a metal precursor (Binod et al. 2018). Trimetallic Fe-Ag-Pt synthesize from *Platycodon grandiflorum* shows excellent catalytic efficiency in reducing 4- nitroaniline to p-phenylenediamine within 25 min and complete decolourization of rhodamine B dye within 15 min (Basavegowda et al. 2017).

6 Remediation Potential of Phyto-Nanoparticles in Wastewater Treatment

6.1 Dye Degradation from Wastewater

Heterogeneous photocatalysis is technique used for purification of wastewater. The mechanism can be classified based upon the type of catalyst. Photo-decolouration involves photo-oxidation and photo-reduction in which dye converted to its original form. Photo-degradation converted the dye into some non-toxic stable product. Photo-mineralization gives the potential to decompose the dye into carbon dioxide, water, nitrate, etc.. Photo-decomposition involves photo-degradation and photo-mineralization. In photocatalytic degradation of dye, excited electron moves from valence band to conductance band, generating electron hole pair which result in oxidative photo-degradation by generating the hydroxyl radical. thus, atmospheric oxygen comes in contact with electron resulting in complete degradation of dye to non-toxic by products such as carbon dioxide, water molecule, etc. (Marimuthu et al. 2020). TiO_2 can be used as potent oxidizing agent because of its high energy gap between valance band and conductance band. A suitable metal which is stable, act as good conductor as well as absorbs light easily can be doped with TiO_2 to reduce the energy gap. Electron captured by oxygen in water forming a free radical. Hole created by due to excitation of electron finally accepts the electron from absorbed dye resulting in reduction of dye. Determination of dye degradation through UV-Vis spectroscopy carried out by evaluating the optical density of nanoparticles. The electron transfer from donor to acceptor held on the surface of nanoparticle thus, it acts as a catalyst for the reaction. The dye degradation is dependent upon the size and shape of synthesized nanoparticle and the target dye chemical structure. Silver and palladium nanoparticle synthesized from *Daucus carota* leaf extract shows high efficiency of removing rhodamine 6-G dye. Catalytic property was evaluated that came to be 98% and 89.4% of rhodamine dye get decolourized within 2 min and 30 min under the treatment of palladium nanoparticle and silver nanoparticle respectively (Joseph Kirubaharan et al. 2020). Silver nanoparticle synthesized from *Albizia procera* shows promising results in removing methyl blue dye. Optimized pH at 11.5 to get removal efficiency of 99.6% of methyl blue dye. Similarly, temperature optimized at 30 degree Celsius and contact time of around 70 min to get removal efficiency of 93.65% and 51.54% respectively (Rafique et al. 2019). Green synthesis of copper nanoparticle is successful in degrading 96% of methyl blue dye from aqueous solution under optimize conditions (Sinha and Ahmaruzzaman 2015). Several studies conducted on applicability of plant-based nanomaterials for dyes and heavy metal pollution, UV–Vis range for dye degradation analysis, and additives etc., have been presented in Table 3.

6.2 Heavy Metal Removal from Aqueous Solution

Adsorption mechanism assisted by electrostatic interaction, complexation and adsorbent nature. electrostatic interaction between metal ion and adsorbent are driving forces for adsorption process (Sarma et al. 2019). plant mediated nanoparticles provide a function group which increases in binding site and form surface complex by electrostatic attraction. Adsorbate interaction with adsorbent determines with the help of different isotherm include Freundlich isotherm and Langmuir isotherm. Kinetic model include pseudo first order reaction and pseudo second order reaction helps in determining the type of adsorption on adsorbent along with reaction pathways. combination of theoretical and experimental calculation obtained from adsorption isotherms and kinetic model explain the efficient removal of heavy metal from the water (Al-Senani and Al-Fawzan 2018). Adsorption capacity of silicon nanoparticle synthesized from plant extracted *Saccharum ravannae, Saccharum officinarum* and *Oryza sativa* found to be above 95% for Pb^{2+} and Ca^{2+}. The adsorption studies of Silica nanoparticle is done by optimizing various parameters like pH, metal ion concentration, temperature, adsorbent dose and contact (Sachan et al. 2021). The adsorption mechanism is understood by various isotherm models like Freundlich isotherm and Langmuir isotherm and thermodynamic studies. Iron nanoparticle synthesized by using tea extract was irradiated with ^{60}Co gamma radiation. The adsorption capacity of Cu^{2+} ions in aqueous solution before and after radiation was observed 81.67% and 97% respectively under optimize condition (Amin et al. 2021).

Table 3 Phytonanoparticles showing removal efficiency of heavy metals and dye degradation from aqueous solution

Nanoparticle	Heavy metal/dye	UV- Vis of dye/NP (obs.)	Reaction time	Additives	pH	Percentage removal/observation	References
Silver nanoparticle	Methylene blue dye	660 nm/420 nm	0–72 h	–	5.6 6.6 7.6 8.6	95.3% at 72 h at 8.6 pH	Vanaja et al. (2014)
Silver nanoparticle	Congo red dye	SPR band—498 (pie-pie) 338(n-pie)	At interval of 1.5 min upto 15 min	Sodium borohydride	–	Not given	Kolya et al. (2015)
Silver nanoparticle	Congo red dye	496 nm dye/426 AgNP	0–13 mintues	Sodium borohydride	–	98.5 upto 5 cycles	Chand et al. (2021)
Silver nanoparticle	Safranin O Methyl red, Methyl Orange, Methyl blue (10 mg/l)	519 nm 415 nm 460 nm 664 nm (AgNP- 419 nm)	0–24 h	–	–	**Degradation rate constant** 1.02×10^{-3}/min 1.03×10^{-3} 1.86×10^{-3} 1.44×10^{-3}	Jyoti and Singh (2016)
Silver nanoparticle	Brilliant blue Tartrazine Carmoisine	629 nm 420 nm 504 nm	**Reaction rate constant-k** 0.2097 0.0076 0.0496	Sodium borohydride	–	–	David and Moldovan (2020)

(continued)

Table 3 (continued)

Nanoparticle	Heavy metal/dye	UV- Vis of dye/NP (obs.)	Reaction time	Additives	pH	Percentage removal/observation	References
Silver nanoparticle	Methyl blue Methyl orange	500–700 350–550 nm	1 mM 5 min 2 mM–5 min 1 mM 5 min 2 mM 5 min	Sodium borohydride	–	27.12% 18.08% 69.79% 42.11%	Chandra Paul et al. (2020)
Silver nanoparticle	Hg2 + detection	Not mentioned (given a graph)	–	–	pH- 3–8 (no effect of pH)	Dark yellow changes to colourless	Roy et al. (2015)
Silver nanoparticle	Detection of Hg2+	Not mention (graph given)	–	–	–	Dark brown changes to colourless	Tagad et al. (2017)
Gold nanoparticle	Toxic metal removal Pd Cd Cu Fe Ni Co Mn Zn Pb	Absorption capacity of adsorbent used in aqueous soln. of toxic metal was determined by batch method	120 min	–	5 6 6.5	Removal% 63.46 60.20 51.50 68.20 42.18 23.47 21.62 35.37 75.76	Baran et al. (2020b)

(continued)

Table 3 (continued)

Nanoparticle	Heavy metal/dye	UV- Vis of dye/NP (obs.)	Reaction time	Additives	pH	Percentage removal/observation	References
SnO_2 nanoparticle	Photodegradation of RhB Removal of heavy metal Co + 2	553.5/370–375 nm	190 min 60 min	–	7 >7 >8	91.7% Adsorption capacity 93.6% Formation of soluble hydroxyl complex in excess of OH Cobalt hydroxide started to ppt from aq. Sol. & adsorption was impossible	Ebrahimian et al. (2020)
Zero valent iron nanoparticle	Adsorption of Cr(VI)	–	30 min 90 min	Oenothein B	–	Adsorption efficiency 98.1% 71.9%	Madhavi et al. (2013a)
Maghemite nanoparticle	Removal of heavy metal in fly ash Pb Cd	–	1 h 24 h 1 h 24 h	–	7	85.56% 90.85% 67.8% Conc. Reached below the detection level of ICP-OES (not defined)	Yadav and Fulekar (2018a)

(continued)

Table 3 (continued)

Nanoparticle	Heavy metal/dye	UV- Vis of dye/NP (obs.)	Reaction time	Additives	pH	Percentage removal/observation	References
Iron based nanoparticle	Removal of Cr (VI) and Cu (II) when co-existed together Cr (VI) and Cu (II) present separately Removal efficiency of Cr Cu (II) Pb (II) Zn (II)	– –	1 h T = 288 K T = 308 K	–	5	Cr (VI)- 58.9% Cu (II)-33% Cr (VI) = 74.2%; Cu (II) = 45.2% Cu-26.8% Cr-50.7% Cu-40.8% Cr-62.6% Cr = from 75.1% to 50.8% Cu 28.3% to 64.2% 44.8 21.5 31.4 10.8	Ajitha et al. (2015)
Zero valent Silver nanoparticle	Cadmium removal from aqueous solution	–	40 min	–	6	85% 75.5%	Al-Qahtani (2017)
Zero valent iron nanoparticles	Removal of Cr (VI) from aqueous solution **TV-Fe** (higher% removal) UD-Fe RD-Fe	Adsorption capacities 466 462 453.7	1–10 1–10 30 min	–	2–9	91.75% 60.95% 97.5% 97.1% 96.94%	Fazlzadeh et al. (2017)
Silver nanoparticles	Chromium removal	350 nm	–	Activated carbon	–	Absorbing pattern was irregular	Samrot et al. (2019)

Iron oxide nanoparticles synthesized from *Ramalina sinesis* extract was successfully able to remove lead and cadmium by following Langmuir adsorption isotherm and Freundlich adsorption isotherm respectively also both removal follows second order kinetic model with removal capacity of 82% for lead and 77% for cadmium under the pH ranges between 4 to 5 and initial ion concentration was 50 mg/l with 70-degree temperature in 1 h (Arjaghi et al. 2021).

Factor Affecting Removal of Heavy Metal

Biomass concentration

Increase in biomass concentration results in increases in number of metal binding sites hence increase adsorption efficiency. Lead, zinc and chromium removal efficiency increases from 94.35% to 100%, 44% to 36.9% and 55% to 81.9% respectively, when concentration of biomass changes from 0.2 g to 2 g (Chandra Sekhar et al. 2003). At very high concentration of biomass, metal removal efficiency decreases because of the reduction average distance available for absorption sites due to the aggregation of biomass. Copper uptake efficiency decreases from 85 to 58%. With increased biosorption concentration from 0.5 g/l to 2 g/l (Chandra Sekhar et al. 2003). From comparative studies, we can conclude that low biomass concentration results in decreased biosorption efficiency. With increase in dosage of zerovalent iron nanoparticle from 0.5 g/l to 2.0 g/l, the removal efficacy of Cu, Zn, Cr and Pb was increased from 76%, 14% 51% and 78% to more than 80% in both Zn and Pb followed by complete removal of Cu and Cr within 30 min. higher pH was obtained at higher biomass concentration for the removal of heavy metal like Zn, Pb, Cr and Cu (Chen et al. 2008). Since metal removal occurs at the surface of the nanoparticle, the maximum removal of heavy metal become unchanged. Also, removal efficiency of heavy metal is inversely proportional to the initial metal ion concentration under a constant biomass concentration. Magnetite concentration was increased from 1 g/l to 4 g/l in order to increases the removal efficiency of hexavalent chromium from 29.1% to complete removal (Ataabadi et al. 2015).

p^H

The important factor is pH, affecting the chemistry of metal ions and biosorbent by influencing their solubility as well as toxicity. Metal uptake related to complexation chemistry of metal ion and behaviour of function group present at the surface of the plant. The carbonyl group of biomolecule provide a negative charge at acidic condition due to which an electrostatic interaction occurs between two cationic which results in biosorption of metal. At low pH, H + ions are occupied in the active sites of adsorption, with increases in pH these sites become free and available for the heavy metal on the surface of adsorbent. In the pH range of 3–7 there is slight increase in removal process of heavy metal like Cr(VI) removal favoured at low pH due to the positive charged surface of nanoparticle at low pH attract the negative charge anions as a result an electrostatic attraction occurred (Weng et al. 2016b). Also, at some point adsorption sites become independent of pH change indicating saturation point which is attributed to formation of hydroxyl complex in excess of OH ion, depends upon the type of heavy metal that is to be removed and the surface of nanoparticle. Adsorption capacity increases with increases in pH until it attains a maximum biosorption at optimum pH because further increase in pH will result in precipitation of metal. Addition of NaOH and HNO_3 leads to increment and reduction of pH respectively. Studies have been be conducted over silver nanoparticle synthesis from different plant extract like neem leaf, sun dried leaf of neem, neem bark, mango leaf, sun dried leaf of mango, green tea and pepper seed extract selectively sense heavy metal like mercury, lead, zinc, cobalt and zinc over a wide range of pH from initial pH and highest pH that maintains acidic and basic environment respectively. At initial pH 4, mercury was detected by neem bark synthesized silver nanoparticle but it was not detected when increasing the pH up to 9 rest other metal like cobalt and nickel was detected (Karthiga and Anthony 2013).

Temperature

Higher temperature increases the solubility of metal ion in water, therefore biosorption of these metal ions become difficult. Temperature depends upon the type of chemical reaction occurring between biosorbent and metal ion. Metal removal efficiency decreases with increase temperature for exothermic biosorption as the absorption of the molecule becomes easier and when temperature rise, desorption of the molecule take place. Sorption of lead and cadmium by Caladium bicolor biomass is affected between 30 to 80 degrees Celsius. Increase in temperature results in weak attractive forces between bisorbent and bisorbate, thinning of the outer boundary layer which help the metal ion which lead to decreases in sorption. Lower temperature helps in enhancing the activation energy and solubility of chemical to increase the rate of the reaction. Uptake of lead ion to biosorbent by peanut shell effectively remove 66% of lead at 20 degrees Celsius. Removal efficiency decreases with increase in temperature from 20 degree Celsius to 40 degree Celsius showed exothermic biosorption (Taşar et al. 2014). In zinc oxide nanoparticle, increase in temperature from 30 to 70-degree result in increase in adsorption capacity of lead from 16.19 mg/g to 19.96 mg/g (Azizi et al. 2017) whereas increase in temperature from 288 to 308 K results in higher adsorption of Cr (VI) and Cu (II) on the surface of iron nanoparticle (Weng et al. 2016b). Interaction between available sites of the absorbent with absorbate efficiently increases the removal rate from 73.8% to 100% of the toxic hexavalent chromium with increase in temperature from 25 degrees Celsius to 40 degrees Celsius respectively (Ataabadi et al. 2015). Therefore, temperature depends upon the nature of the process.

Initial metal ion concentration

Adsorption capacity increases with initial metal ion concentration. When all binding sites occupied with metal result in increase in concentration slope. There would be decrease in adsorption rate with further increasing the concentration of metal ion as all the obtainable nanoparticle sites are filled. Removal efficiency of chromium is 86% with initial iron concentration 30 mg/l. increase in iron concentration up to

150 mg/l result in deduce the adsorption efficiency from 86 to 70% (Al-Qahtani 2017). 100 mg/ml is the concentration of metal ion where the removal efficiency of chromium is highest that is 42.37%. Further increasing the metal ion concentration up to 200 mg/ml, the removal efficiency of chromium decreased from 42.37% to 33.75% as all available site occupied which result in closing of the pores and hence metal ion preventing penetrating deep into adsorbent pore (Shaik et al. 2020b). Zerovalent iron nanoparticle effectively remove hexavalent chromium with adsorption efficiency of 98.1% observed within 30 min when initial metal ion concentration is 200 mg/ml. adsorption efficiency decreases to 71.9% with increase in metal ion concentration up to 400 mg/ml (Madhavi et al. 2013b).

Contact time

Contact time indirectly effects the rate of adsorption and the data was analysed by kinetic models namely pseudo first order and pseudo second order model. It has similar affect as of initial metal concentration, biosorption increases with increase in contact time. When all the binding sites become fully saturated, the reaction become independent of time. Cadmium removal from aqueous solution carried out at optimum contact time of 40 min. over that time, no further increase in cadmium removal efficiency (Al-Qahtani 2017). Absorption capacity of hexavalent chromium decreases from 98.1% to 72.9% with increase in contact time of 30 min to 90 min (Madhavi et al. 2013b). Maghemite nanoparticle effectively remove lead and cadmium from fly ash with respect to time. The removal process observed upto 0 to 24 h. It has been observed that initially, 85.56% of lead is removed and cadmium removal was not defined by ICP-OES. After 2 h, 67.8% removal of cadmium was detected followed by detection of 90.85% lead at 24 h (Yadav and Fulekar 2018b). Most of the adsorption site are occupied initially that's why it attains an equilibrium and removal percentage does not increase rapidly.

6.3 Desorption Analysis

Desorption studies refer to the removal of absorbed metal from the surface of absorbent. There are various types of desorbing agents including tap water, sodium hydroxide, hydrochloric acid, deionized water, sulphuric acid, ammonium hydroxide, potassium hydroxide. For example, the desorption efficiency of HCl and H_2SO_4 is very high in removing the Cr (VI) and Pb (II) ions respectively from the adsorbent. It was found that adsorbent exhibited good removal efficiency of chromium and lead up to five consecutive cycles (Bayuo et al. 2020). Sodium hydroxide is considered best for the removal of chromium up to five consecutive cycles as its desorption efficacy reduces from 98 to 89% after the fifth cycle (Al-Haddad et al. 2020). Chromium desorption from red peanut skin synthesized iron nanoparticle was done by using 16 M hydrochloric acid and distilled water as desorbents. Iron nanoparticle dried by vacuum under 40 degrees Celsius and 60 degrees Celsius as well as air dried at room temperature. Maximum chromium efficiency that is 100% within 1 min was achieved by vacuum dried at 60 degrees Celsius then followed by vacuum dried iron nanoparticle at 40 degrees Celsius. It has been hypothesized that with increase in temperature of vacuum drying, reduction of radius of iron nanoparticle occurred due to which at higher temperature chromium removal occurred more rapidly. Minimum removal efficiency was 90% in 4.5 h given by air dried iron nanoparticle due to formation of ferrosoferric oxide (Pan et al. 2019). Bimetallic silver- copper nanoparticle at zinc oxide surface efficiently able to degrade rhodamine B to leuco rhodamine B dye and Congo red dye within 12 s and 9 s respectively up to five consecutive cycles because after that leaching of metal started which can be determined by ICP-AES (Manjari et al. 2020).

7 Challenges and Future Prospects

Nanoparticles have been successfully found active in remediating the waste water but at the same time, they also face some critical challenges in their synthesis. Since we discussed the green synthesis of nanoparticle, first of all there is need for the selection of appropriate plants which are rich in phytocompounds like polyphenol, flavonoids, terpenoids, alkaloids etc. Identification of particular biochemical compound present in the plant that acts as a reducing capping and stabilising agent of the nanoparticle is also necessary. After selection of suitable plant with exact targeted active phytochemical compound, the selection of suitable metal precursor is needed. Nanoparticles have small size and larger surface area due to which they have more interaction sites available on their surface with cells. This gives several toxic biological responses. Smaller the size of the nanoparticle, greater the toxicity attributed with it. Toxicity of nanoparticle depends upon various parameters including structure, shape, hydrophilicity, composition, concentration, reaction temperature and surface chemistry. The toxicological effects of nanoparticle affect humans and environment. Acute and chronic toxicity occur upon oral exposure of several metal nanoparticles. Due to their small size, some nanoparticles pass the blood brain barrier which may lead to dangerous neural diseases. Reuse of nanoparticles is another challenge.

Nanotechnology is establishing in every field, be it agricultural engineering, drug delivery, X-ray imaging, dentistry, cosmetics or other environmental aspects. From past studies, we can conclude that nanoparticle synthesis by traditional methods requires heavy machine equipment and toxic chemicals with complex handling which is a very costly procedure. To overcome these disadvantages, we moved to green synthesis of nanoparticles from plant, algae, bacteria and fungi. This chapter presents an insight into nanoparticle synthesis from plant extract. Exact mechanism of the targeted biochemical process occurs at cellular level for increased production of nanoparticle, which can be discovered in further studies. More studies can be carried out on capping agents which prevent the further reaction aggregation and result in providing stability for a longer period of time. We have to expand the area of the sources of nanoparticle synthesis so that they can be synthesized from waste materials like algae and several other plants which are cheap, easily available and suitable. Very limited studies have been carried out on the toxicity of phyto-nanoparticles. We need to develop several strategies for detoxification of green nanoparticles. Research cannot be restricted to water pollutant removal only. We can explore new opportunities in the environmental air purification also, to reduce the amount of particulate matter (PM), ozone, sulphur, etc.

8 Conclusion

Pollutants such as heavy metal and various organic or synthetic dyes contaminate the water bodies leading to water pollution thus creating many problems related to human and animal health and affecting the environment. Therefore, there is an immediate requirement of water treatment. Phyto-nanotechnology holds an enormous potential to remediate wastewater as it offers inexpensive, eco-friendly and efficient way. In this chapter, we have discussed the various types of plant synthesized nanoparticles which can be used to remediate wastewater. The exploration of biotechnological prospects for nanoparticles synthesis at industrial scale remediation of wastewater is needed. The article concludes that phytonanoparticles can be a potential, inexpensive and ecofriendly agents for environmental pollution remediation.

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Bacteria and Pollutants

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Abstract With the immense technological advances in the twenty-first century, regarding the twenty-first century as the technological era would not be an exaggeration. These advances have revolutionized almost all the arenas be it in terms of automobiles, electronics, medical and healthcare, agriculture and food crops, etc. However, recent decades have focussed on the sustainability of the environment to a similar extent as to human advancements and benefits. In this regard, waste management in terms of both solid as well as liquid waste, is one of the most important sectors which needs and has gained immense value. Compared to traditional physical and chemical treatment methods, bioremediation technology utilising microorganisms and their aggregates is acknowledged as an efficient and economic green treatment (biological origin) method. These microbes have been used in various ways some of which include direct mixing, immobilisation or encapsulation. Analysis of these microbes in different set ups including wastewater treatment plants or solid waste removals have been studied extensively and plethora of knowledge has been gained in this regard suggesting that bacterial populations differ greatly depending on the operating circumstances of the waste disposal and the properties of the waste. It has also been observed that variations in the organic or nutritional composition of the waste might potentially impact the bacterial community. Emerging contaminants (ECs), which include endocrine-disrupting compounds, pharmaceuticals (lipid regulators, antibiotics, diuretics, non-steroid anti-inflammatory drugs, stimulant drugs, antiseptics, analgesics, beta blockers), detergents, disinfectants, and personal care products, have become a grave concern owing to their bioactive presence on environmental matrices. It is therefore vital to undermine the potential of microbes in the proper disposal of both solid and liquid waste. The current chapter, in this regard, is about the microbes that have been identified as potential waste disposal mechanisms. In addition, the chapter also focuses on the novel technologies that have been employed using microbes for waste disposal.

Keywords Pollutants · Bacteria · Emerging contaminants · Green treatment · Novel technologies · Solid waste

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1 Introduction

Since time immemorial we have observed that every discovery brings with itself a new set of advantages and challenges opening even more scope for research and development and breaking the boundaries that were once created. Needless to say, the inventions and discoveries in the twenty-first century have been immense and in this regard the challenges too have been extensive. One of those challenges include the generation of large amounts of waste products.

The acknowledgement of the excessive rise in the waste generated has been done via almost all sections of the society and affected the economy of the world altogether. Some of the instances from the same are shared here. “Poor waste management is contaminating the world’s oceans, clogging drains and causing flooding, transmitting diseases, increasing respiratory problems from burning, harming animals who unknowingly consume waste, and affecting economic development, such as through tourism,” said Sameh Wahba, World Bank Director for Urban and Territorial Development, Disaster Risk Management, and Resilience (Ayilara et al. 2020). According to the World Bank’s What a Waste 2.0 report, the world produces 2.01 billion tonnes of municipal solid waste per year, with at least 33% not being managed in an environmentally sound manner. Take, for example, plastic garbage, which is choking our oceans and accounts for 90% of all marine debris. In 2016, the globe produced 242 million tonnes of plastic waste, which is roughly equivalent to 24 trillion 500-mm, 10-g plastic bottles (Ayilara et al. 2020).

The recent years have shed light on the essentiality of environmental sustainability for the survival of humans and other life forms on earth leading to the generation of Sustainable Development Goals commonly called as SDGs in 2015 by the United Nations of which SDG No. 6 majorly focuses on waste disposal and treatment. A decade back these were considered as the sources of infection spread in addition to creating sub-standard conditions for living. However, research in the last decade has shed light on the role of microbes particularly bacteria in the removal or disposal of these waste products referred to as bioremediation. This chapter is therefore focused on the waste generated owing to the plethora of advancements and industrialization in the twenty-first century and the traditional strategies employed for the disposal of this waste. Also, the chapter described the role of microbes employed in the disposal of solid as well as liquid waste emphasizing on the novel strategies employed for the same.

2 Novel Waste Generated in the Twenty-First Century

Waste is usually referred to as the materials or substances that are to be discarded after primary use. However, there is no accurate definition of waste products since waste for one can be the raw material for the other and thus a huge amount of focus is on the reuse and recycling of the same. Similarly the classification of the waste can also be done on various parameters such as on the basis of physical state (solid and liquid), the toxicity (hazardous and non-hazardous) and the source (municipal or industrial waste). All these waste types have been described briefly in the upcoming section.

3 Solid and Liquid Waste

As per the physical state, waste is categorized as liquid or solid waste wherein liquid waste include dirty water, organic liquids, wash water, waste detergents, industry effluents and even rainwater. Thus, this waste can be sourced via households or industries both and depending on the components present can be hazardous or non-hazardous. However, it is quite clear that the household waste is usually non-hazardous. On the other hand, solid waste includes the waste from household such as municipal waste, plastic waste etc. or industrial waste such as chemicals, construction or demolition waste. The solid waste is usually categorized as:

- **Plastic Waste**: This includes bags, containers, jars, bottles and a variety of other items found in any household. Though plastic is non-biodegradable, it can be recycled in various forms.
- **Paper Waste**: Packaging materials, newspapers, cardboards and other items are included in this category which can be readily recycled and reused.
- **Tins and Metals**: This includes sheet metal, siding, roofing, rebar, flashings, pipes, window frames, doors, furnaces, duct work, wire, cable, bathtubs, fencing, bicycle frames, automotive body components, machines, garbage cans, metal furniture, tyre rims, propane cylinders and other ferrous and nonferrous metallic items.
- **Ceramics and Glass Waste**: Ceramic waste powder (CWP) is produced during the polishing of ceramic tiles and is thrown in landfills, where it can pollute the soil, air, and groundwater, causing major environmental issues. Another waste product that is produced in great amounts and is difficult to eliminate is waste glass. It is well known that the majority of waste glass, particularly container glass, is collected, remelted, and utilized to make new glass. However, not all waste glass can be used in the manufacture of new glass and thus becomes an accumulated waste.

4 Hazardous and Non-Hazardous Waste

As per toxicity of the generated waste, it can be segregated into hazardous and non hazardous waste. As per conventional definition, hazardous wastes are the ones which are potentially harmful to the humans and the environment and thus needs to be controlled with utmost care. These are described as the ones which are ignitable, corrosive or reactive and meet one of the following criteria: ignitability, corrosivity, reactivity, or toxicity along with ecological, geological, and environmental calamities that last a long time well. These are usually produced owing to manufacturing operations. These include radioactive materials, medical wastes, combustion waste, waste from mining etc. These categories are described briefly in the upcoming section.

- **Medical Waste**

According to US EPA 2022 (United States Environmental Protection Agency) this includes any solid waste generated during diagnosis, treatment or biological research and is produced by hospitals, private clinics, nursing homes for elderly people, blood banks, autopsy or mortuary facilities, research institutes or laboratories and usually contains blood, body fluids or other infectious elements. As per WHO reports about 85% of the waste generated by health care activities are non-hazardous but the remaining 15% is infectious, chemically reactive or radioactive (Pujara et al. 2019). It is further segregated in the following categories:

- Infectious waste including waste contaminated with blood and other bodily fluids (e.g. discarded diagnostic samples), infectious agent cultures and stocks from laboratory work (e.g. waste from autopsies and infected animals from laboratories), and waste from infected patients (e.g. swabs, bandages, and disposable medical devices)
- Expired, unused and infected medications and vaccines are among the common pharmaceutical waste.
- Waste containing genotoxic compounds (i.e. substances that are mutagenic, teratogenic or carcinogenic), such as cytotoxic medications used in cancer treatment and their metabolites
- Products polluted by radionuclides, such as radioactive diagnostic or radiotherapeutic materials, are examples of radioactive waste.
- Human tissues, organs or fluids, body parts, and contaminated animal carcasses constitute pathological waste
- Syringes, needles, disposable scalpels and blades, and other sharp objects
- Solvents and reagents used in laboratory preparations, disinfectants, sterilants and heavy metals found in medical devices (e.g. mercury in broken thermometers) and batteries are examples of chemical waste.

- **Radioactive Waste**

Radioactive waste is the waste consisting of ionizing radiation-emitting chemicals. As per the regulations of US EPA, these are categorized as high-level, low level and transuranic wastes on the basis of their source and content of the radioactive materials.

- *High Level Radioactive Waste (HLRW)*: This includes the one obtained from production of nuclear materials especially for defense purposes and is held as sludge, liquid or pellets which needs to be solidified before being disposed of. The other sources of this waste include the laboratories or diagnostic and therapy labs which use the radioactive materials in surplus.

- *Low Level Radioactive Waste (LLRW):* The sources for these wastes include nuclear power sector, medical and academic institutions where small amounts of radionuclides are being used in the respective processes such as medical tracers.
- *Transuranic Waste (TRU)*: These include the waste consisting of radioactive elements with atomic numbers higher than uranium such as plutonium and any items contaminated with such elements are included in the category (Gupta et al. 2021). These are further divided into remote handled (RH TRU) which emit penetrating radiation and must be shielded and contact handled transuranic waste (CH TRU) in which the radiations are not penetrating and need not be shielded. 96% of the TRU is composed of CH TRU (Lee et al. 2013). This type of waste is particularly harmful over long periods of time.

Though the waste removal strategies are discussed in great detail in the upcomings sections, radioactive waste disposal requires extra attention particularly the HLRW. To dispose of HLRW, various methods have been proposed, including outer space disposal, ocean disposal, and subsurface burial. For the prospective storage and disposal of HLRW, deep geological disposal in subterranean repositories/tunnels 500–1000 m deep is usually considered. For deep geological HLRW disposal, multi-barrier methods have been proposed, with three consecutive barriers: waste canisters (made of carbon steel, copper, copper steel, and titanium), an engineered structure (made of cement or clay), and a third barrier (known as buffer and/or backfill). Deep geological HLRW disposal's ultimate purpose is to isolate radioactive waste from humans and the environment until residual/leaked radioactive waste (also known as radwaste) has no meaningful impact on humans or the environment. When researchers are attempting to safely dispose of HLRW, they must consider a number of factors.

- **Combustion Waste**

Coal is one the most commonly used fuel in the present scenario and is responsible for innumerable facilities such as electricity and power production, heating, transportation and thus is the major source of air pollution. It can be used alone or in combination with other fuels such as diesel, gasoline oil, natural gas, etc. but is particularly significant in the cases where coal constitutes about 50% of the total fuel (Gupta et al. 2021). This not only is a source of air pollution but also affects the health of the individuals particularly children during and after birth.

- **Extraction and Mining Waste**

Intensive mining activities have resulted in a massive amount of hazardous wastes all over the world which are commonly associated with high levels of acid-generating sulfide minerals as well as potentially toxic metals and metalloids (PTMs) such as As, Sb, Cu, Pb, Cd, Zn, Hg, Ag, Sn, Fe, Al, Mn, Tl, U, Th, and W (Anawar 2015). The extraction and mining waste majorly includes the water used for infiltration of the mine during the extraction process and also the soil and rock created during the process. The entire mining process is a combination of multiple steps such as crushing, grinding, dissolution, calcining etc. that may either lead to an intermediate or a final product (Gupta et al. 2021). The toxic components produced during the process are distributed in the aquatic and terrestrial systems via means of erosion, dispersal, leaching or air. In spite of multiple strategies such as burial, pedogenesis, chemical weathering etc., the potentially toxic metals and metalloids (PTMs) and acid mine drainage (AMD) fractions cause significant damage to the aquatic and terrestrial systems ultimately posing a serious health concern (Anawar 2015).

- **E Waste**

E waste also known as Waste Electrical and Electronic Equipment (WEEE) includes any electrical or electronic device that has been thrown, surplused, obsoleted or broken. Needless to say, with the advancements in technology and industrialization, this is the fastest growing waste throughout the world and thus has become a major public health concern. Besides, the inadequate information regarding its correct disposal has led to its accumulation in households, especially in developing countries like India. This ever-increasing trash is extremely complicated in nature, and it's also a rich source of metals like gold, silver, and copper which may be recovered and reintroduced into the manufacturing process. As a result, e waste trade and recycling coalitions employ a diverse range of people (Mishra et al. 2019). The composition analysis of e waste suggests that metals account for about 60% of the total waste while plastics account for about 30% with about 3% hazardous pollutants (Kishore 2010). Many toxic metallic pollutants, such as lead, cadmium, and beryllium, as well as brominated flame-retardants, can also be found in electronic equipment. Lead is the most extensively utilized dangerous heavy metal in electronic devices for a range of reasons, resulting in a variety of health risks due to environmental contamination.

- **Industrial Waste**

The expansion of industrial sectors such as agro-, food-, paper-, and pulp industries is the need of the hour to meet the demands of the growing population. These industries produce hazardous waste, the majority of which is organic in nature and so is deposited or processed in the environment. These wastes cause increased contamination resulting in high mortality in addition to the physical and morphological changes in the organisms/animals that come in contact. Although the waste generated is toxic, it is primarily composed of macromolecules and bioactive chemicals, making it suitable for extraction and manufacturing of value-added goods. The creation of value-added products such as bioplastics, biofuels, and biosurfactants at the same time improves the process' economics and contributes to environmental sustainability (Gaur et al. 2020).

5 Non-Hazardous Waste

After a detailed description of the hazardous waste, in the upcoming section, the chapter focuses on the non-hazardous waste generated in the twenty-first century. Though it does not appear to be a concern by definition, this form of garbage can cause considerable environmental damage. Non-hazardous waste can be generated during the manufacturing of goods and products, especially in the industrial sector. Electric power generation and the production of materials such as pulp and paper, iron and steel, glass and concrete are other examples. Ash, sludges, antifreeze, grinding dusts, and liquids polluted with non-hazardous chemicals are examples of common industrial pollutants that are deemed non-hazardous in most jurisdictions. This can further be categorized broadly in three types including municipal waste, agricultural waste and construction and demolition waste which are briefly described below:

- *Municipal waste:* It is the most common and socially significant source of the waste and an enormous source of waste particularly in urban areas owing to high population density. Depending on the physical state of the waste, it can be both solid or liquid in nature.
  - Solid waste: A recent study has suggested East Asia and Pacific region to be the source of major amounts of municipal solid waste with China and India accounting for 15% and 11.94% of the global waste respectively (Kishore 2010). Besides, owing to expanding populations and ongoing migration from rural to urban regions, and other factors, many cities in lower-income nations in Africa and Asia are expected to double their MSW generation within 15–20 years (Vikaspedia). According to a study, only 6–7% of MSW gets converted to compost in India, with the rest disposed of through landfilling with almost 50% content as the organic matter thereby further complicating the disposal process (Sharma and Jain 2019).
  - Liquid waste: This includes human waste, kitchen and food processing trash, pathogens, parasite eggs, inorganic materials, sand and grit, and other organic waste components. All hazardous components must be removed from waste, leaving an inert residue that may be properly disposed of. Sewage and sludge are the most common types of liquid waste. Sewage is wastewater that contains human waste, whereas sludge is wastewater that is generated by everyday activities, such as washing and cooking residue, but does not contain human waste. Though it is considered to be non-hazardous, it may contain toxic elements which in the case of developing countries are frequently dumped in natural ecosystems while purified by certain processes in developed countries before disposal.

- *Agricultural waste:* Waste generated during the production and harvesting of crops or trees, as well as animal raising belongs to this category. Animal waste is a subcategory of agricultural waste that includes waste from cattle, dairy and other animal-related agricultural and farming practices (e.g., feed waste, bedding and litter, and feedlot and paddock runoff) (Gupta et al. 2021). This waste is specifically harmful owing to the accumulation of N2O, SO2, CH4, and smoke owing to improper disposal of agricultural waste. Every year, India produces over 350 million tonnes of agro-industrial waste (Katare et al. 2020) which is traditionally burned/incinerated or left to rot in the fields, resulting in the production of smoke, toxic gasses, carcinogens (for example, polycyclic aromatic hydrocarbons, furans, and dioxins), and greenhouse gasses, all of which have negative health and environmental consequences (Sharma et al. 2020). This traditional disposal particularly of pesticide waste results in degradation of natural resources in addition to posing a threat on human health (Ghosh et al. 2018). To combat this waste disposal, research in 4 major categories have been focused including low-carbon and energy utilization of agricultural waste explored by conversion of straw, wood, and other waste into bioethanol to develop the biomass industry, reducing the discharge of agricultural waste contributing to the mitigation of greenhouse gas emissions, the material and energy flow of agricultural waste and the prevention and control of agricultural waste pollution (Ghosh et al. 2018).

- *Construction and demolition waste*: Commonly called the "C&D waste", it is referred to as the waste generated during construction, renovation and demolition activities including land excavation or formation, civil and building construction, site clearance, demolition activities, roadwork and building renovation. Multiple adverse impacts of C&D waste generation are observed including usage of a large amount of land resources for waste landfilling, harming the surroundings by hazardous pollution, and wasting natural resources (Kornberg et al. 2019). It is worrisome to note that India alone produces about 530 million tonnes of this waste being the second largest in the world (According to a report by Earth5R Organization in 2020) and thus must build a comprehensive system to monitor and utilize the same including government-led popular awareness campaigns.

6 Traditional Strategies of Waste Disposal (Solid and Liquid Waste)

The previous sections have described the waste generated in the twenty-first century in great detail therefore this section focuses on the disposal strategies of the same. Multiple strategies have been employed for the same since long ago and these have been varied in terms of the country's economic and technical developments as well as the kind of waste generated. Besides, over the course of time, innovations have been made in the same strategies. This section describes the traditional strategies of waste disposal and treatment focusing separately on the methods for solid and liquid waste disposal. Certain major strategies for the disposal of waste have been described below:

- **Disposal of solid waste**:

Although a majority of the solid waste produced in India is dumped in landfills without appropriate sorting or pretreatment ultimately leading to greenhouse gas emissions endangering both human health and environment (Mohanty et al. 2020), several other strategies have been developed. The major factors to be kept in mind for developing integrated solid waste management include environmental friendliness, cost-effectiveness and social acceptability. Besides, "zero-waste production" and “waste prevention” attempts are the highlights for the same in order to reduce gaseous emissions, solid leftovers and pollution, also contributing to climate and environmental protection (Mohanty et al. 2020). The common methods included in this section include composting, incineration, landfills, mechanical biological treatment and pyrolysis and gasification which are briefly described below:

- *Composting*: It is a way of transforming garbage into a useful product rather than a final disposal method thus ultimately reducing waste. Compost has been used as a soil conditioner in both rural and semi-urban regions for a long time for growth of vegetables and crops. The technologies involve simple processes such as windrow composting involving shredded plant material to automated enclosed vessel digestion of mixed home trash. The aerobic process is termed as composting while the anaerobic is termed as digestion and their hybrids have also been attempted. It is one of the safest ways to dispose off the trash leading to the conversion of organic waste under aerobic conditions and production of useful products like biogas, biofertilizers, etc. The prepared compost is useful for a variety of purposes including boosting agricultural productivity, bioremediation, plant disease control, weed control etc. It also boosts soil biodiversity while lowering the environmental dangers of synthetic fertilizers. Rather than being a natural and uncontrollable process, composting is started and managed in a controlled atmosphere and this is how it is different from decomposition. Though the process is coupled with all these advantages, it also possesses certain disadvantages such as long time duration, disagreeable odor and may contain infections that can tolerate high temperatures to some extent, i.e. thermotolerant pathogens, as well as having insufficient nutrient content (Ayilara et al. 2020). The detailed description and the role of microorganisms in composting is discussed in upcoming sections.
- *Incineration*: Waste destruction via burning and energy generation is referred to as incineration which is also known as “energy-from-waste” (EfW) or “waste-to-energy” though the terms are misleading. This process has been used many times for getting rid of medical waste and recovery of metals and energy in addition to reduction in the volume of waste. Though it is still one of the most widely used methods, release of toxic elements such as dioxins and furans limit its use. Besides, it has also been a technical challenge in developed countries owing to the high amount of organic matter and construction wastes (Kornberg 2019).
- *Landfill*: It is one of the most traditional ways of trash disposal which began with building landfills in abandoned quarries, mining voids and borrow pits. Though the method minimizes environmental problems and was a relatively economical strategy of waste disposal, the increase in generated waste and reduction in available land over the time has forced it to shift to other strategies. Additionally, wind-blown litter, vermin attraction and pollutants like leachate that drain into ground water and the landfill gases, including methane and carbon dioxide are negative impacts of the process. The major anions observed in the leachate include chlorides, nitrates and sulphates owing to sewage, agricultural and animal waste deposits which ultimately contaminates nearby water bodies, underground and surface waters.

- *Mechanical Biological Treatment*: It is a combinatorial method particularly used for organic waste via both mechanical sorting and biological treatment. Depending on the processing order, it can be referred to as MBT or BMT. This is particularly helpful in developing countries where the source-point waste classification cannot always be guaranteed (Fei et al. 2018). The mechanical stage involves the separation of the recyclable materials such as metals, plastic or glass for fuel generation referred to as refuse derived fuels (RDF). The biological method includes digestion or composting depending on whether the method is anaerobic or aerobic, leading to the production of biogas or green energy (optional, only with digestion). Studies have also shown that coupling MBT to a biogas purification system gives about 40% energy efficiency with the lowest amount of pollutants (Fei et al. 2018). Many metro and urban cities in India including Delhi, Mumbai, Ahmedabad, Hyderabad, Indore, and Rajkot, have RDF plants that create fluffy solid fuels and pallets that can be used as an alternative fuel in the industry. This approach is a technically validated MSW disposal technology that minimizes environmental and land consequences. RDF has a calorific value of 8 to 14 MJ/kg, and the generated energy/electricity can be used for district heating and industrial purposes. As a result, RDF is a viable alternative to fossil fuels in the manufacturing of steel and cement kilns. In India, 36 RDF plants are operational, with many more in the development stages to generate segregated combustible fraction (SCF), which accounts for around 10% of total waste generation. The Indian government has mandated that industries use at least 5%–15% RDF replacement fuel. SCF/RDF applications are environmentally friendly since they divert trash from landfills and reduce greenhouse gas emissions (Pujara et al. 2019). High economic costs is one of the limitations of the method.
- *Pyrolysis and gasification*: These are two types of thermal treatment involving heating materials at high temperatures with little oxygen in a sealed vessel. This method has a higher efficiency than direct incineration. Pyrolysis, carried out between 673 to 973 K, is the conversion of solid waste to solid, liquid and gaseous products of which liquid and gaseous counterparts are burned to generate energy while solid residue is processed to form activated carbon and other goods. An example of the same is a model unit created by TERI and located in Gwal Pahari, Gurugram, Haryana. However, owing to being an energy-intensive and a non-self-sustaining process, little investments have been made in this technology. Another reason for its failure to conquer the market is the unpleasant odor evolution that occurs during the procedure. Gasification, on the other hand, converts organic molecules into synthetic gas (syngas) containing carbon monoxide and hydrogen which is further burned to generate power and steam as a renewable source of energy. The aforementioned plants primarily used biomass as a feedstock. There are two types of gasifier designs available in India: the NERIFIER model and the TERI model. Navreet Energy Research and Information installed the NERIFIER model in Nohar, Rajasthan (NERI) and Sawdust, agricultural debris, and forest trash are used in the NERI model. TERI has deployed over 650 thermal biomass gasifier units in various micro, small, and medium businesses (MSME) and rural electricity access across the country. The TERI unit, on the other hand,

is a two-stage unit that also seeds on biomass. So far, no successful MSW gasification projects have been reported, owing to the greater moisture content of Indian OFMSW. The OFMSW gasification technique, when used as RDF pellets, is predicted to be successful in the near future. The good news is that, following a successful experiment in Dubai, an Indian business is attempting to develop an MSW gasification plant (Ghosh et al. 2018).

- **Disposal of liquid waste**:

Specific care for liquid waste disposal has to be taken focusing majorly on environmental protection, human health protection and aesthetic concerns. The environmental concerns include destabilization of aquatic ecosystems and destruction of marine animals. Improper liquid waste disposal leads to contamination of both ground and surface water ultimately affecting human health via numerous diseases. Similarly, aesthetic concerns include unpleasant odor making life uncomfortable. As already mentioned in the previous section multiple types of liquid wastes are obtained such as municipal sewage, industry effluents, storm sewage, etc. thereby requiring different strategies for the same. Some of the most commonly used strategies are described in the upcoming section.

- *Dewatering*: Elimination of water from the waste leading to its condensation and further landfilling is one of the attractive approaches for non-hazardous waste. The eliminated water can then be filtered and treated for secondary uses. The process initiated with transfer of waste in strong plastic bags for water elimination and has moved up to centrifugation in a cylindrical tank for the same.
- *Sedimentation*: Similar to dewatering the purpose of this method is to eliminate water from the waste via gravity instead of centrifugation in dewatering. The liquid waste is deposited in a sedimentation basin designed in such a way to reduce the velocity of water thereby helping sedimentation of the suspended particles. Eventually, the liquid and the solid components can then be separately treated via appropriate strategies.
- *Composting*: This is another strategy that can be employed to get rid of non-hazardous waste wherein organic materials with nutrients such as nitrate, potassium etc. can be separated and used for agricultural purposes. The strategy is eco-friendly and relatively economical as compared to other strategies of liquid waste disposal.
- *Incineration*: A strategy employed for the disposal of hazardous waste wherein the liquids consisting of acids, chemicals, oils, etc. are removed via heat in specialized furnaces leaving just the water behind. For this process, two types of specialized furnaces are used including fluidised bed furnace and multiple hearth furnaces. In a fluidised bed furnace, waste coupled with a bed of solid particle matter or solid–fluid mixes made up of sand, ash or limestone under pressure are heated in the presence of oxygen for complete and efficient burning of the waste. On the other hand, a multiple-hearth furnace incinerates massive volumes of garbage at

different stages at a uniform rate using multiple stacked chambers. The chambers are layered and thus compact and easy to fit into tight spaces and are reasonably inexpensive to produce and install. However, this method also has a negative impact on the environment via release of hazardous pollutants and greenhouse gases affecting both human health and climate change.

- *Root Zone Treatment*: This strategy is particularly useful for domestic wastewaters which are relatively clean and involves a multistep procedure consisting of sedimentation followed by filtration process ultimately leading the water to the roots of growing plants. The series of steps employed in this process include pretreatment sedimentation, anaerobic reactor, anaerobic filter and plant filled gravel filter. Pretreatment sedimentation involves removal of solid particles in the sedimentation basis which is followed by pumping the water through baffled design in an anaerobic reactor. The numerous internal compartments of the reactor possess accumulated microorganisms on the surface which decompose most of the suspended particles in water. This is followed by further treatment of the wastewater by microorganism colonies growing in the media present in the anaerobic filter. It is at this stage that the majority of the suspended solids are being consumed by the bacteria and the water is ready to be transported to the roots of the plants present in the gravel in a plant filled gravel filter. The plants are usually tough reeds that provide resistance to the passage of the water. Plants respire, supplying oxygen to the effluent and assisting in the removal of any leftover pollutants. The method has multiple advantages. It usually relies on gravity because the water flows downhill from stage to stage, reducing the need for pumps and valves. It's also extremely eco-friendly, as root-zone technology requires only 20% of the energy that a standard sewage treatment facility does. In addition, a well-established plant bed usually requires relatively little maintenance. Root-zone therapy, on the other hand, can be costly to conduct since it comprises multiple parts, and its complicated installation means may not be possible in some places.
- *Solidification*: Adding binding chemicals to wastewater in order to solidify the waste to a compact, stiff, and easily disposable solid is the process of liquid waste solidification. Solidification changes the physical qualities of garbage, making it harder, stronger, or less permeable, as well as enclosing any dangerous materials. The most common solidifying agents used in the process are lime ash, sawdust, cement kiln dust, lime kiln dust, gypsum, fly dust, asphalt or cement. The follow up strategies after this conversion include landfills or incineration. Solidification is a reasonably inexpensive and simple process, however the extra solid material produces a lot of waste. The extra weight and mass can result in increased shipping and disposal expenses, as well as a disproportionate amount of landfill space.

7 Soil Microbes Associated with Municipal Solid Waste

The soil is home to a sizable colony of bacteria involved in the bioconversion of waste. In the form of single cell proteins, enzymes, antibiotics, and other precious molecules, microorganisms provide a wide range of benefits. Municipal trash is made up of a variety of components that serve as a substrate for a wide range of microorganisms to flourish. A variety of microorganism species, including cellulolytic fungal strains, which have been employed to transform cellulosic materials into significant components like alcohol and organic acid, thrive in the biologically rich environment provided by municipal solid trash. These fungi come in both mesophilic and thermophilic types. Typically, compared to their mesophile counterparts, the enzymes produced by thermophiles are more dynamic at high temperatures and more thermally stable (Lo et al. 2009; Gautam 2011).

By using waste materials as a carbon source for themselves, bacteria are able to make a number of straightforward and useful compounds that are essential for the health of the soil, plant growth, and the maintenance of the natural ecosystem (Barman et al. 2011) An essential biological component of excellent farming practises and the bioconversion of wastes from the kitchen includes bacteria and some fungus. According to the findings of their study, W.M.F. WanIshak et al. (2011) concluded that municipal solid waste should be sanitised before being released into the environment in order to decrease microbe activity and thereby prevent or delay the release of hazardous chemicals into the environment as well as reduce odour production.

8 Role of Bacteria in Treatment Systems

The fundamental biological components of aerobic waste treatment systems are bacteria. The majority, if not all, of the organic chemicals included in industrial wastes can be metabolised by bacteria thanks to their complex biochemical makeup. In all aerobic waste treatment systems, obligate aerobes and facultative bacteria are present. Any species' capacity to compete for a portion of the system's available organic material determines how quickly it can reproduce. Typically, bacterial predominance will separate into two main groups: those that use the organic waste compounds and those that use the lysed byproducts of the first group of bacteria. The most significant group of bacteria will influence the characteristics of the treatment by utilising the organic components in the waste.

The majority of the bacteria eventually die and lyse after the organic substrate is depleted. Other bacteria can thrive because the bacteria's biological components are released. Secondary predomination will happen because biological treatment systems are typically over designed for safety. The ability of the bacteria to flocculate is by far the most significant trait, after their metabolic traits. For full stabilisation, all aerobic biological waste treatment systems rely on the separation of the microorganisms from the liquid phase through flocculation.

Initially, it was believed that *Zoogloea ramigeria*, a single bacterial species, was responsible for flocculation, but more recent research has revealed that numerous other bacterial species are also capable of flocculation. All bacteria may be able to flocculate in specific environmental conditions, according to a theory. The primary influences on flocculation are the energy level and surface charges of the bacterium. It has been demonstrated that the electrical surface charge on bacteria cultured in diluted organic waste systems is less than the 0.020 V threshold charge for auto-agglutination. This means that when two bacteria approach one another, Brownian movement generates enough energy to overcome the electrical forces that repel them, allowing the Van der Waal forces of attraction to prevail and hold the two together.

9 Novel Strategies of Waste Treatment by Using Bacteria

1. **Aerobic Composting**

The process of composting is a natural decomposition of solid organic waste by the local microbial community under the ideal environmental conditions of hot, humid air. It is a pathogen-free procedure that significantly reduces waste by up to 85%. The process produces CO_2, water, mineral ions, and stabilised organic matter, commonly known as compost or "humus." Compost is a stable nutrient-enrichment material that is helpful to plant growth (Riddech et al. 2002).

Compost undergoes in the following stages:

(i) An initial rapid stage of decomposition
(ii) Stabilization stage
(iii) Incomplete humification stage

The bacterium acts as an energy transducer in this process, which is entirely microbe-driven. A significant quantity of energy is produced during this process, some of which is utilised by bacteria. The leftover energy is released as heat, which raises the temperature of the pile and speeds up the regular composting process. Here, a rise in temperature acts as a sanitizer, while microbial activity promotes the mineralization of organic materials and lowers the C: N ratio. The finished item was reliable and secure for gardening and farming. It boosts plant development, conserves water, minimises soil erosion, soil acidity, pathogen assault, and dependence on agrochemicals, to name just a few of its numerous advantages (Rastogi et al. 2020).

Commonly occurring bacteria in composting mixture are *Alcaligenes faecalis*, *Arthrobacter* sp., *Brevibacillus brevis*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus sphaericus*, *Bacillus subtilis*, *Clostridium thermocellum*, *Flavobacterium* sp., *Pseudomonas* sp., *Thermus* sp., and *Vibrio* sp. whereas, fungi involved in composting are *Aspergillus fumigatus*, *Basidiomycetes* sp., *Humicola grisea*, *H. insolens*, *H. lanuginosa*, *Malbranchea pulchella*, *Myriococcum thermophilum*, *Paecilomyces variotii*, *Papulaspora thermophilia*, *Penicillium* sp., *Scytalidium thermophilum*, *Termitomyces* sp., and *Trichoderma* sp., and actinobacteria are *Streptomyces* sp., *Frankia* sp., and *Micromonospora* sp.

Multiple variants of composting that are used in the current scenario are described as:

- **Static Pile Composting**

The aerated static pile composting method uses either positive or negative ambient air. Along with organic wastes and bulking agents, air is circulated through the compost pile. Layers of bulking agent are layered into the pile to improve air flow and add porosity. The aerated static pile method is nearly identical to the windrow composting method in terms of structure, with the exception that the aerated static pile method does not require rotating to produce aeration. Aerated static pile composting can produce compost in as little as three to six months and is suited for a wide range of organic wastes, including yard trimmings, papers, and food scraps. Furthermore, when compared to the windrow composting method, it requires less land. This composting method is expensive and ineffective at decomposing animal manures and fats (Palaniveloo et al. 2020).

- **Windrow Composting**

Windrow composting is a popular approach because it can handle a significant amount of organic waste. This form of composting entails piling organic waste into long, narrow heaps known as "windrows," which have a triangular or circular cross-sectional area. The piles are then either manually or mechanically turned. The temperature of raw materials is used in the turning process, as a turning signal, the windrows will be rotated if the temperature reaches a certain level. As a result, the pile is aerated. This method can break down large amounts of organic wastes like grease, liquids, and animal manures. Because the pile is large enough to generate adequate heat and maintain the temperature, windrow composting is ideal for restaurants, cafeterias, and markets that produce enormous amounts of food waste. However, to accommodate the enormous equipment, this composting method necessitates a large amount of land and is time-consuming (Palaniveloo et al. 2020).

- **In-vessel Composting**

In-vessel composting is often utilized in the industry as well since it can also treat a large amount of organic wastes. This approach encloses all organic wastes in various containers or vessels, which are then manually or automatically spun to ensure that the wastes are aerated. When compared to windrow composting, in-vessel composting requires a smaller amount of land and manual labor. This procedure has disadvantages in that it is pricey and may necessitate equipment knowledge (Palaniveloo et al. 2020).

- **Vermiculture Technology**

Vermiculture technology is a system that uses earthworms to convert organic waste into vermicompost. It has a wide range of applications in waste management and sustainable organic farming, and has proven to be one of the most efficient and cost-effective ways to handle organic waste. For the biodung and vermicomposting processes, a mixture of grass clippings, water hyacinth, and cattle dung was used as organic waste. The results shows the organic wastes were successfully treated over a 60-day period using partial bio-dung composting and vermicomposting. It contains growth-promoting chemicals including auxins and cytokinins and adds to the delivery of important micronutrients.

2. A**naerobic Digestion**

By simultaneously creating biogas as a source of energy, Anaerobic digestion (AD) demonstrates a promising sustainable method for treating MSW (McCarty 2001). Its potential for expansion is constrained by its sluggish digestion rate and the production of chemicals that hinder methanogenesis (Stams and Plugge 2009; Shah 2021a, b). However, due to its capacity to effectively reduce the chemical oxygen demand (COD) and biological oxygen demand (BOD) from the waste streams and convert them into biogas/methane, the AD process has recently been used to treat diverse agricultural wastes, food residues, and wastewater (Kwietniewska and Tys 2014). AD is a promising method for treating MSW and generating CO_2 and CH_4, which, coupled with the manure produced after decomposition and the ability to be utilised as a biofertilizer, can satisfy the energy demand.

AD process can be further subdivided into four distinct steps:

1. Enzymatic hydrolysis: Extracellular enzymes convert complicated organic stuff (protein, lipid, and carbohydrates) into more easily soluble molecules (amino acids, fatty acids, and sugars).
2. Fermentation: By the presence of fermentative bacteria, reduced end products from hydrolysis are transformed into a combination of short-chain volatile fatty acids (VFAs) and other products such CO_2, hydrogen, and acetic acid during fermentation.
3. Acetogenesis and Methanogenesis: Using acetogenic bacteria, organic acids are changed in this stage into acetate, CO_2, and hydrogen. *Clostridium*, *Ruminococcus*, and *Eubacterium* genera contain both acetogenic and non-acetogenic bacteria that are crucial for acetogenesis. *Acetobacterium* and *Sporomusa* are two exclusively acetogenic bacteria.

3. **Nitrogen Fixation**

A vital natural supply of reactive nitrogen for the wetland environment is the nitrogen fixation by bacteria. The rhizosphere's enrichment of nitrogen-metabolizing bacteria is facilitated by the roots' supply of oxygen and organic matter (Lamers et al. 2012). Bacteria change nitrogen in the rhizosphere of wetland plants through nitrification, denitrification, absorption, and anaerobic oxidation of ammonia by nitrate and nitrogen fixation (Bañeras et al. 2012). This process' metabolic energy comes from the oxidation of organic materials and lithotrophy. Microbes perform an N-fixation of non-reactive N2, and nitrogen is produced. The heterotroph and autotroph prokaryotes contribute toward the production of a large amount of reactive nitrogen by nitrogen fixation.

Cyanobacteria in wetlands must have access to light in order to fix nitrogen. In wetlands, the significant genera of N-fixing bacteria include *Enterobacter*, *Azospirillum*, *Pseudomonas*, *Klebsiella*, and *Vibrio*. Typically, the roots and the heterotrophic nitrogen fixer form a mutually beneficial symbiosis, and the roots' carbohydrates are traded for the ammonia the bacteria create.

4. **Degradation of Organic Pollutants**

Because they can degrade almost all kinds of organic contaminants, microbes are referred to as bioremediators (Fenchel et al. 2012; Shah 2020). The organic contaminants are broken down by microbes through a co-metabolism process. In this process, the complex carbon-based compounds are broken down by microorganisms in the rhizosphere of aquatic and terrestrial plants to produce organic carbon and electron acceptors. The microbial population and amount of xenobiotics in natural water determine the biodegradation rate, and the macrophyte species has a significant impact on the microbe population. Microbes in the rhizosphere receive organic carbon from plants, which helps them break down complex organic molecules like hydrocarbons and aromatic hydrocarbons. In order to promote plant growth, bacteria also emit indole acetic acid (IAA).

Many bacteria that were isolated from aquatic plants also exhibited activities that promote plant growth and pollution breakdown. Organic substances including phenolics, amines, and aliphatic aldehydes can be broken down by the biofilms that are connected to aquatic plants. These biofilms can also break down dissolved organic materials, including polychlorinated biphenyls (PCBs) and atrazine.

5. **Metal Biosorption and Bioaccumulation**

In general, bacteria execute passive and active processes for metal ion biosorption within their cell walls. The cell walls of both active and dormant bacteria facilitate passive biosorption through a variety of metabolic activities. Metal ions adhere to the cell surface as a result of their reactivity with functional groups (such as amine, amide, carbonyl, hydroxyl, and sulfonate) in the cell wall. Different mechanisms, such as ion exchange, sorption, complexation, chelation, and micro-precipitation, may each play a separate or complementary role in the metal ion binding process.

On the other hand, live cells take up metal ions during the active biosorption process. Metals that enter live cells have different outcomes depending on the species and particular elements. The elements may be delivered to a particular structure and may be bound, stored, precipitated, and sequestered in certain particular intracellular organelles. The endophytic bacteria displayed exceptional capacities for heavy metal bioaccumulation and detoxification.

Additionally, bacteria create biosurfactants and exude them as root exudates. By interacting and complexing with insoluble metals, these biosurfactants increase the bioavailability of metals in the soil and aquatic media. A crucial role in the complexation of metals is played by extracellular polymeric molecules, which are mostly made up of proteins, polysaccharides, nucleic acids, and lipids. This decreases the bioavailability of the metals. For instance, Azobacter sp. reduced the uptake of metals by *Triticum aestivum* by producing extracellular polymeric substances (EPS) that formed complexes with chromium and cadmium. In plants, the production of several metabolites, including siderophores and organic acids (such as citric acids, oxalic acids, and acetic acids), affects the bioavailability and translocation of heavy metals (Visioli et al. 2014).

6. **Bioventing**

Any substance that is aerobically degradable can be broken down using this method. In bioventing, nitrogen and phosphorus as well as oxygen are injected into the contaminated area (Rockne and Reddy 2003). The texture of the soil affects how these nutrients and oxygen are distributed in the soil. In bioventing, a modest air flow rate provides ample oxygen for bacteria. Bioventing is nothing more than the process of pushing air into a well that had previously drawn air into contaminated soil above the water table. When the water table is far below the surface and the location is hot, bioventing is more effective. It is primarily used to remove petroleum, oil, and other hazardous materials. From one place to another, various chemicals are removed at varying rates.

7. **Biosparging**

In biosparging, air is injected under pressure beneath the groundwater to raise the oxygen concentration. For the microbial breakdown of the contaminant, oxygen is injected. The aerobic decomposition and volatilization are increased by biosparging. To stop volatile material from being transferred into the environment while injecting oxygen at the polluted site, pressure must be under control. By decreasing the diameter of the injection point, the cost can be decreased. It is important to understand the permeability and texture of the soil before infusing oxygen. This technology was used to measure the extent of remediation accomplished in terms of both mass removal and reduction in mass discharge into groundwater at a known source of gasoline contamination. Underground storage tank (UST) locations can reduce petroleum products by using biosparging. Mid-weight petroleum products, such as diesel and jet fuel, are most frequently used at sites where biosparging is employed; lighter petroleum products, such as gasoline, have a tendency to volatilize more easily and be removed more quickly by air sparging.

8. **Bioaugmentation**

To speed up waste decomposition, microorganisms with appropriate metabolic capabilities are introduced to the contaminated location. Bioaugmentation is used to ensure that in situ microorganisms can completely break down chlorinated ethenes, such as tetrachloroethylene and trichloroethylene, to ethylene and chloride, which are non-toxic, at areas where soil and groundwater are contaminated.

9. **Biopiling**

It is a synthesis of farming and composting. A treatment bed, an aeration system, an irrigation/nutrient system, and a leachate collection system make up the fundamental biopile system. Moisture, heat, nutrition, oxygen, and pH should all be under control for effective breakdown. Underground irrigation equipment uses vacuum to deliver nutrients and air. The soil is coated with plastic to avoid runoff, which also prevents evaporation and volatilization and encourages sun heating. The biopile therapy method takes 20 to 3 months to finish (Niu et al. 2009).

10. **Landforming**

Make a layer of excavated earth sandwiched between clean soil, clay, and concrete while constructing land. The two uppermost layers should be the concrete layer and the clear soil at the bottom. After that, let nature take its course. Additionally, add oxygen, nutrients, and moisture, and use lime to keep the pH level close to 7. Land formation is primarily beneficial for pesticides.

10 Bacteria and Liquid Waste Management—Novel Strategies

The primary goal of wastewater treatment is to prevent water sources from being contaminated and to protect the general public's health by preventing the spread of illness. This is accomplished using a number of treatment technologies, including onsite and offsite treatment systems. Therefore, the purpose of this part is to describe the off-site wastewater treatment technologies (activated sludge, trickling filters, stabilisation ponds, built wetlands, and membrane bioreactors) (USEPA 2005).

1. **Activated sludge**

The goal of the activated sludge process is to remove organic materials from wastewater by using a high concentration of microorganisms, primarily bacteria, protozoa, and fungi, which are present as a loose clumped mass of tiny particles that are kept in suspension by stirring (Templeton and Butler 2011). Sewage that contains active microorganisms that aid in the decomposition of organic waste is known as activated sludge. Of all the wastewater treatment technologies, it is the most flexible and efficient.

The microorganisms in the aeration tank of an activated sludge system can break down the organic stuff in the wastewater. 70–90% of the microbes' weight is made up of organic material, and 10–30% is inorganic. The various cell types change according to the chemistry present and the unique traits of the organisms that make up the biological mass. A clarifier, also known as a settling or sedimentation tank, separates the suspended solids from the treated wastewater by gravity after the mixed liquor is emptied from the tank. In order to maintain a concentrated population of microorganisms to treat the wastewater, the concentrated biological solids are subsequently returned back to the aeration tank. Because the system is constantly producing microbes, a means of removing the extra biological solids must be supplied. A bigger volume of sludge must be handled since the waste solids from the aeration tank are less concentrated than those from the clarifier. It's feasible to maximise or reduce the creation of solids depending on how the process is set up and run.

The oxidation of sewage organic matter into carbon dioxide and water is facilitated by the action of the microorganisms. Prior to allowing the sewage to travel through the settling chamber, which aids in the removal of sand and other materials, while the floating debris is shred and ground, during the early stage of treatment, the large floating materials in the wastewater are first screened out. The wastewater is first treated by passing air through it in the system's aeration tank. According to research, an activated sludge system is effective in removing 75–90% of the biological oxygen demand (BOD) from sewage (Tortora et al. 2010).

2. **Trickling filter**

A trickling filter is a commonly used method of secondary wastewater treatment. It is made up of a filter bed that contains a highly permeable media (gravel or plastic material etc.), which has a layer of microorganisms on the surface that leads to the formation of a slime layer. In a trickling filter system, the microorganisms are attached to the media in the bed and form a biofilm over it. As the wastewater passes through the media, the microorganisms consume and remove contaminants from the wastewater.

One typical technique for secondary wastewater treatment is a trickling filter. It is constructed of a filter bed with a highly porous media (gravel, plastic, etc.) that has a layer of microorganisms on the surface that causes a slime layer to form. The microorganisms in a trickling filter system cling to the medium in the bed and create a biofilm on top of it. The microorganisms consume and eliminate pollutants from the wastewater as it moves through the media.

A septic tank, a clarifier, and an application system make up trickling filters. The application system aids in distributing the treated wastewater to the correct location, the clarifier helps the biological materials settle out of the wastewater, and the septic tank aids in the removal of wastewater solids. To prevent them from covering the thin layer of microorganisms present and from killing them, solid and oily debris must first be removed from wastewater before it is processed and transferred to a trickling filter.

Depending on the amount of hydraulic or organic loading, trickling filters can be categorised as high rate or low rate. Low rate filters involve straightforward processing that results in consistently high-quality effluent. 80–85% of the applied BOD should be able to be removed by the low rate trickling system. Higher organic and hydraulic loadings than low rate filters are often characteristics of high rate filters. Recirculation happens with high rate filters but not with low rate filters. Filter effluent is returned and put to the filter once more through a process called recirculation. This wastewater recycling increases the amount of trash that is applied with microorganisms, causing the effluent to undergo adequate treatment. (Van Haandel and van der Lubbe 2007).

3. **Membrane bioreactor**

In a membrane bioreactor, direct solid–liquid separation by membrane filtration using micro or ultrafiltration membrane technology is combined with the biological degrading process of activated sludge. The technique enables total physical retention of all suspended particles and bacterial flocs inside the bioreactor. A membrane bioreactor has advantages over other treatment systems, including high effluent quality, effective disinfection, increased volumetric loading, and less sludge generation.

In order to separate the solid from the liquid components of the sludge suspension, a membrane bioreactor (MBR) uses membrane technology instead of the gravity settling used in the traditional activated sludge process. Biologically active wastewater inputs from municipal or industrial sources are treated in membrane bioreactors. There are two possible MBR configurations: internal/submerged and external/side stream. While in the external/side stream, the membranes are a separate unit process requiring intermediate pumping stages, in the submerged, the membranes are immersed in and integral to the biological reactor. (Lofrano et al. 2013).

Polymers or inorganic materials are used to create the membranes in membrane bioreactor systems. They are composed of numerous tiny pores that can only be seen under a microscope. Only minuscule particles and water can flow across the membrane due to its microscopic hole size. Although there are other membrane forms, the hollow fibre, flat sheet, and tubular membranes are the most often used ones in membrane bioreactors. While the tubular membrane is often put outside the bioreactor, the hollow fibre and flat sheet membranes are typically submerged in water.

The conventional activated sludge system and other biological wastewater treatment methods are competitors of the MBR process. Conventional biological methods can struggle to achieve treatment standards for discharge into sensitive settings, even when they perform well in meeting typical discharge norms and are economical. Additionally, it has been noted that using conventional methods to reuse wastewater is not cost-effective unless ultrafiltration or microfiltration membranes are utilised as a post treatment (United Nations Environmental Programme, Wastewater and stormwater Treatment (2012).

11 Roles and Dynamics of Microorganisms in Wastewater Treatment Systems

Bacteria, protozoa, viruses, fungi, algae, and helminthes are the main microbiological species identified in wastewater treatment systems. The majority of these organisms are found in water, which promotes the spread of diseases.

11.1 Bacteria

Bacteria are crucial to the conversion of organic materials contained in wastewater treatment systems to less complicated molecules. Bacteria that range in size from

0.2 to 2.0 mm in diameter are in charge of the majority of the wastewater treatment in septic tanks. Even though not all bacteria are hazardous, some of them do cause illnesses in people and animals that are tied to water. Cholera, dysentery, typhoid fever, salmonellosis, and gastroenteritis are a few of these ailments (Akpor and Muchie 2010).

As in the case of activated sludge, bacteria can be discovered entangled in flocs. While some bacteria, such as filamentous bacteria, are essential in biological treatment, others, such as these, can seriously interfere with settling and foaming. There are several reports of waterborne gastroenteritis with no identified cause, and bacteria are the vulnerable agent. This illness may be caused by specific bacteria of *Pseudomonas* and *Escherichia coli* that can harm newborns. These microorganism strains have also been linked to epidemics of gastrointestinal diseases. The most significant number of bacteria in wastewater treatment systems. The majority of organisms are facultative, meaning they can survive with or without oxygen. Although heterotrophic and autotrophic bacteria can both be found in wastewater treatment systems, heterotrophic bacteria are more common.

The carbonaceous organic materials in wastewater discharge is often where heterotrophic bacteria get their energy. The energy produced is used to create new cells as well as to release energy by converting organic material and water. *Achromobacter*, *Alcaligenes*, *Arthrobacter, Citromonas*, *Flavobacterium*, *Pseudomonas*, *Zoogloe* and *Acinetobacter* are a few notable bacterial species that are present in wastewater treatment systems (Oehmen et al. 2007).

11.2 Protozoa

Protozoa, which are tiny, unicellular creatures, are also present in wastewater treatment facilities. They carry out a variety of helpful tasks during the treatment process, including clarifying the secondary effluent by removing bacteria, flocculating suspended debris, and acting as bioindicators of the sludge's health. In at least one stage of development, the protozoa that live in wastewater treatment systems can move. They are unicellular creatures with organelles that are enveloped in membranes and are 10 times larger than bacteria.

Protozoa have an advantage in wastewater because they feed on pathogenic bacteria. Depending on how they move, they can be divided into five groups: free swimming ciliates, crawling ciliates, stalked and sessile ciliates, flagellates, and amoeboid. Protozoa are helpful biological markers of the health of wastewater treatment systems. Although some protozoa may survive for up to 12 h without oxygen, they are typically classified as obligate aerobes, making them ideal markers of an aerobic environment.

They can also display more sensitivity to toxins than bacteria and act as markers of a toxic environment. The absence or immobility of protozoa in a treatment system is a sign of potential harm. It is suggested that a sign of a well-run and stable system is the presence of significant numbers of highly developed protozoa in the biological mass in a wastewater treatment system. Depending on how they move, they can be divided into five categories. The free-swimming ciliates, crawling ciliates, stalked/sessile ciliates, flagellates, and amoebae are the members of these categories. Free-swimming ciliates, crawling ciliates, and stalked ciliates are the three different types of ciliates. These three all have cilia, which resemble small hairs and beat in unison.

Aspidiscacostata, *Carchesiumpolypinum*, *Chilodonellauncinata*, *Operculariacoarcta*, *Operculariamicrodiscum*, *Trachelophyllumpusillum*, *Vorticella convallaria*, and *Vorticella* microstoma are the ciliated protozoa species that are most frequently seen in wastewater treatment operations. There is evidence that the free-swimming ciliates, which contain cilia on every surface of their bodies, are often found suspended or swimming freely in the bulk solution, including *Litonotus* sp. and *Paramecium* sp. In contrast, the crawling ciliates, like *Aspidisca* sp. and *Euplote*s sp., only have cilia on the surface of their belly, or ventral, where the mouth opening is situated. The stalked ciliates, including *Carchesium* sp. and *Vorticella* sp., have their cilia only around the mouth opening and are attached to floc particles, as opposed to the crawling ciliates, which are typically found on floc particles. Their anterior portion is larger, while their posterior portion is thin. Dispersed bacteria are drawn into the mouth opening by a water vortex created by the beating of the cilia and the springing motion of the stalk.

There are two main forms of amoebae that are found in wastewater systems: naked amoebae like *Actinophyrs* sp., *Mayorella* sp., and *Thecamoeba* sp., and shelled amoebae or testate amoebae like *Cyclopyxis* sp. The shelled amoeba has a protective covering made of calcified material, whereas the naked amoeba has no protective covering at all. Protozoa that have flagella have an oval form and one or more whip-like flagella. The flagella of flagellated protozoa help drive them through wastewater treatment systems in a corkscrew pattern of movement.

11.3 Viruses

In particular, human viruses that are heavily discharged in faeces can be discovered in wastewaters. Although bacterial viruses may also be present, native animal and plant viruses can be found in wastewater in lesser amounts. They are the responsible parties for a number of water-related illnesses in people, including conjunctivitis, meningitis, and gastrointestinal and respiratory infections. According to reports, enteric viruses were the primary cause of the majority of aquatic illnesses with unknown sources. When present in wastewater, they are very notorious and persistent and can continue to be an active source of infection for months.

11.4 Fungi

The microorganisms present in wastewater treatment systems include fungi as well. Multicellular organisms called fungi are also found in activated sludge. They may effectively compete with bacteria in a mixed culture under specific environmental conditions and metabolise organic molecules. Additionally, only a few fungi have the ability to oxidise ammonia to nitrite and even fewer to nitrate. The sewage fungus species *Sphaerotilus natans* and *Zoogloea* sp. are the most prevalent. Although they can also metabolise organic materials, a variety of filamentous fungi are naturally found in wastewater treatment systems as spores or vegetative cells.

11.5 Algae

Algae are a type of biological plant that contributes to the overall stabilization of organic wastes. Because algae get their energy for synthesis from sunlight, they don’t need to digest organic substances like bacteria and fungi do. The inorganic components of wastes, including ammonia, carbon dioxide, phosphate, magnesium, potassium, iron, calcium, sulphate, sodium, and other ions, are predominantly used by algae to make protoplasm. Because algae and bacteria do not need the same waste components, it is possible for algae and bacteria to coexist. The bacteria break down the waste’s organic components and release some of the inorganic components that the algae need. The bacteria use the oxygen released by the algae during protoplasm synthesis to complete aerobic stabilization of the organic matter. In the absence of sunlight, algae, like bacteria and fungus, must rely on the metabolism of organic materials to receive the energy they require to stay alive. This organic substance is generally derived from stored food within the cell, but it can also be derived from organic waste in some algae species (Adebayo and Obiekezie 2018).

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Biofilms in Porous Media

Esha Garg, Ajit Varma, and M. S. Smitha

Abstract Biofilms are microbial communities that are attached to a surface in three dimensions. Biofilms formed when microorganisms like bacteria adhere, proliferate and enclose themselves in extracellular polymeric polymers (EPS) which is self-produced. It is formed in natural ecosystem or engineered systems and plays remarkable role in hydrodynamics in porous media. Microbial biofilms are resistant to environmental factors like temperature, pH, and water activity, mechanical stress. Microbial biofilms can impact the hydrodynamics of porous medium in both natural and artificial systems. Porosity, permeability, dispersion, diffusion, and mass transfer of reactive and nonreactive solutes are all influenced by biofilm development in porous media. Understanding and regulating biofilm development in porous medium will maximize the potential value of porous media biofilms while minimizing the negative consequences. Beneficial porous media biofilm uses include subsurface cleanup, improved oil recovery, and carbon sequestration, to name a few. The objective of the chapter is to focus on the various aspects of biofilm development in porous media.

Keywords Bacteria · EPS · Hydrodynamics · Microbial biofilms · Permeability · Porous media

1 Introduction

Biofilms are microbial communities that are adhered to a surface in three dimensions. A biofilm is mainly produced by microorganisms like fungi, bacteria, protozoa, algae that are attach to a surface and encapsulated in an EPS array that they have self-produced. Biofilms can be found on a variety of surfaces as well as in a variety of industrial, environmental, and medical systems. Depending on the culture conditions, biofilm communities are found to form continuous films and distinct colonies (Halan et al. 2012). The huge amount of EPS associated with biofilms provides

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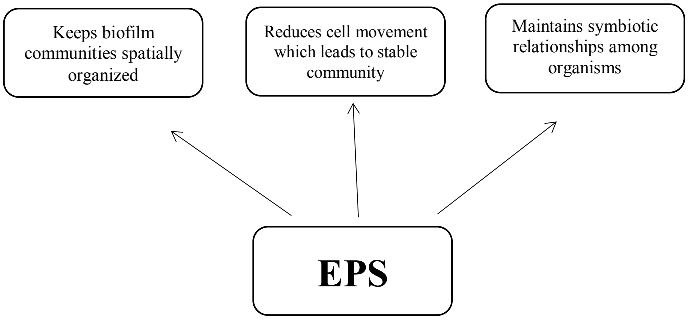

Fig. 1 Functions of EPS

structural support and is distinguish feature between biofilms and suspended cells. The presence of EPS helps to keep biofilm communities organized spatially. EPS immobilize or drastically reduces microbial cell movement, leading to the creation of stable microbial community which is less rigid (Fig. 1). Its presence provides an edge because of the symbiotic or mutualistic relationships among organisms.

A porous medium is a solid matrix (Flemming and Wingendet 2010) whose pores are of several micrometers in size and provides an amiable environment to biofilm forming microbes because of its high specific surface. The formation of biofilms in porous media is of immense application in bioremediation of soil (Shah Maulin 2020) and aquifers (Meckenstock et al. 2015), sequestration of carbon dioxide, bio-assisted oil recovery etc.

Biofilms in porous media can have a wide range of morphologies, including continuous films of varying thickness (Ye et al. 2015) or patchy, colony-like biofilms. Biofilm structure formation is greatly influenced by following factors like growth regulating factors, existence of inhibitors and the hydrodynamics (Hobley 2015). The comprehensiveness of formation of biofilm depends on the ability of growth of adhered microorganism and the rate of reproduction. The subsequent factors that influence biofilm formation are described (Fig. 2).

(1) Availability of nutrients and energy
(2) Conditions that are appropriate in terms of geochemistry
(3) Presence of inhibitors
(4) Several biofilm-eating microorganisms, and
(5) Influence of Hydrodynamics on solute mass transport which causes mechanical stress on biofilms.

2 Morphological Features of Biofilms in Porous Media

Biofilms appear opaque in natural and synthetic porous media. Planar pore networks etched in glass and flow chambers jammed with beads of glass have been employed

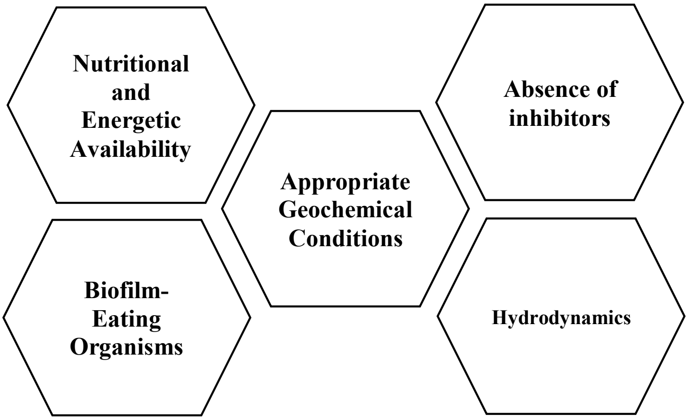

Fig. 2 Major factors that influence biofilm formation

as porous media model to review the expansion of biofilms, allowing direct optical examination of core and pore scale development (Wildenschild and Sheppard 2013). According to some studies, methods like magnetic resonance (Vogt et al. 2013) and confocal microscopy fully supported the investigation of biofilm growth in porous media. Paulsen et al. discovered the three different biofilm growth stages when he photolithographed the sandstone having a porous structure on glass plates- (a) formation of smooth biofilms on the pore walls, (b) Non-uniformity in biofilms over time, and (c) strands of biofilm eventually spanned the pores (web-like structure). Patchy biofilms, smooth biofilms continuous biofilms and irregular aggregates were discovered by McCarty and Dupin. By using a chamber-and-throat pore network etched on glass, Vayenas et al. investigated the biodegradation of a combination of organic toxicants at the time of growth of biofilm. According to one of the study's intriguing findings, the average width of biofilms had a concrete interaction with the pore size.

3 Bacterial Interactions with Porous Media

The deposition of planktonic cells to the substratum is the first step in biofilm formation and growth. Deposition is commonly thought of as a series of transport and adhesion steps. The adhesion step is controlled by cell substratum interaction, whereas transport is determined by hydrodynamics.

Between the cell and the substratum is a Gibbs energy barrier. Bacterial cell surface macromolecules can break through this energy barrier, allowing them to reach the substratum with high ionic strength. As a result, interactions between cell surface macromolecules can break through this energy barrier and reach the substratum with high ionic strength. At high ionic strength, adhesion is determined by interactions between cell surface macromolecules and the substratum, which are referred to as steric interactions. Bridging, which promotes adhesion and lowers the activation energy of adhesion, and, steric hindrance, which inhibits adhesion and thus are the two types of steric interactions that commonly occur.

Modeling biofilm growth is one of the main tools used to improve current understanding of the correlation between the hydrodynamics of porous media and bacterial biofilms. Over geological time scales, this approach can provide predictions that are difficult to observe experimentally.

4 Bacterial Transportation in Porous Media

Convective movement in the aqueous phase, slowed by attachment to surfaces and straining or trapping into interstitial pores are administered by bacterial transport on porous media (Xiong et al. 2016). Several factors influence bacterial transport through porous media, including bacterial cell properties, solution chemistry, porous media characteristics, and interspatial fluid acceleration (Kone et al. 2014). The presence of molecules such as proteins or polysaccharides on the cell surface, as well as motility and chemotaxis, can influence bacterial transport, retardation, and adhesion to surfaces.

Cell size and shape, as well as cell surface charge and cell by drophobicity, have all been linked to transport through porous media. Bacterial transport and adhesion to surfaces have been shown to be influenced by solute characteristics which includes ionic strength, pH, temperature, concentrations of dissolved organic matter, surfactants, and nutrients. Attachment of a bacterial will increase with increase in iconic strength according to many studies.

In the presence of high ion concentrations, this effect is usually attributed to compression of the electrostatic double layer. Porous media properties such as pore water velocity, typore size, the presence of Fe minerals, the organic matter content, and grain and pore size distribution have been reported to influence bacterial transport and adhesion. Some combination of the aforementioned parameters may influence bacterial transport through porous media.

To predict bacterial transport through porous media, experiments should be conducted with the aquifer material of interest and under conditions as close to those expected in the field as possible. The convection–dispersion equation for bacteria has been used in many basic bacterial transport models. To account for the extent of bacterial attachment to surfaces, empirically determined collision efficiency factors are typically used.

The bacterial transport can be explained by the convection–dispersion transport model. Due to the numerous interactions occurs within porous media, transportation of bacteria in the subsurface (Sams et al. 2016) notably in the vadose zone, is a complicated task. Depending on the prevailing interactions of the bacteria in the pore system, bacteria may be captured at the media-air–water three-phase interface, at the air–water interface or on the media surface, when transported within the vadose zone. By using column experiments, transportation of *Bacillus subtilis* (Wilking et al. 2013), *Pseudomonas fluorescens* and *E.coli* in silica sand under water-unsaturated conditions was reviewed in one of the research. To ascertain media surface thermodynamic properties and bacteria, interfacial tension measurements were used to interpret bacterial interactions within the system.

5 Effect of Growth of Biofilm on Porous Media Hydrodynamics

Hydrodynamics in porous media is affected by EPS and microbial cells. Formation of discrete clusters marked as an initial phase of biofilm accumulation. The local hydrodynamics, effective porous media particle size and availability of nutrients all influence biofilm distribution in porous media. Carbon availability also influence the formation of biofilm in porous media.

The method of measurement and the pore size of media influence the porosity in media. The permeability of porous media is thought to be reduced as biomass and polysaccharides accumulate.

With increased biomass production, permeability decreases are usually not uniform, but rather vary spatially, and temporally. In biofilm-affected porous media, dispersivity increases over time, if the biofilm reaches to a pseudo steady phase, it often reaches to semi-stable values.

Nondestructive, spatially and temporally resolved measurements of hydrodynamic dispersion and velocity in porous media (Holzner et al. 2015) as well as limited biofilm imaging, are well-suited to magnetic resonance microscopy (MRM) techniques. Growth of biofilm in porous media significantly reduces space in pores for advective flow.

Carbon availability is known to impact the formation of biofilm in porous media (Hand et al. 2008). However, increased biofilm growth has been reported in untreated sewage areas with trace of carbon (Dixon et al. 2018; Godzieba et al. 2022). Another limiting growth factor, such as oxygen, may have become more available, which could explain this. Microbial activity growth takes place when oxygen is introduced into column systems that are repeatedly tried to open or attached to gas-permeable piping system, for example silicon piping system.

Biofilm-induced mineral formation, such as divalent or trivalent (e.g. Ca,Fe,Mg) sulphate, phosphate minerals, carbonate and sulphide frequently causes transition in hydrodynamics in biofilm-affected porous media (Li et al. 2015). In one of the investigation, it was found that debris substance majorly was calcium carbonate which has an impact on growth of biofilms in porous media (Zhang and Klapper 2014).

6 Visualization of Biofilms in Porous Media

Counterproductive and non-invasive measurements is used to evaluate the circulation, presence and framework of biofilms in porous media. Examples of such techniques include microscopy and high-resolution photography, transmission electron microscopy or scanning electron microscopy (TEM or SEM), nuclear magnetic resonance (NMR) spectroscopy, ultrasound-based imaging techniques, X-ray tomography. Microscopes and image analysis software can be used to measure the dimensions of stained or unstained biofilms.

Optical techniques for monitoring biofilms in porous media are limited by the fact that porous media particles are not smooth (e.g. glass beads, sand grains). Background signal and image chromatic aberration are increased on porous media surfaces because they are typically irregularly shaped (Chen 2002). Porous media are also opaque, and because high-resolution microscopy objectives have a defined working distance, thus the detailed analysis is restricted.

The thickness of surface biofilms is measured by using electron microscopy methodologies such as TEM and SEM. Microscale imaging of biofilms in porous media (Neu and Lawrence 2014) is continuing, despite the emergence of X-ray tomography techniques for imaging porous media (Beltran et al. 2011). NMR, ultrasound, and complex conductivity imaging are non-optical techniques for visualising biofilms in opaque media that have a lot of upside.

7 Biofilm Systems in Porous Media as Models

The model used for understanding the process of biofilms in porous media should take into account the impact of classifications and microscale heterogeneities. Admittedly, modelling an outsized scale system on the microscale are often computationally intensive.

Bulk-scale models based on logical or fast analytical simulation are computationally intensive (Peszynska et al. 2015) and perform well in situations where generous bulk is adequate and it is, when microscopic mechanisms are minimal in comparison to total system conduct. As a function of formation of biofilm in porous media, these models can imitate bulk changes in specifications such as specific surface area, porous structure, dispersivity and permeability. Microscale models, one on either hand, treat porous media affected with biofilms as multi–faceted on the pore scale, permitting them to analyze regionalized obstruction, which impacts the total device hydrodynamics.

Multiscale models have recently been described that numerically overcome the Brinkman and Navier–Stokes equations which incorporate the approach with Lagrangian-type simulations or cellular automaton of detached segment pathways (Kapellos et al. 2007a, b).

8 Nature and Innovation of Biofilms in Porous Media

Biofilms have been found in a wide range of manufacturing, ecologic, and medical configurations. At first, study focused on eliminating biofilms by employing antimicrobials, but several characteristics of biofilms have recently been recognized as potentially beneficial for engineered applications.

Due to their elevated degree of environmental pressure and toxicants, biofilm reactors are quite often suggested for the intervention of recalcitrant compounds. Such compounds involve dyes, surfactants, organic solvents and herbicides (Mondragón-Parada et al. 2008). Biofilm hinders colloidal transport (Majumdar et al. 2014). Healthcare system depends on micropollutants (colloid-mediated contaminant) transport and infectious agent (biological colloids) through porous media. Formation of established biofilm communities is proved to be advantageous despite the fact that its development and organization is still unexplained. Biofilms are enticing to innovators because of their near vicinity to one another, which enables for cell–cell interaction, genetic component transfer (DNA, RNA), colocation of physiologically various organisms, which can enhance metabolite exchange, and enhanced tolerance to ecological stresses.

Because of their increased resilience to toxic compounds and environmental stress, biofilm reactors are oftenl postulated for the cure of recalcitrant compounds. Subsurface biofilm barriers prefer maximum porosity, permeability reduction and groundwater flow control. Adequate thickness is preferred in bio trickling filters (used in sewage treatment) having an impact on removal of excess of solute with high consistent permeability. Grid of flow channels is regarded as a special type of porous medium, biofilm growth (bio-fouling) in such processes can have a significant impact on flow, as evidenced by increased back-pressure in (bio-) fouled membrane processes. Membranes including reverse osmosis filtration cartridges are also precluded from evaluation.

Biofilms builds up solutes and potentially vast quantities of minerals in environment and manufacturing in industries. Calcium carbonate, sulfur-containing minerals and iron oxides are most prominent inorganic components of biofilms in porous media. Mineral precipitation results in semi-permanent to permanent biofilm cell encrustation, along with clogging of key locations in a porous medium (Thullner 2010).

8.1 Profound Subsurface Biofilms for Improved Oil Recovery and Carbon Capture

Selective plugging of high-permeability networks in reservoir rock to strengthen sweep efficiency, manufacturing of biosurfactants or gases to improve mobility of oil and in situ biocracking, that incorporates microorganisms that breaks down long alkane chains and produces higher-solubility alkane with short chains, all these are instances of microbially improved oil recuperation (Armstrong and Wildenchild 2012).

Biofilms are beneficial at preferentially plugging high-permeability water-filled residential areas as crook zones. In high-permeability areas, lowered permeability grant fluids such as water or supercritical CO_2 ($scCO_2$) used for improved secondary or tertiary oil recovery to construct remaining oil in areas with low-permeability and this marks for establishment which is quite successful.

8.2 Biofilm Reactors with Porous Media in Industry and Waste Treatment

In sewage water treatment and water treatment, biofilms in porous media plays vital role (Wingender and Flemming 2011). Microorganisms adhered to water filtration can have significant effect on water treatment processes as they either grow inside just at inffluent or sewage waters of filtration apparatus.

Microorganisms, fairly immobilized in porous media, have for quite some time been utilized in wastewater expulsion of toxicants from wastewater (Shah Maulin 2021) in trickling filters and infiltration systems. Role of biofilms in large-scale applications and primary analysis are currently in progress (Qureshi et al. 2005; Iliuta and Larachi 2004; Shah 2020, 2021; Mauclaire et al. 2006).

Porous media biofilms, on the other hand, have been witnessed to aid in the oxidative precipitation of metals like reduced manganese and iron, sorption or degradation of organic particulates and solutes, in water.

8.3 Biofilm Barriers in the Subsurface for Contaminated Groundwater Control and Remediation

Growth and expansion of microbial activity helps in advancement of subsurface biofilm barriers. In order to monitor and govern the groundwater and environment soil, semipermeable, permeable and impermeable biofilm barriers have been suggested (Hiebert et al. 2001; Komlos et al. 2006; Cunningham et al. 2003).

By stimulating dense growth of biofilms, these barriers are able to limit the permeability and thus it allows to reduce or direct groundwater flow through specific subsurface areas. These subsurface biofilm barriers are quite competent in removing solutes.

9 Conclusion

Biofilm development in porous media involves a complex set of biological, physical and chemical interactions. Microbial biofilm produces Extracellular Polymeric Substance (EPS) when it adheres to the porous media and starts proliferating. Its growth affects porous structure, absorption, diffusion, dispersion, and transport systems of reactive and nonreactive solutes in porous media. Many latest techniques and technologies have enabled unveiling the biofilms formation in porous media.

The application of microbial biofilm in the areas of soil improvement, pollutant restoration, waste treatment etc. without the complete elucidation of biofilm formation displays its eco-social impact. The difficulties associated with biofilm in porous media need to be analysed in detail using sophisticated instruments and improvised work plan. In future, we anticipate that new knowledge would be generated describing the interactions between biofilms and their adaptation in porous habitats.

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Removal of Heavy Metals from Industrial Wastewater Using Bioremediation Approach

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Abstract Heavy metal contamination has developed great attention throughout the globe as an effect of their persistent and recalcitrant nature which results in toxic effects on the environment, shelf lives of animals and plants, estimating chronic diseases in individuals. Heavy metals exist a wider scope for science and innovation, with pressure on cost-effectiveness technology and to minimize the effect of anthropogenic accomplishments on the environment, and exploration of innovative, eco-friendly methods for ecological restoration. Bioremediation is known to be promising and eco-friendly technology for the remediation of contaminated sites and for a huge range of pollutant removals this treatment has been applied. Bioremediation technology has the potential to treat various types of waste and it is one method through which contaminant or toxic metals are treated by using living cells like bacteria, fungus, and algae, and this bioremediation method is better over than conventional methods. In this book chapter heavy metals and their types, effects of industrial discharge heavy metals on the water body and human beings are explained. This chapter also reviews the conventional treatment for the treatment of heavy metals that existed in wastewater. Then further bioremediation and its mechanism, types of bioremediations, the interaction between microorganisms and metals, and advantages of bioremediation methods for industrial wastewater are also discussed.

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1 Introduction

The presence of heavy metals in industrial emission leads to solid deposits, apoptosis along wastewater body streams and in faunas that live near dumping sites. In daily life exposure to contaminated wastewater is extensive, specifically in populated areas and where for agriculture purposes wastewater is used (Elgallal et al. 2016). Earlier research revealed that effectual metal waste management is a significant worldwide concern. Several industrial activities, like paper pulp, tanneries industries, batteries, electrical gadgets cause a severe impact on marine life. Heavy metal pollution is a worldwide issue and its level changes from source to source. Sewage and industrial wastewater released into waterways are a substantial source of heavy metal pollution in the water bodies (Van Oosten and Maggio 2015). The metals substantially bind to stripy materials, accumulate in the bottom of a river, and then are disposed into the water surrounding, pretending a secondary contamination source, and putting ecosystems at threat. Anthropogenic sources of metallic compounds in the natural ecosystem include acid rain leaking from the soil, lead in gasoline, runoff from non-point sources, atmospheric and industrial pollution, landfill, mining, manufacturing operations, and processing of nuclear fuel (Ahmed et al. 2017).

Industrial wastewater affects the fauna and flora of the water body with groundwater eminence. Metals like zinc (Zn), cobalt (Co), copper (Cu), selenium (Se), nickel (Ni), cadmium (Cd), vanadium (V), arsenic (As), mercury (Hg), lead (Pb), and chromium (Cr) are dangerous to humans and other living organisms. The hazardous chemicals should be eliminated from the industrial water to restore valuable compounds and to avoid negative significances. In their elemental forms, several metals like Cr, Cd, Pb, Ni, and Hg are severely toxic. (Patil and Bohara 2020). Thus, the heavy metals leached quickly into water and it gets accumulated into the human cell or tissues. Heavy metals are found in the gills, tissues, liver, and muscle of various species of fish in contaminated marine bodies. Metals get accumulated in various parts of the human body organs when they enter the food chain. Though most heavy metals are frequently used in industries, people and employees living close by facilities are possibly exposed and get contaminated. Heavy metals above the permissible limit, may have negative signs on people, other organisms, and the whole ecosystem. In food, the acceptable, permissible level of heavy metals has been allied to fewer human health threats. Heavy metal pharmaceutical contaminants, phosphorus, bisphenol-A, hydrocarbons, cations, textile dyes, detergents, nitrogenous elements, and pesticides are chemical impurities that commonly exist in wastewater (Koedrith et al. 2013; Gurav et al. 2019, 2021a; Choi et al. 2020a, b; Gurav et al. 2022).

With the growing number of contaminated sites worldwide, it is necessary to use an effective approach to control the spread of pollutants and reduce toxin concentrations. Innovative technologies should develop to harness the microbe's capacity to extract pollutants from contaminated sites. Biological treatment includes the utilization of indigenous microbes for the removal of organic matter in wastewater (Patil et al. 2019a, b; Shah Maulin 2020; Liu et al. 2015). Bioremediation microbes-based technologies are a substitute treatment and are flexible, safe, economically feasible, low-risk, and environmental friendly (Gurav et al. 2017). Microorganisms mostly fungi, and bacteria, which degrade environmental contaminants into fewer damaging types are identified as bioremediation. In bioremediation radioactive pollutants are enhanced for remediation by altering them into less poisonous forms to eliminate or immobilize the contaminants. The treatment needs naturally existing microbes that decompose and utilize dangerous pollutants as a source of food for their progress (Patil et al. 2019a, b). The process of bioremediation can be efficacious in environments that consent to the activity of microorganisms. Depending on its working, bioremediation is stated to be in-situ and ex-situ bioremediation. In-situ bioremediation treatment of wastewater is carried out on spot, with negligible excavation. In ex-situ bioremediation, the pollutant clearing and removing process are done after it has been shifted to a suitable location by excavation or pumping. In ex-situ the wastewater is collected from the contaminated site and in a distinct location it is treated using physicochemical and biological treatment (Ali et al. 2013, Shah 2021a, b). This book chapter presents heavy metals and their types, effects of the industrial discharge of heavy metals on the water body and human beings, conventional methods for heavy metals wastewater treatment. Bioremediation, types of bioremediations, and advantages of bioremediation are also discussed in this book chapter.

2 Industrial Wastewater and its Current Status

The universe is transitioning to a sustainable age. The wastewater which is produced not only disturbs the environment but also reduces the pure water concentration that will harm to the upcoming generations. To recover and reuse the wastewater there is a need for eco-friendly and innovative wastewater treatments. In this universe, there is a persistent need to overcome the water pollution problems. The concerns of industrial discharges are far-extensive, and they are affecting living creatures. For the working purpose, almost all industries require a huge amount of water (Sharma et al. 2021a, b). Water gains contact with toxic pollutants, hazardous waste, toxic metals, and biotic sludge through several processes. This polluted water contaminates the soil and negatively impacts the cropland, and also destroys the diversity of species that existed in that area. Individuals who comes in contact with contaminated soil or water also suffer prolonged health issues. Due to air pollution, there is a prominent growth in numerous diseases, and it seems to affect us frequently. Air and water pollution is affecting the atmosphere and health of individuals due to the arrival of medium, small, and huge industries. Pollution due to industries damages the normal patterns and cycles, signifying severe biodiversity consequences. Industries emit greenhouse gases and smoke into the environment, which subsidizes the hazardous products, global warming, chemical waste, and other pollutants that continue to destruct the universe and its inhabitants. Industrial emission also enriches the concentration of heavy metals present in soil like cadmium, iron, etc. A comprehensive range of heavy metals has been shown by polluted topsoil. The pre-treated industrial water is generally discharged to the surface water body. The concentration of metals in the bottom sediments and riverbanks is usually high. Unintended industrial development, deficiency of pollution control guidelines, a huge number of enterprises, use of outmoded machinery, and improper waste disposal are the significant causes of industrial pollution (Ali et al. 2020; Gurav et al. 2021b, c).

2.1 Distillery Wastewater

In distillery wastewater sugarcane juice, di-butyl phthalate, benzenepropanoic acid, and 2-hydroxy caproic acid are being exist. Few of these contaminants like 2-hydroxy caproic acid, di-n-octyl phthalate, and butanedioic acid dibutyl are responsible for endocrine disruptors in living creatures. The several contaminants that existed in distillery wastewater are genotoxic, cytotoxic, and carcinogenic and they should be removed before disposing it into the environment. Generally, untreated disposal of these contaminants into aquatic ecosystems results in high chemical oxygen demand, biochemical oxygen demand, sulfate, high nitrogen, and phosphate content that causes eutrophication. If these contaminants are disposed on the terrestrial ecosystem results in inhibition in seed germination, soil acidification, and less crop growth (Zhang et al. 2021).

Advancement in technology has resulted in the development of organic treatment methods these treatments significantly reduce numerous problems of the environment but are ineffectual in treating inorganic waste existing in wastewater. To cope with the hazardous pollutants numerous physical, biological, and organic approaches can be applied individually or in a mixture. Flocculation, coagulation treatment will reduce biological load, color, and suspended solids but it is not solely effective for inorganic materials. Organic treatments are known to be eco-friendly, and they can be applied aerobically and anaerobic for the treatment. The anaerobic treatment is effective for reducing the organic load from wastewater, but an appropriate sample and microorganism's requirement is essential. The microbe bio-transfers the contaminants that are existed in wastewater into comparatively less hazardous elements. Anaerobic digestion can be done with conventional reaction methods like up-flow anaerobic sludge blankets or with continuously stirred tank reactors, anaerobic batch reactors, single and biphasic systems, anaerobic filters, etc. (Singh et al. 2021).

2.2 Pulp and Paper Industry

Wastewater from the paper and pulp industry is a huge source of heavy metals adulteration in the environment all-round the universe. The extensive quantity of wastewater has been produced from the paper and pulp industry due to the elaborate processing of material at a diverse stage prominent to difficulty maintaining the contaminant release levels laid by numerous environmental protection policies. Subsequently, the discharge of wastewater into and environment have adverse impacts like scum formation, increase in temperature, slime production, odor, and decrease in aesthetic beauty. In recent years due to advance technology the amount of wastewater discharge has been reduced. The production scale also impacts wastewater quantity as compared to bigger mills medium and small-scale mills generate less concentration of wastewater. Thus, the pulp and paper industry wastewater lead to ecotoxicity so there is a need to improve the manufacturing process and treatment amenities to overwhelm the problem. Approximately a ton of paper production discharges 190–200 m^3 of wastewater with huge suspended elements and dissolved solids. In 2016–2017, the Central Pulp and Paper Research Institute has reported that there are 850 pulp and paper industries, comprising the minor paper industry. It characterizes the significant quantity of lignocellulosic waste and toxic metal contamination produced by these productions in India (Zhang et al. 2021).

These treatments are known to be better in terms of performance and expense in addition to being environment companionable, nevertheless, organic treatments are not predominantly effective for reducing recalcitrant pollutants and color of wastewater. The efficiency of these treatments is based upon the approaches like biodegradability index, nitrogen to carbon ratio of generated wastewater. Hence, more research is needed to express the norms for approving any treatment system and attain current outcomes from the designated approach. Therefore, research is also needed to implement strategies to eliminate recalcitrant contaminants from the wastewater with the concurrent generation of energy like the bioelectricity, biogas by sustainable technique (Gurav et al. 2020; Gurav et al. 2021d). Heavy metal contamination in surroundings poses risk to zooplankton, microbes, phytoplankton, human health, and wildlife due to recycling in the food chain. The paper factories generate kraft paper, writing paper, and hardboard produces numerous metals, principally Mn, Cu, Fe, Pb, Cd, Cr, and Zn as well as a huge concentration of unwanted waste. These toxic metals have a robust binding propensity with lignocellulosic waste due to their cations, causing an organometallic complex. Due to their composite composition, these chemicals perform as insistent biological contaminants. As a result of their lasting persistence and damage caused by continuing bio-concentration in animals and plants tissue, these toxic metals and persistent organic contaminants create a negative impact on the ecosystem, disturbing the food chain, food web by bioaccumulation and biomagnification. Mostly in industrial sludge, these heavy metals are being existed, which are enormously harmful to living creatures, and the environment (Singh et al. 2021).

2.3 Tannery Industry

In-universe, yearly tannery industry produced approximately 300 million tonnes of wastewater. China individually produces around 1.2 hundred million tons of liquid waste from approximately 1.4 hundred million tons of water disbursed in the tannery processes yearly. Currently, liquid waste reduction and the development of a sustainable tannery industry are the main significant factors to be pursued. Because of its huge pollution, tanneries trades are levied upon severe guidelines concerning the discharge of the contaminants. Countries like Southeast Asia, the USA, Europe, China, and Brazil are more conscious and effective in following rules and regulations about pollution. These nations have implemented and placed standards regarding the limits of biological oxygen demand (BOD), chemical oxygen demand (COD), pH, chromium, sulphide, etc. There are numerous processes in the tannery industry, which contribute to generating a huge amount of wastewater generated like production, processing, and manufacturing of leather each single step generates a specific quantity of wastewater with a diverse concentration of pollutants. The generated water from the tannery industry is extremely alkaline, with high COD, BOD, suspended solids level, saline and contain a pungent odor, with dark color (Gurav and Jadhav 2013). In the tannery industry from the individual process, the wastewater is treated distinctly before merging with the integrated liquid waste (Singh et al. 2021).

The primary treatment eliminates suspended solids, scarves, hairs, and other biological matter intending to decrease the biological load for the secondary treatment. By several methods, the primary treatment is performed like neutralization, electro-dialysis, air floatation, adsorption, coagulation, micro-electrolysis, etc. Every treatment system confirms the toxic element removal that is existed in the liquid waste and is ready to be progressed to the biological treatment. Secondary treatment methods involve anaerobic and aerobic treatment systems applied to tannery liquid waste followed by primary treatment methods. The numerous treatments systems are included in secondary treatment methods like sequencing batch reactor, oxidation, oxidation ditch, activated sludge, moving bed biofilm reactor, microbial remediation, constructed wetland, and anaerobic treatment method with biogas production. Almost all these treatments are eco-friendly and stable even in medium, small, and big scale tannery industries but they can handle the low organic load. Secondary treatment methods do not efficiently eliminate phosphorus and nitrogen from wastewater, and numerous other contaminants require strong and advanced innovative technology. In tertiary treatment, by applying membrane filtration and oxidation the contaminants are removed. Above both treatments, effectively eliminate the lasting pollutants to comply with the set level of standards. Still, they are expensive, suffer drawbacks like membrane fouling, and need further expansion to develop their performance (Ayele and Godeto 2021).

To decrease wastewater, various tanneries throughout the world have applied procedures to make appropriate resources use like liquid waste recycling or applying it for washing ground. Few recognized uses of tannery-treated liquid waste are irrigation to sunflower fields and tannery liquid waste in microbial fuel cells. To ensure the lower discharge of tannery liquid waste, to reduce the pollutants, and for effective consumption of tannery waste, research and measures are still needed to be executed throughout the complete tannery industry process. Approaches to ensure cleaner manufacture are required, including hair preservation, salt reduction, low chrome tanning, and salt reduction (Gurav et al. 2016). Lessening in COD, BOD and concurrent mitigation of phosphorus and nitrogen must be safeguarded while treating the environmental balance of the receiving aquatic body or land (Meruvu 2021).

3 Types of Heavy Metals

Pollutants exits in the industrial effluent are classified as organic and inorganic contaminants that exist a huge range of toxic levels in it. Chemical, physical, and biological treatments are commonly applied in treating organic pollutants (Lyu et al. 2016; Zhang et al. 2016). But the above methods are not appropriate for treating inorganic contaminants like heavy metals. Since they have qualities like oxidation, reduction, complex formation, and solubility, the decomposition of heavy metals plays a primary concern (Lu et al. 2020). The element which has an atomic mass between 63 and 200 and specific gravity of more than 5.0 specifies that the compound is heavy metal. In the environment, they exist as a natural compound. The heavy metal word refers to the compound whose density is higher and toxic or harmful even at less concentration. In current years, heavy metal pollution has become a major threat to the environment, because even at a very minute level high risk relates to human health and the ecosystem. Because of its accumulation, endurance, magnification, and flexibility, it is a huge burden to the environment. Toxic metals either exist in a mixed or chemical form that is challenging to eliminate from the wastewater (Zhang et al. 2021). The presence of heavy metals in the open aquatic bodies tends to algal blooms, oxygen deficiency, and the end of flora and fauna. The discharge of heavy metals into rivers converts into hydrated ions which have an extremely toxic property than metal elements. The hydrated compounds interrupt the enzymatic process and immersion is more rapid (Soliman and Moustafa 2020). So heavy metal removal is obligatory to lower the risks of living organisms. To limit the pollution levels of water, World Health Organization (WHO) and Environmental Protection Agency (EPA) have recognized the most allowable discharge of heavy metal levels into the surrounding. So far if the discharged effluent comprises heavy metals with high concentrations, then the allowable limits will cause environmental and human health problems (Sharma et al. 2021a, b).

3.1 Cadmium

Cadmium exists in natural deposits, industrial effluent and is known to be the most toxic metal. It has a key role in plating, nickel–cadmium batteries, alloys, stabilizers, and phosphate fertilizers industries. In the eco-system, even at less concentration, the compound of cadmium is harmful. Due to cadmium "Itai-Itai" disease is induced by accumulation of cadmium in the aquatic bodies and occurs bones softening and fractures to individuals (Sharma et al. 2021a, b). Furthermore, they bring out special effects like lung cancer, hepatic toxicity, and produce harm to the liver, respiratory system, reproductive organs, and kidney. So efficient and economically consistent treatment is necessary for cadmium removal from the wastewater (Ali et al. 2020).

3.2 Chromium

On earth the utmost existing seventh element is chromium. It occurs in the ore form, which is composed of crocoite ($PbCrO_4$), chrome ochre (Cr_2O_3), and ferric chromite ($FeCr_2O_4$). In tanning industries, textile industries, electroplating industries, and leather industries are the major sources of chromium. The above manufacturers generate hexavalent chromium Cr (VI), and trivalent chromium Cr (III) but for animals, plants, and other organisms the hexavalent chromium is more injurious than trivalent chromium. Cr (VI) exists mostly in salt chromate production and Cr (III) is beneficial in fat metabolism and shows a chief part in sugar (Ayele and Godeto 2021). The above-mentioned forms are used in the chrome plating, glass industry, steel production, wood conservation, plating and electroplating, pigment fabrication. Chromium metal functions as a titrating agent, cleaning agent, and additive in-mold construction and fabrication process in magnetic tape. When humans are exposed to chromium it causes kidney and liver damage, skin inflammation, ulcer creation, vomiting, and pulmonary congestion. So, from wastewater chromium should be significantly removed before it reaches the environment or it should be modified into a low toxic form (Singh et al. 2021).

3.3 Nickel

Nickel is a hard silver metal with 28 atomic numbers. It is non-biodegradable and persistent heavy metals mostly exist in industrial wastewater. The industrial sources of wastewater are silver refineries, printing, electroplating industries, alloy industries, and battery manufacturing industries. Nickel is also used in several applications like jewelry, catalysis, alloys, batteries, coins, machinery parts, and resistance wires (Goswami et al. 2021). The utilization of nickel in various appliances creates a threat to the environment and humans. The human effects of nickel are chest pain, nausea, dry cough, breathing problem, skin eruption, renal edema, gastrointestinal ache, and pulmonary fibrosis. To avoid environmental and health risks, a striking and innovative technology is necessary to recuperate the nickel metal (Meruvu 2021).

3.4 Lead

Lead is a soft and heavy metal that occurs in the cerussite, galena sulfite form. The chief source of the lead association in the industrial effluent is primarily due to lead-acid batteries. Lead exists often in wastewater from electroplating industries, steel industries, electrical industries, and explosive manufacturers (Aibeche et al. 2021). It affects DNA and protein synthesis and cell replication. It is known to be hazardous and in the body of humans, it gets readily collected. It generates illnesses like damaging the nervous system, kidney damage, cancer, and mental retardation. To animals and plants, exposure to lead is dangerous and causes environmental pollution. So, numerous researchers around the globe are focusing their ideas on treatment for lead removal from wastewater (Sharma et al. 2020).

3.5 Copper

Copper is considered toxic when it is at a high concentration. It is the vital element needed for living organisms and plays a necessary role in the synthesis of an enzyme, in bone and tissue development. There are different forms of copper like cuprous ions, metal, and cupric ions from these cupric ions are more hazardous and toxic for humans than that for others (Zvab et al. 2021). The chief contributors of copper are metallurgy, mining industries, electroplating industries, chemical manufacturing, printing circuit, steel industries, fertilizers, and paints. The human effects of copper are anemia, hair loss, headache, kidney damage, brain, and liver damage, and even death. So, for copper recovery from wastewater satisfactory treatment technology is necessary (Wang et al. 2019).

3.6 Zinc

Zinc controls the physiological mechanisms and biochemical operations of human tissues. For other metals, zinc functions as a decorative and protective layer for steel zinc acts as an anti-corrosive agent. In various coal combustion, steel processing, and mining industries zinc is exploited. Although it is necessary for trace levels in organisms if it is more than limited health disputes such as vomiting, pain, anemia, skin inflammation, and fever are detected. The zinc industrial sources, accountable are pulp and paper, steel making industries, electroplating industries, and brass work industries. The above-stated things were collected with the desirable for zinc removal active treatment (Areco et al. 2018).

3.7 Mercury

Global awareness regarding mercury pollution has enhanced due to the hazardous incidents that occurred in Japan. The extremely toxic and hazardous heavy metal contamination in the water stream is mercury. In various forms, mercury exists in the environment like mercurous ion, elemental mercury, and mercuric ion. The transportation of mercury metal will contaminate, damage, disturb the entire water stream (Zhao et al. 2021). As it is available from industrial sources that pollute the environment so to preserve the environment and human health in 2013 the Minamata convention is weighed up. This convention has synchronized the materials comprising mercury fetch out stricter standards emission. Methyl mercury disturbs the synthesis of protein and harms the sites of enzymes. Mostly, from plastic industries, pulp and paper, oil refineries, pharmaceutical and chloro-alkali industries more concentration of mercury is discharged into the environment. The probable mercury consequences to humans are damage to the brain, kidney, respiratory and reproductive systems. Hence, the researchers have gained more devotion to removing mercury from industrial wastewater (Matsuyama et al. 2021).

3.8 Arsenic

Arsenic exists in rock, topsoil, midair, and water. Organic arsenic composites are chiefly exited in fish. Inorganic arsenic exits in groundwater further consumed for domestic and drinking purposes. Arsenic human exposure is significant via food consumption and potable water. Foodstuff is known to be the imperative arsenic source, inorganic arsenic is departures in potable water and through potable water, and individuals get wide-open to arsenic. Arsenic disclosure occurs through polluted soil like mine tailings (Ostermeyer et al. 2021). Contingent upon the solubility and particle size of arsenic the inhale in airborne absorption is dependent. Arsenic is the soluble form that is effortlessly immersed by the gastrointestinal tract and arsenic is ethylated and metabolites through urine are evacuated. Arsenic concentrations in nails, blood, hair, and urine, are used as exposure biomarkers. Great absorption of arsenic in the human body damages the liver, kidney, rises cardiovascular, immunological, metabolic disease (Sher et al. 2020).

4 The Effects of Heavy Metals Released from Industries on Natural Water Bodies and Living Organisms

Heavy metals like nickel, arsenic, antimony, zinc, cadmium, chromium, etc. are hazardous at greater concentrations and induce toxic sound effects on the biotic components. Ionic forms of metals react with biological molecules in the living body and form more toxic compounds. The toxic characteristics of these elements depend on the bioavailability, and critical concentration which is elevated through ecological progressions like bioaccumulation, and biomagnification. In many cases, biomagnification affects ecosystem composition and services. Oxidized forms and the ligand state of heavy metals play a vital role in the accessibility of toxic metals. When the heavy metal levels are beyond the threshold level, the heavy metal becomes toxic and disrupts metabolic reactions at the cellular level which affects the organ and organism. The injuriousness of metals touches the delicate organs and disturbs the nervous role, harms the blood content, affects kidneys, lungs functions, and other organs (Ahmed et al. 2012). This consequently results in a softness, loss of memory, an upsurge of allergies, raise blood pressure. Death of cells takes place due to the free radicals formation which is significant for the oxidative mechanisms in the body. Thus, heavy metals can change the biological composition of ecosystems and how living organisms interact with one another and their surroundings. Subsequently, it may result in the threatening of ecosystem services. Several regulatory bodies have adopted the permissible limits for the discharge of heavy metals. The researchers have concentrated on the development of innovative treatment techniques. If not properly treated, the fate of heavy metal-loaded wastewater will be entered the ecological food chains from the polluted water and soil resources (Damtie et al. 2018).

The entry of these elements in the biotic communities and ecological food chains is determined by various environmental factors. Uptake of these elements depends on the chemical property, solubility, bioavailability, chelation, properties of the medium, etc. The problem associated with heavy metals is that they may accumulate in the creature's bodies (microbes, floras, and animals) without killing them. If this living organism receives small quantities of toxic metals in food, then they will not eliminate, their concentration within the body of the organism increases. This process of accumulating higher and higher amounts of material within the body of the organism is called bioaccumulation. The small quantity of floras is called hyperaccumulators that effortlessly absorb the great levels of heavy metals from the surrounding. If the above floras are harvested the much more concentration of toxic metals will get reduced. Soil acidity (pH) is responsible for plants metal uptake. If the acidity is high the mobility and solubility of metals are more, ultimately, they are more accumulated and uptake by plants (Carlos et al. 2018).

The unusual changes in the coloring and growth patterns are signs that indicate the plants are polluted and adulterated with metals. In the body of carnivorous and herbivorous organisms, the metals enter through eating food, fish, meat, leafy vegetables, and by drinking milk, water with an eminent level of metals. From the creature body, the toxic metals are not eliminated, they get accumulated in the organisms organs, bones, skin, and hair. Lowest feeders predominantly accumulate these toxic metals and with sediments, the metals are ingested. The seaweed from sediments, and aquatic surroundings accumulate the metals. Thus, when they enter the food chain, this phenomenon of increasing levels of metals in the bodies of higher trophic level creatures is recognized as biological amplification. As humans are at the end of food chains, they are more exposed to metal adulteration from soil and aquatic water bodies. Due to their persistence, their effects on organisms at higher trophic levels, concerns about long-term human health problems (Liu et al. 2019).

4.1 Effect of Heavy Metals on Human

The Environmental Protection Act (EPA) has revealed that cadmium and its compounds, mercuric chloride, lead, methyl mercury are probably suspected to be carcinogens. To all the mercury forms the individual's nervous system is sensitive. Great concentration exposure permanently harms the brain, developing fetus is damaged, and kidney failure. The brain effect may result in tremors, irritability, memory problems, vision change, hearing loss, shyness. Metallic mercury high-level exposure in short term causes diarrhea, lung damage, high blood pressure, eye irritation, increase in heart rate, nausea, skin rashes, diarrhea. Disclosure to cadmium diarrhea, vomiting, breathing difficulties, abdominal cramps, high or low blood pressure, weakness in the muscle, and face numbness. Large amounts of barium intake can cause, high blood pressure, changes in heart rhythm or paralysis, and life loss (Wu et al. 2016). The arsenic is odorless and tasteless. Minor level exposure decreases the production of red and white blood cells, irregular heart rhythm, nausea, blood vessels injury, vomiting, and deadness of 'pins and needles in hands and feet. Lead can affect every body organ. Lasting lead exposure results in decreased performance of a nervous system, weakness in fingers, wrists, or ankles, small increases in blood pressure, and anemia (Kondzior and Butarewicz 2018). Disclosure to high lead levels can severely damage the kidney and injure the brain and ultimately cause death. In pregnant women, great levels of exposure to lead may cause miscarriage. High-level exposé in men can damage the organs responsible for loss in sperm generation. Ingesting high concentration and long-term acquaintance even to low concentration severely leads to and build-up possible kidney disease, lung damage, and fragile bones. Table 1 and Fig. 1 represents impact of heavy metals on humans and ecosystems is represented (Singh et al. 2017).

5 Conventional Treatment and Technologies for Heavy Metal Removal

The discharge of contaminants in water can be reduced by following adequate procedures. Due to its inhibitory characteristics, to remove the contaminant from wastewater high elimination enforcement treatment is required. The industries are facing more difficulties in reducing the contaminants from discharge. Therefore, to conserve and protect the surrounding, various technological methods have been innovated by researchers like ion exchange, dissolved air flotation, membrane bioreactors, reverse osmosis, etc. Depending upon the effluent type, and heavy metal concentration, the treatments is applied to the wastewater (Chai et al. 2021).

Table 1 Impact of heavy metals on humans and the ecosystem (Jia et al. 2018; Damtie et al. 2018; Kondzior and Butarewicz 2018)

Heavy metals	Impact on humans	Impact on ecosystem
Cu	Damage liver and kidney, headaches, dizziness, nausea, stomach cramps, vomiting	Affects the water purity, root growth
Cr	Hyperaemia, renal failure, cancers, necrosis, acute tubular necrosis, histiocytic infiltration, and lymphocytic	Damage the nutritional cycle of the ecosystem
Hg	Behavioral and neurological illnesses like tremors, memory loss, and insomnia	Seed germination in the plant is reduced, chlorosis; reduces the plant height
Co	Effect on pulmonary functions, and eye	Declined the starch, amino acids sugar, and protein content
Cd	Damage the bones and kidney	Affect the rate of germination and declined the quality of water
As	Pregnancy damage, cancer, and skin problems	Reduced the plant and leaf health, bio-accumulate, and biomagnifies in organisms
Hg	Behavioral and neurological illnesses such as tremors, insomnia, and memory loss	Seed germination in the plants is reduced, chlorosis, improper in development plant
Ni	Reduced the function of the lung, respiratory ache Syndrome, lung and nasal sinus cancer	Enzyme activity is decreased which affects the CO2 fixation and Calvin cycle
Mn	Parkinson's diseases affect the rate of mortality	The concentration of chlorophyll is decreased
Zn	Hair loss, necrosis, etc	Sugar concentration is declined, carotenoid, decrease in starch, amino acid, and chlorophyll content
Pb	Abdominal pain, hypertension, birth flaws fatigue, mental retardation, sleeplessness, hallucinations, paralysis, weight loss, vertigo, alteration in the function of the nervous system, and renal dysfunction	Effect on the enzyme action of organisms; biomagnifies in living organisms, declines the quality of water

5.1 *Ion Exchange*

Ion exchange is the separation treatment, where the ions are replaced with other ions, and metal ions are removed from the industrial effluent. The deposition of sludge is low in the process of ion exchange as compared to coagulation treatment. Ion exchange resin is used to eliminate the material from water. The resin is designed in stress-free and strain form to avoid natural decomposition. Through cross allied polymer, matrix resins are designed which are attached with functional groups and covalent bonding, and space in resin structures permits the ions to shift applicably.

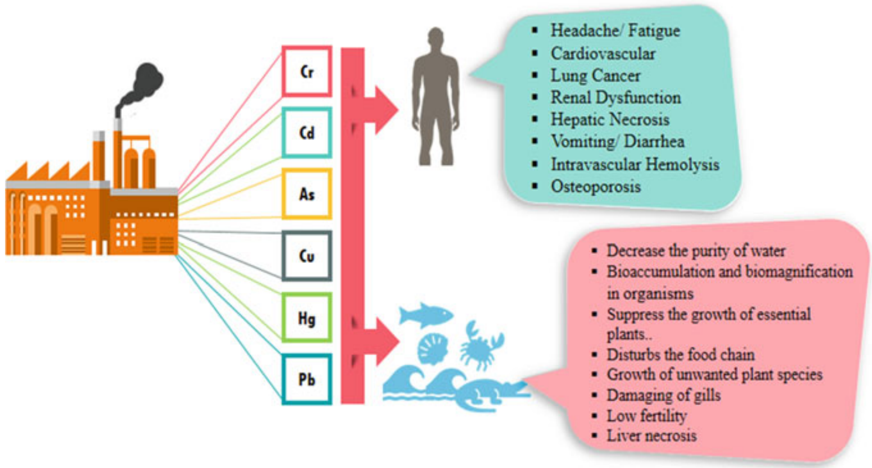

Fig. 1 Effects of heavy metal on humans and the environment

The resins are categorized into two types natural and synthetic resins from these two in the treatment process one resin has been utilized to interchange the metal ion with cation (Bashir et al. 2019). For the separation of metals from effluent than natural resins, synthetic resins are extensively preferred. The synthetic resins fore mostly remove the arsenic metals from a waste liquid. The cationic exchanger is widely preferred which includes weak basic and strongly acidic resins. In acidic resins, the sulfonic acid is an exit and in basic resins carboxylic acid is present. The metal cations and transmutable ions are delivered by hydrogen ions. Natural zeolites have great cation exchange capability for heavy metal removal from the waste liquid. The structure of zeolite is in crystalline form which includes silicate and aluminum atoms linked with oxygen bridges. Zeolites remove a great amount of chromium metal from wastewater, and it is also applicable for Pb, Cu, Zn, Cd. From the effluent 98% of heavy metals are eliminated by zeolites. Hence the ion exchange technology has been applied in various industries and an abundant quantity of effluent is treated for efficient heavy metal exclusion (Pan and An 2019).

5.2 Dissolved Air Flotation (DAF)

In dissolved air flotation the accumulation of suspended particles has raised by transiting the air bubbles in the liquid that can be effortlessly separable from the shallow of the water. Surfactants are supplemented in the process to surge the performance and enhanced the accumulation between negatively charged flocks with positively charged air bubbles. In DAF organic polymers are extensively utilized and on the particle surface during the agglomeration phase, the monolayer is formed which is signified by the polymer chain length and molecular weight. Various types of polymers are utilized in DAF like polyvinyl alcohol (PVA), chitosan, and polyethylene glycol in the DAF, modified PVA for Cd, Mn, Zn, Ni, Pb (Pooja et al. 2021). By applying chitosan to waste liquid in DAF 29% of Cd, 29% Pb, 31% Mn, and 27% Ni, were removed. In modified PVA treatment extensively Ni and Zn are removed and this treatment help in the elimination of other heavy metals. In DAF treatments the micro and nanobubbles are utilized from heavy metals exclusion. With the help of DAF treatment exclusive concentrations of heavy metals are eliminated from the industries so, this treatment has the prospective to confiscate the heavy metal overloaded with wastewaters (Azevedo et al. 2018).

5.3 Membrane Filtration

In membrane filtration, pressure-driven departure treatment is applied to waste liquid. Rather than heavy metal elimination, disinfection also occurs in this method. In membrane filtration, the particles are separated based on their concentration, pressure, size, and pH. By merging the membrane with chemical solutions, the mechanism of filtration is enhanced. The membrane consists of a precise porous surface which has a crucial role in eliminating heavy metals from polluted water. The material of the membrane is categorized into two types namely, polymer and ceramic. The ceramic membrane is preferred mostly in treating industrial effluent than polymer membrane because it is expansively resistant to a chemical due to its hydrophobic capacity. In polymeric membrane polypropylene, polyethylene, and polyvinylidene fluoride materials are mostly used due to their hydrophobic nature and they can foul effortlessly (Almasian et al. 2018). The inter collaboration between the heavy metal and polymeric membrane is great. Dependent upon the membrane pore size, the permeability of the membrane is achieved. The solute is on one cross and a pure solution is on the other side. This treatment is considered an optimistic technology due to its ease of operation, efficiency, and it requires less space. Supplementary organic elements and suspended solids are also eliminated in this treatment. The organic matter (OM) and dissolved organic matter (DOM) are major exits in the membranes. To enhance the membrane performance and to remove the OM, DOM effluent should be pretreated. So, to separate non-polluting material some focused membrane filters are exploited like nanofiltration, microfiltration, reverse osmosis, and ultrafiltration to segregate the heavy metal from effluent depending upon its size. These methods can grip the extent volume of aqueous liquid for heavy metal elimination (Efome et al. 2018).

5.4 Reverse Osmosis (RO)

In reverse osmosis, the charge exclusion and size exclusion principal work. The semi-permeable membrane is used for dissolved species removal and waste liquid is passed through the membrane. The range of pore size in RO is from 0.1 to 1.0 nm. For the process of desalination, it is greatly used. In treating industrial waste solutions for heavy metal removal reverse osmosis has extensively been used. In RO membrane various modifications have been carried out to treat the electroplating of an aqueous solution. The treatment efficiency of RO membrane is reliable on pressure, pH, membrane material, temperature, and clogging properties of the membrane (Thaçi and Gashi 2019). To prevent membrane fouling, wastewater should be pretreated to remove the colloidal and surface particles from the waste solution. For the last ten years, this method has been applied for treated wastewater. Before RO treatment ultrafiltration is applied to the waste solution to eradicate the clogging problem. This RO technology removes 92.3–99.8% of the organic pollutants, heavy metals, and inorganic materials from waste solutions. The RO removes most of the contaminants from the waste solutions hence it is preferred in extensive industries and for commercial purposes (Wang et al. 2016).

6 Bioremediation and its Mechanism

Bioremediation is a biotechnological intervention for the cleanup of the polluted sites, which can be done in situ as well as in ex-situ form. It relies on the integral characteristics and abilities of indigenous plants, fungi, and bacterial species. It has applications on the global, regional and local scale for preventing future pollution. For groundwater protection and soil conservation in current years bioremediation has been used. Bioremediation is a grouping of technologies that utilizes microbiota mostly, fungi, and heterotrophic bacteria to decompose or to modify the hazardous contaminants into other substances which are fewer hazardous byproducts than the parent substance. The beginning of bioremediation is the massive natural ability of microbes and plants to degrade organic forms and to accumulate inorganic forms of pollutants (Abatenh et al. 2017). The removal mechanism called bioaccumulation is the accumulation of substances or chemicals in an organism. There are a larger number of plants called hyperaccumulators that significantly absorb high concentrations of heavy metals from the soil environment. If such plants species are grown the living organisms will get more explored to these harmful metals, so most caution should take while utilizing these plants for animal and human use. The treatment site should be protected from the entry of wild animals. Thus, the entry of these materials into the food chain and unintended environmental consequences can be avoided (Bhatt et al. 2020).

The mechanisms used for microbial bioremediation are toxic metals sequestration by components of the cell wall or by intracellular metal-binding peptides and proteins like phytochelatins and metallothioneins (MT) along with elements like bacterial siderophores which are generally catecholate, related to fungi that generate hydroxamate siderophores. Altering biochemical pathways to block metal uptake may be a second mechanism. Transformation of metals by enzymes and reduction of intracellular concentration of metals using precise efflux systems is the consequent mechanism (Bhatt et al. 2020).

The capacity can be improved by applying genetically modified microbes and plants. As the toxic waste material remains in the vapor, liquid, or solid-state, the bioremediation technique differs depending upon the type of waste, the concentration of waste, and the site. When site conditions are not suitable, bioremediation requires the construction of engineered systems to supply materials that stimulate microbial as well as plant growth. Physiochemical conditions are optimized in engineered bioremediation (Kumar and Dwivedi 2021).

Various advantages are offered by applying the bioremediation process. Bioremediation solely relies on natural processes, and the threat to the ecosystems is reduced. In bioremediation to clean up the pollutants or impurities, the microbes are emended in ex-situ and in situ way. In the bioremediation process, the organisms or habitat is not disturbed and it is known to be a cheaper clean method because in this method there is no need for substantial labor or equipment. This process does not create any pollution or hazardous substance. Mostly the contaminant and hazardous products are converted into a sustainable form. In the case of inorganic bioremediation inorganic metal elements are collected, after being concentrated by the accumulators, and can be reused, or properly disposed of (Azubuike et al. 2016).

7 Interaction Between the Metals and Microorganisms

Microbes are involved in the surrounding fate of heavy metals, inducing transitions between insoluble and soluble phage through a range of biological and physico-chemical mechanisms. These methods are significant components of normal biogeochemical cycles for metalloids and metals, and a few of them may be applied to treat contaminated material. Through reduction, methylation, dealkylation, and oxidation microbes can alter metalloid and metal species. Metalloids undertake two major alterations: (i) decrease of metalloid oxyanions to chemical form like $SeO_2{-}3$ to SeO, and $SeO2^{-4}$ (ii) metalloid methylation, organometallics to methyl products, metalloid oxyanions, like AsO^{-2}, methylarsonic acid to (trimethylarsine) $(CH_3)_3As$, AsO^{-2}. The surface phenomena, bio-sorption retaining dead biomass, and application by alive biomass are examples of microbe metal interaction. The existence of dissimilar resistance mechanisms in microbes is a decent survival strategy for microbes to alter metal in the existence is even a great application in the soil and aqueous system (Azubuike et al. 2016). Economically, innovative practical biomass restoration and transformation of recovered metal into functional form are the outstanding selections for biosorption treatment. Additionally, remediation of metal from polluted soil demanded a great assimilated approach combining in situ and ex-situ techniques. To cope with these complex environmental issues, advanced development and supreme practicable innovative technologies are needed. The genetic and physiological foundation of microbe metal interactions, the microbial treatment seems to be the solution. Methods are often biogeochemically crucial because they alter the toxicity and stability of metals and contain biotechnological probable in bioremediation. Due to early toxic metal exposure, numerous microorganisms are presumed to have advanced metal resistance. The resistance of metal in the last 50 years is the outcome of increased exposure to metal contamination. Due to human activities, the heavy metal concentration has enhanced in the environment, but active microbes have developed the metal tolerant capacity and detoxifying approaches. A greater number of metal resistant and tolerant microbes were found in contaminated water, and soil. In recent years for the removal of excess chemical, physical parameters, and harmful toxic metal, the application of metal resistant and tolerant bacteria has attracted great interest (Kumar and Dwivedi 2021). Metal resistance elements in microbes are found on plasmids, which are certainly transmissible and extent by horizontal transfer. The essential resistance genes can be introduced into the appropriate area by genetic transfer method for manufacturing commercial inoculants, or use of enzymes for soil and water bioremediation. Bacterial adaptive responses to ecological stress and molecular processes of variation have been researched using molecular (PCR-based) and phenotypic methods. In the above section recent advances in microbe metal interaction, with special reference to metal toxicological effects on living creatures, responses of microorganisms in developing metal resistance and tolerance mechanisms, and applications of microorganisms in metal-contaminated environment bioremediation is explained (Verma and Kuila 2019). In Table 2, possible microbes for the great tolerance of heavy metals are described.

Table 2 Probable microbes for the great tolerance of heavy metals (Sharma et al. 2021a, b)

Sr. no	Heavy metals	Microbes
1	Cu	*Kocuria sp.* CRB15
2	Pb, As, Cu	*Microbacterium sp.* CE3R2, *Curtobacterium sp.* NM1R1,
3	Cd	*Flavobacterium sp., Rhodococcus sp., Klebsiella pneumoniae*
4	Hg, Cd, Ag	*Pseudomonas putida*
5	Cd	*Enterobacter, Klebsiella, Leifsonia, Bacillus*
6	Ni	*Bacillus licheniformis*
7	Cr, Co, Mn, Pb	*Pseudomonas moraviensis, Bacillus cereus*
8	Fe, Ni, Cr, Zn	*Bacillus sp.* PS-6
9	Zn, Cd	*Chryseobacterium humi, Ralstonia eutropha*
10	Pb	*Bacillus sp.* MN3-4
11	Pb, Cd	*Azospirillum*
12	As, Cd	*Mesorhizobium huakuii*

8 Types of Bioremediations

Various types existed in the bioremediation process. Bioremediation can be done by applying bacteria, fungi and in the phytoremediation process, numerous plants species have been used for the treatment of contaminated sites or to purify the pollutant material. In treating the hazardous substance or site the above methods are combined and the researchers have modified the process and treatment is implemented (Sharma 2021). In Fig. 2 types of bioremediations are represented and the details of the methods are deliberated under the subsequent heads.

8.1 Microbial Bioremediation

The heavy metal-microbe interactions are the basis of bioremediation. Microorganisms are ubiquitous and play a key role in the environmental fate of heavy metals. All types of microorganisms like bacteria, protozoa, fungi, algae, and lichens can mediate some types of interactions with heavy metals which can be explored in bioremediation. During their contact, heavy metals strongly affect the activities and survival of these organisms. High concentrations of many heavy metals produce harmful effects on many microbial species, and heavy metals are toxic to many microorganisms even

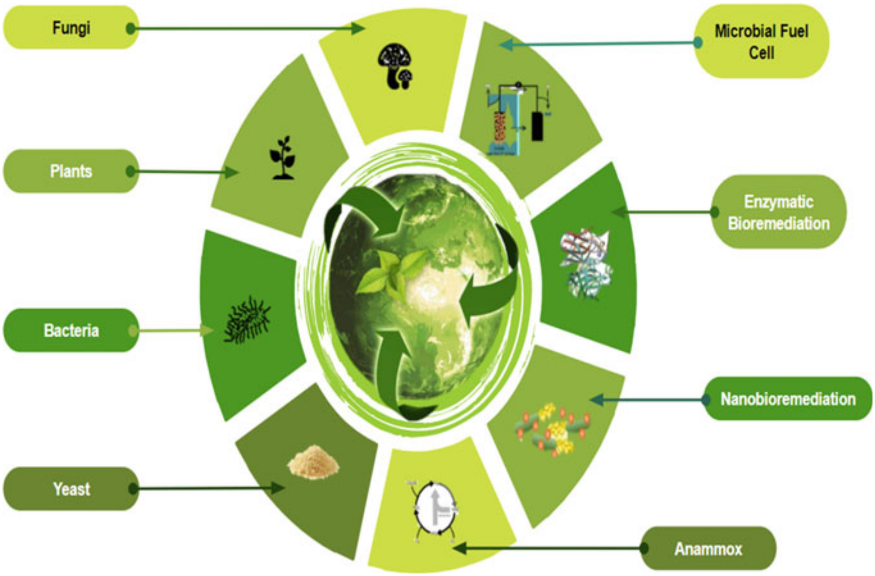

Fig. 2 Types of Bioremediations

at very lower concentrations. On the other hand, heavy metal-resistant microorganisms with their varying phenotypic expressions also influence the heavy metals in the environment (Verma and Kuila 2019).

Unlike bioremediation of oil, solvents, and pesticides which works as a source of food, energy, and hydrolyze contaminants which relies on stimulating the growth of certain microbes, in the case of heavy metals these are absorbed and accumulated inside the microbial body. Bioremediation requires a combination of the factors like nutrients, moisture, pH, osmotic balance, aeration, and the right temperature. The absence of these elements may prolong the process. Conditions that are unfavorable for bioremediation may be improved by adding amendments to the treatment site, such as molasses, vegetable oil, or simply air. These amendments optimize conditions for microbes to flourish, thereby accelerating the completion of the said bioremediation process. The presence of heavy metals at elevated concentrations of toxic chemicals, high salt concentration adversely affect bioremediation. So, the technology is feasible only for diluted metal contaminants (Ojuederie and Babalola 2017).

Bacterial diversity, ubiquitous nature, augmentation, and easy handling of bacteria have a bright future in heavy metal remediation of contaminated sites and wastewater. Acidic wastewater containing heavy metals, rich in sulphates is fed to the bioreactors which are specifically designed to facilitate microbial bioremediation. Bacteria in the bioreactors produce hydrogen sulfide, which is circulated to the metal precipitation tank where metal ions bound to sulfur forms metal sulfides which are least soluble and precipitated out from the water. These sulphides are collected and processed further to harvest minerals of economic value. The treated water after bioremediation becomes pure enough to meet safety standards so safely discharged and clean enough to use for agriculture and other related activities (Bharagava et al. 2019).

Biosurfactants are compounds produced by microbes like bacteria and fungi, which act as biological complexing agents for various heavy metals. Both biosurfactants and biosurfactant-producing microorganisms can be explored at heavy metal-contaminated sites for their removal by washing and flushing for ex-situ bioremediation. Bio-volatilization is a process that converts heavy metals into volatile derivatives using the catalytic activity of microorganisms. This process offers treatment for As and Hg contamination. Moreover, bio-volatilization has also been reported for other heavy metals like Bi, Sb, and Tc. Biosorption is a passive uptake process to bind heavy metals on the cellular structure of the biological mass. It involves physical and chemical attachments with some selected bio-functional groups, where ion exchange, covalent bonding, complexation, electrostatic attraction, Van der Waal's force, and microprecipitation play important roles in their interactions. For this process, a great variety of inactive and nonliving microbial biomass has been explored with a diverse range of biosorption capabilities. But pH, temperature and ionic strength of the solution, porosity, prehistory, and pretreatment of biosorbents, concentration and speciation of heavy metals strongly influence the capacity, and adsorption rate. Chemical modifications of biomass with accelerated binding capacity and affinity for heavy metals have a promising future. Table 3 represents the bacteria and fungus used in the bioremediation process (Wang et al. 2020).

Table 3 Microorganisms used in bioremediation process (Wang et al. 2020; Bharagava et al. 2019; Verma and Kuila 2019)

Heavy Metal	Microorganisms	Fungus
Pb	*Bacillus subtilis, Micrococcus luteus, B. firmus, Aspergillus niger, B. megaterium, Brevibacterium iodinium, Penicillium species, Streptomyces spp., Staphylococcus spp., Pseudomonas spp.*	*Candida sphaerica*
Cd	*Alcaligenes faecalis, B. megaterium, Pseudomonas aeruginosa, Bacillus subtilis*	*Coprinosis atramentaria*
Cu	*Streptomyces sp., Staphylococcus sp., Enterobactercloacae, Methylobacterium organophilum, Desulfovibrio desulfuricans, A. faecalis* (GP06)*, Enterobactercloaceae, Flavobacterium spp., Arthrobacter strain, Micrococcus sp., Gemella spp., Pseudomonas aeruginosa Micrococcus spp., Pseudomonas sp., Flavobacterium spp.*	*Aspergillus versicolor, Sphaerotilus natans, Aspergillus niger* (pre-treated with Na_2CO_3), *Candida spp. Aspergillus niger*
Ni	*Acinetobacter sp., Micrococcus sp., Desulfovibrio desulfuricans Pseudomonas spp.*	*Aspergillus spp., Aspergillus niger, Aspergillus versicolor, Aspergillusniger* (0.2 N) pre-treated with Na_2CO_3), *Candida spp.*
Hg	*Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio parahaemolyticus (PG02), Vibrio fluvialis, Bacillus licheniformis*	*Candida parapsilosis*
Cr	*Acinetobacter spp., Bacillus cereus,* and *Arthrobacter sp.*	*Aspergillus niger, Saccharomyces cerevisiae, Rhizopus oryzae, Aspergillus versicolor, Penicillium chrysogenum, Sphaerotilus natans, Phanerochaete chrysosporium, Saccharomyces cerevisiae, Hansenula polymorpha, Yarrowiali polytica, S. cerevisiae, Rhodotorula pilimanae, Rhodotorula mucilage,* and *Pichiaguillier mondii*
Zn	*Pseudomonas spp., Bacillus firmus*	
Co	*Enterobacter cloacae*	

8.2 Algal Bioremediation (Phycoremediation)

Algal bioremediation the known to be an innovative treatment for heavy metal elimination from wastewater applying chiefly inactive and non-living biomass and algae. Live algae have limited sorption capacity as the heavy metal ions often adversely affect the living cells and numerous factors of the environment which directly impact the absorption capacity of ions. Additionally, the sorption method shows high differences based on the development phase of algae. Absorption mechanisms in living algae are more complex than in non-living algae. The non-living algae cells absorb metal ions from the cell membrane surface, and it is a kind of extracellular process where metal recovery becomes easy. Non-living algal biomass is an assemblage of polymers (such as complex carbohydrates, cellulose, pectins, and other associated glycoproteins, etc.) that is capable of binding to heavy metal cations as adsorbents with the potential for cost-effective wastewater treatment (Zeraatkar et al. 2016).

8.3 Phytoremediation (Plant bioremediation)

Phytoremediation is a process that uses various types of vegetation (green plants) for situ treatments to remove, transfer, and stabilize, the metal contaminants. In this innovative treatment, the natural properties of phytoplanktons are utilized in engineered methods to remediate toxic waste. The advantage of the plant nutrient utilization process is to absorb nutrients and water through roots, emerge water by leaves; and performs as a transformation structure to metabolize to biological element (Patil et al. 2022). The plant bio-accumulate and absorb heavy metals including trace elements. For plants, the eagerly bioavailable heavy metals are nickel, selenium, cadmium, copper, arsenic, and zinc. Temperately bioavailable heavy metals are cobalt, chromium, manganese, lead, and iron. Uranium is mostly not available metal. By applying chelating agents much more lead can be accessible. Similarly, to enhance the accessibility of radio-cesium 137 and uranium ammonium nitrate and citric acid should be used.

Factors affecting phytoremediation other than bioavailability are the selection of proper accumulator which is responsive to agricultural practices that allow repeated planting and harvesting. Production of sufficient biomass and accumulation in shoots are also essential criteria. More than four hundred species of plants are considered suitable for use in phytoremediation (Gerhardt et al. 2017).

Phytoaccumulation (phytoextraction) is the process, where plant roots of selected hyperaccumulator plant sorb (absorb/adsorb) store the metal contaminants along with other nutrients and water. The mass which is contaminated is not devastated but ends up with the leaves and plant shoots. This treatment is primarily used for wastewater containing a lower concentration of metals. The metals are stored in the plant in aerial shoots, which are harvested and either smelted for potential metal recycling/recovery or are eventually disposed of as hazardous waste (Pandey and Bajpai 2019).

Phytoextraction includes the absorption and uptake of polluted metals which is existed in the soil through roots and underground elements of plants, by hyperaccumulation mechanism. From the polluted soil, the hyperaccumulator plants uptake huge concentrations of heavy metals, and accumulate and transfer them in other body organs beyond the ground at from 100 to 1000 times greater concentrations than those existing in non-hyperaccumulating plant species. These hyperaccumulators will not have any phytotoxic effect or disease hence they are very suitable for phytoremediation. The hyperaccumulators are usually grown in metal and toxic contaminated soil areas and also generate plentiful biomass. These plants are unique because of the characteristics like the much greater capacity to take up heavy metals from the soil; enhanced root-to-shoot translocation of metal ions; a much greater ability to detoxify and sequester extremely large amounts of heavy metals in the shoots, and the ability to grow fast with the profuse root system (Suman et al. 2018). Rhizofiltration is related to phyto-accumulation, but the phytoplankton applied for cleaning are elevated in greenhouses with their origins in water. This system can be used for ex-situ treatment. That is, wastewater is impelled to the plant's surface irrigation. For hydroponic systems, the artificial medium soil like sand is submerged with perlite or vermiculite are used. As the roots become saturated with contaminants, they are harvested for valuable metal recovery and disposed off if they are useless (Pajević et al. 2016).

8.4 Nano-Bioremediation

The nanomaterials are categorized as substances having sizes 1–100 nm. The size of nanoparticles (NPs) is precisely tiny that has quantum special effects by restraining their electrons. Due to the NP's size, they hold numerous exclusive and special properties. NP's have been applied in several fields like electronics, biomedicine, catalysis, and photonics. NP's signify changeable properties with the help of bulk counterparts, so there are various opportunities to develop innovative materials that can be utilized in several industrial applications. By utilizing the single-step treatment method the NP's can treat the wastewater efficiently and remove the contaminants that existed in wastewater. In industrial wastewater treatment (WWT) the NP's are applied as adsorbents. Due to its unique structure and properties like adsorption capacity and high selectivity, it is significant to remove heavy metal exited in wastewater. Their high volume and surface ratio permits them to absorb heavy metals and other contaminants. Nanomaterials can increase reactivity, penetrate deeper, and eliminate heavy metals efficiently. Current research highlights the possible use of materials such as nanocomposite, nanowires, nanotubes, and nano-spheres merged with conventional WWT which is beneficial to eliminate several inorganic and organic contaminants, including heavy metals. Depending upon the existence of heavy metals and the external factors dissemination potential of nanomaterials is influenced. External factors affect the nano-adsorbents properties like sorbents size, shape, surface chemistry, chemical composition, fractal dimension, solubility, crystal structure, agglomeration state. Nanomaterials bargain atomic level modification, unlike bulk materials that have several innovative characteristics and properties which bulk materials are not offered (Tripathi et al. 2022).

9 Advantages of Bioremediation for Industrial Wastewater Treatment

Bioremediation using indigenous microbial and plants species is one of the waste treatment technologies that utilizes the natural state of the environment. It is an engineering and scientific application to the processing materials through biological agents which are renewable. It is an eco-friendly method that needs very low energy consumption. As biotic factors are explored in the treatment process it is an aesthetically pleasing and cheaper option. In the ex-situ process, easy monitoring of microbes and plants can be done. Periodically harvested material can be used for the recovery of valuable precious metal species for further use. Toxic and hazardous metals can be properly disposed of at the disposal site (Pajević et al. 2016).

Thus, the use of plants for the bioremediation of water offers a wide range of advantages. In phytoremediation, solar energy has been utilized and in an undisturbed condition, without excavation, the huge concentrations of contaminants are removed. The numerous metals are treated and removed with the phytoremediation method. For the polluted sites, phytoremediation delivers a valuable tool, mostly these sites are not remediated by other treatments like huge extension sites at shallow depths with fewer impurities concentrations. The plant species utilized in the phytoremediation process fits studied crop plants hence there is extensive knowledge available for the management, harvesting, and cultivation, of these plant species (Gerhardt el at. 2017).

In phytoremediation, the ground area is covered by the plants, so the waste and air pollutants are restricted by the plants, which decreases water and wind-based erosion and steadies the soil. The wind erosion exposes the direct pathway of air contamination inhalation and contaminated food ingestion by suspended particle deposition onto the leafy vegetables and plants. If we used hyperaccumulators then its biomass can be incinerated which declines the waste volume and mass. As compared with other remediation treatments phytoremediation is a cost-effective method. In contaminated site removal, many steps are included like removal, excavation, isolation, and returning of the residue to the specific site. The human–plants relationship in the evolutionary past is rooted deeply. With the help of remediation, the contaminated site or the barren land can get converted into an aesthetic green field. The social and public acceptance of phytoremediation is also high. Huge research has still to be done to increase the efficiency of bioremediation treatment, to enhance the production and number of organisms for the treatment. Another limitation long time duration of the site clean-up (Pandey and Bajpai 2019; Suman et al. 2018) (Fig. 3).

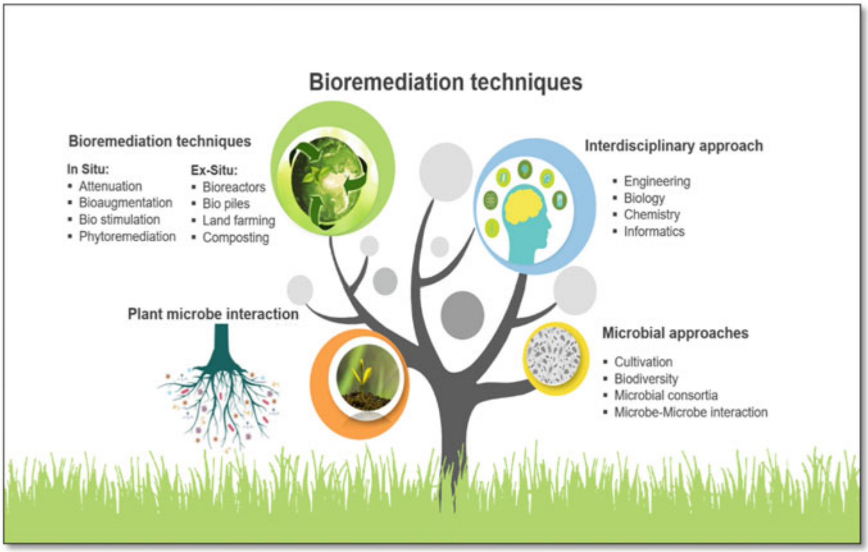

Fig. 3 Advantages of Bioremediation

10 Perspectives, Challenges, and Opportunities

Bioremediation is a low maintenance cost, highly selective, less contaminated, and low water generated, microorganism-based promising and remediated technology. This process is mostly employed to exclude toxins from the surroundings and the adsorption of harmful pollutants from wastewaters, pesticides, or fertilizers. The several advantages are offered by biosorption, microbes the probable potentials of enormous scale application seem encouraging when limited costs are considered. In bioremediation selection and screening of microbes, strain is very important. Constant mechanisms study of sustaining heavy metal biosorption, and bioaccumulation by microbes, development in equilibrium and kinetic model to upgrade the process of bioremediation. Also, the commendation for encouraging toxic metal elimination, utilization of biomass including chemical modification treatment, and combining or incorporating this method with conventional for heavy metal removal is needed. Microorganism role, identification, catabolic and metabolic pathways have been promoted from advanced molecular treatments like proteomics, genomics, transcriptomics, and metabolomics (Pandey and Bajpai 2019). Contaminant degrading microorganisms often existed in contaminated sites; the types and pollutants concentration may stimulate their growth, development, and metabolic activities, but it can be applied for agro-industrial waste, which encompasses high content of phosphorus, nitrogen, and potassium, and act as a nutrient source for a most extremely contaminated site. Then the pure isolates microbial consortium degrades contaminated material more effectively. Optimizing the application of genetically engineered microorganisms (GEM) to enhance the capability of bioremediation is a promising approach. This is outstanding to generate biocatalyst target pollutants, which contain resistant elements by combining efficient and novel metabolic pathways, spreading the substrate level of prevailing pathways, and increasing the stability of catabolic activity. GEM multiplication and parallel gene transferrin a bioremediation application has become a successful strategy. Additionally, applying biological, organic, and eco-friendly methods, smearing GEM to a target polluted material could enhance the efficiency of bioremediation (Pajević et al. 2016).

11 Conclusion

Bioremediation of metals from wastewater uses indigenous as well as genetically modified strains of microbes as well as plants. It is ecological, economical, and socially acceptable technology where renewable biological resources are explored. Phytoremediation enhances photosynthesis through cultivated plants for phytoremediation. The activity can be intervened by modifying hyperaccumulators for their luxurious growth, disease resistance, other environmental stress resistance as well as herbicide resistance features as per the requirements. These Genetically Modified Organisms (GMOs) contain foreign genes implanted into the genome of another creature of the different or same species by applying recombinant DNA technology. These genetically engineered microbes and plants have been applied to acquire capable strains for bioremediation of polluted environments by having the enriched capability to break-down abundant contaminants. Considering the importance of transgenic microbes in heavy metal contaminants removal, more research must be done to enrich the survival rate of microbes when released into the ecosystem for bioremediation, because their survivability is poor in the current situation. Many environmental factors like temperature and nutrient concentrations and other related factors, can hamper their utilization and need to be controlled for the effectiveness of the bioremediation process. Anticipation of horizontal gene transmission from engineered microorganisms to indigenous microbes, use of anti-sense RNA and suicidal genes, antibiotic genes markers should be avoided and can be replaced with other selectable markers to avoid unintentional transfer of antibiotic resistance genes. However, research is essential to appreciate the metabolic pathways of microbes and transgenic plants applied in bioremediation to discover their efficiency. High biomass hyper-accumulator plants should be identified, and they should be improved through genetic engineering. The capacity of the microbes applied in bioremediation to compete with the indigenous microbial population is essential for the success of bioremediation.

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Membrane Reactor and Moving Bed Biofilm Reactor for Tannery Wastewater Treatment

C. Raja, J. Anandkumar, and B. P. Sahariah

Abstract Tannery effluent is one of most complex industrial effluent, huge amount of wastewater rich in organic and inorganic pollutants including heavy metals and toxic elements. High COD along with presence of chromium and sulphides is basic characteristics of tannery wastewater (TWW). Research community emphasizes on tannery effluent treatment using individually or in various hybrid processes owe to complexity and requirement of non-harmful dischargeable limit of this wastewater. For preserving water in its natural state, researchers all over the world develop numerous new tools and technologies such as membrane systems, advanced oxidation system, sorption systems and moving bed biofilm reactor etc., along with conventional bioremediation/physicochemical treatment processes. Membrane reactor (MBR) follows principle of separation of pollutants using organic and inorganic membranes developed from kaolin, clay, polyvinylidene fluoride, poly sulfone, poly ether sulfone material etc., also has drawn attention for tannery wastewater treatment. Moving bed biofilm reactor (MBBR) uses microbe's pollutants remediation potential providing a substratum for its settlement, high density, speedy growth and shock tolerance. This chapter discusses fundamentals of MBR and MBBR, their applicability in individual and hybrid system configuration for achieving successful treatment of wastewater from tanneries.

Keywords Tannery wastewater treatment · Membrane reactor (MBR) · Moving bed biofilm reactor (MBBR) · Hybrid process · Chromium

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1 Introduction

Tannery industries are one of the most important commercial successful industries in the world. In leather processing, tanning is the major and essential operation for producing various leather products. Leather processing is divided into a few basic operations such as pre-tanning, tanning, post tanning, and finishing. The average consumption of water for raw material processing is from 25,000 to 80,000 L per unit operations (Mannucci et al. 2010). In pre-tanning operation includes soaking, unhairing, liming, bating, and deliming. Degreasing, pickling, tanning, pressing, and drying are the major operations in the tanning process. Chrome and vegetable tanning processes are important steps in tannery process and produce a huge volume of wastewater and responsible to cause pollution in the aqueous system (Lofrano et al. 2013). Reports states that for more than 2,500 tanneries in India, the waste bio-sludge generation in TWW is as high as 165,200 tons/year and the scenario for treatment becoming financially heavy (Bharagava et al. 2017; Sodhi et al. 2021).

1.1 Characteristics of Tannery Wastewater

TWW is diverged in characteristics from tannery to tannery due to the raw material used and the processing. Type of tanning process, organic loading, and size of the tannery highly influences the characteristics. TWW contains organic & inorganic biomolecules such as blood, fats, hair; processing chemicals such as calcium sulfide, trivalent chromium, tannins, sulfonated oil, spent dyes, resulting in chlorides, sulfates, and sulfide, etc., in significant concentrations; and a high quantity of total solids, BOD, and COD (Korpe and Rao 2021; Lofrano et al. 2013; Chowdhury and Mostafa 2015; Sawalha et al. 2019). Re-tanning and dyeing operations can produce wastewater with 71,040 mg BOD/l and 2400 mg COD/l.

General composition of TWW observed in the different studies is summarized in Table 1.

Pickling and chrome tanning operations produce a huge quantity of solids (TS and TDS). The discharged wastewater from the liming and unhairing operations has high pH conditions. Post tanning process consumes as high as 8.6 m^3 of water on averagely which has high conductivity due to the presence of chloride and sulphate ions. High sulphide is characteristic of liming process with a discharge of as high as 0.12 m^3 WW/ton. Dye, metal complex, fat liquoring agents, and tannins are important chemicals present in the TWW that can cause toxicity to the environment (Hansen et al. 2021). Significant amounts of heavy metals such as Cr, Ni, Cd, Pb, Cu, Zn (Abdel-shafy et al. 1997) are regular in the TWW. Sivagami et al. (2018), Shah (2020) reported that after primary and secondary treatment failed to meet the discharge limit of sulfate and total dissolved solids from tannery wastewater containing 3980 mg/l of COD and 4000 sulfates.

Table 1 Characteristics of tannery wastewater reported in different literature

pH	BOD (mg/l)	COD (mg/l)	Chloride (mg/l)	Chromium (mg/l)	Sulphate (mg/l)	Dissolved solids (mg/l)	Suspended solids	Total solids	References
7.5	1000 ±	7200 ± 20	–	–	–	8100 ± 50	1240 ± 40	9340 ± 70–	Chowdhury and Mostafa (2015)
7.8–8.8	150–250	1500–2500	1360–1740		2100–2760	3000–5000	200–1000		Sivagami et al. (2018)
7.5	920 ± 15.81	3980 ± 29.66			4000 ± 14.32	14,000 ± 50.99	6800 ± 35.35	20,800 ± 55.01	Chowdhurty et al. (2013)
7.15	–	35,200	43,100	–	–	52,000	16,000	–	Sawalha et al. (2019)
4.12–4.68	2099 ± 1185	4767 ± 2422	1302 ± 420		2671 ± 1582	7677 ± 4084	–	–	Boopathy et al. (2013)
4.62–8.13	900–1200	1100–3000	–	0.360- < 0.001	–		58–200	–	Rangel et al. (2007)
7.5	2000	> 10,000	–	–	–	–	4000	45,000	Song et al. (2000)
8–12	1243–1984	6593–7267		14–26	–	3250–7489	1217–2847	–	Aregu et al. (2021)
10.2	–	10,500	4620	8.2	2560	7420	–	–	Pal et al. (2020a)

(continued)

Table 1 (continued)

pH	BOD (mg/l)	COD (mg/l)	Chloride (mg/l)	Chromium (mg/l)	Sulphate (mg/l)	Dissolved solids (mg/l)	Suspended solids	Total solids	References
–	–	55,000	–	198	0.14	–	985	–	Stoller et al. (2013)
5.11	–	21.305 ± 150.58	–	–	–	–	4831 ± 131.16		Yang et al. (2021)
7.2–8.5	–	541–6309	–	–	–	9900–14,090	–	–	Vo et al. (2021)
5.65 ± 0.86	–	992 ± 21.5	–	–	1.165 ± 14	–	–	–	Serkaran et al. (2013)

1.2 Conventional Treatment Process

The presence of large molecular organic and inorganic compounds, diverse heavy metals at high concentration in TWW are prone to cause serious pollution issues to the environment if released without any proper treatment. Conventional treatment methods used for the TWW include bioremediation in activated sludge process (ASP), up-flow anaerobic sludge blanket (UASB), chemical precipitation, adsorption, coagulation-flocculation, floatation, ion exchange and wetland systems etc. The following section briefly describe a few conventional processes in view of TWW.

Bioremediation

Bioremediation is the process of removal /reduction /transformation of pollutants into less harmful or elemental component with the help of biological species. Generally, bioremediation involves biodegradation, biosorption, bioleaching and bioaccumulations where microbial species and numerous plant species and live/dead biomass fix the pollutants; and the same are applicable for the treatment of TWW (Ghumra et al. 2021). In microbial -based bioremediation process of TWW, microbes degrade organic compounds originated from animal skin/ leathers; colouring agents like dye material and process biosorption of heavy metals present in the TWW. Efficiency of microorganisms such as bacteria, fungi, yeast, and algae are well documented for TWW bioremediation provided suitable operating and environmental conditions. Similarly, the phytoremediation process where plants are used to treat contaminates, the treatment plants follow mechanisms of phytostabilization (fixed state/ location of pollutants), phytoaccumulation (absorb and accumulate in plant body parts) phytovolatilization (evaporation), phytodegradation (breakdown) and phytoextraction (extract and absorb) of the pollutants. Specific organic acids, enzymes and root exudates consists of phytosiderophores (metal solubilizing substances) present in plant species play the major role in phytoremediation.

The bioremediation is governed by the cellular and molecular components present in the microbial cells that releases enzymes and other functional proteins suitable for remediation mechanisms. Numerous conventional reactors with various environment such as activated sludge process (ASPs), up flow anaerobic sludge blanket (UASBs) and *Constructed wetland system* are frequently employed for TWW treatment. ASP is one of the prominent processes aerobic biological processes used in the secondary treatment of TWW efficient for removal of BOD and COD in the continuous treatment process along with sludge separation. It is an easy to operate and economical viable method. Excess activated sludge is the major issue of this process. Bera et al. (2012) evaluated the efficiency of ASP and achieved 95.58% of chromium removal from the tannery wastewater. Ballén-segura et al. (2016) achieved removal of chromium, nitrate, phosphate, and BOD using. *Scenedesmus* and observed that growth of the algae species is directly proportional to pollutant removal efficiency. Sekaran et al. (2013) reported efficiency of immobilised *Bacillus* sp. and algae species

Synechocystis to remove BOD, COD, VFA in a chemo autotrophic activated carbon oxidation reactor with complete sulphide removal. *Serrata marcescens, Bacillus aryabhattai HU-39, Wickerhamomyces anomalus M10, Saccharomyces cerevisiae, Aspergillus flavus CR500* are a few important microorganisms frequently recognised for heavy metal removal in the aqueous solutions (Kumar and Dwivedi 2021). Removal of 4-n-nonylphenol, mono- and di-ethoxylated nonylphenols from conventional tannery effluents using plants are well recognised (Gregorio and Ruffini 2015). Kassaye et al. (2017) observed chromium removal using *Polygonum coccineum, Brachiara mutica* and *Cyprus papyrus*.

Constructed wetland system is another conventional method highly considered for treatment of tannery wastewater. Rangel et al. (2007) *applied Stenotaphrum secundatum, Canna indica, Typha latifolia, Iris pseudacorus and Phragmites australis for treatment of the tannery wastewater in construct wetland system where Typha latifolia,* and *Phragmites australis*, exhibited satisfactory performance. (Younas et al. 2022; Shah 2021a, b) reviewed the importance of plant species and microorganism in constructed wetland system for the treatment of chromium rich tannery treatment wastewater and wet land plants and growth media are the important factors for high removal efficiency of chromium. However, the different wetland methods are used in the lab scale level only and further development is needed. (Aregu et al. 2021) reported *Chrysopogon zizanioides* and pumice as a species in wetland system that can significantly remove more than 98% of chromium concentration at HRT 5 h. Adsorption, filtration, microbial activities, and plant uptake are the prime mechanism of the process.

The presence of perilous organics, various tannins and several inorganic elements upshots high strength and complexity of the TWW which are many a times responsible for inhibition of microbial activities (Bharagava et al. 2017; Sodhi et al. 2021). Therefore, advanced and combine/ hybrid treatment options are recommended for remediation of complex wastewaters before releasing to receiving environment.

Physicochemical Methods

The conventional physicochemical methods used for wastewater treatment are precipitation, coagulation-flocculation, Ion exchange, and Floatation etc. to name a few. Precipitation method require addition of precipitating agents to form precipitates with the pollutants is generally used to reduce COD, chromium, sulfate from the tannery industries (Fettig et al. 2017). Reyes-Serrano et al. (2020) observed more than 99% chromium removal along with COD while using calcium hydroxide as precipitant for chromium removal from TWW. Precipitation is also employed for the removal of suspended solids present in the tannery effluent. The major advantages of the system are its efficiency to reduce the settling period of particles present in the wastewater (Ghumra et al. 2021). Aluminum sulphate, ferric chloride, ferrous sulphate are the few important coagulants can be used as precipitant in the TWW treatment process (Lofrano et al. 2013). Chowdhury et al. (2013) observed 150 mg/l ferric chloride coagulant dosage can provide removal of COD, and Cr removal as 92 and 96% respectively. Time, pH, and coagulant dosage were influencing the treatment process. Elsheikh (2009) reported the 98.8% chromium and 31% removal of COD using calcium hydroxide for precipitation of chromium in the coagulation method. Ion-exchange process, resins are used to remove pollutants from solutions which are insoluble substances and exchange their own radicals from the solutions (Fu and Wang 2011). Low consumption of chemicals, less sludge production, and low cost are the few advantages of this process. A macroporous carboxylic resin exhibited 90% removal of chromium from other metals and organics (Tiravanti et al. 1991).

Flotation is based on imparting the ionic metal species in wastewaters hydrophobic by use of surfactants (Fu and Wang 2011). Flotation is an easy and faster method comparatively than coagulation and sedimentation used for heavy metal removal. Bubble attachment, dissolved air floatation, ion floatation and precipitation floatation are the few important floatation methods used in the mineral processing. (Fu and Wang 2011). Medina et al. (2005) reported, precipitate floatation method for the removal of Cr (III) and achieved 96.2% at pH 8.0. The advantages and limitations drawbacks of different conventional system are listed in Table 2.

Table 2 Limitations and advantages of conventional treatment methods

Methods	Advantages	Disadvantages
Activated sludge method	Low capital cost Easy to operate	Sludge formation
Phytoremediation	Low cost eco friendly	Slow process, not suitable for higher concentration
Constructed wetland	Eco friendly Cost-effective	Controlled environmental condition is required
Precipitation	Ease to operate Suitable for high pollutant load Integrated physiochemical process	High sludge formation High chemical consumption Handling and disposal issue
Coagulation	Simplicity in operation Low capital cost	Huge sludge generation High chemical consumption
Floatation	Low retention time Suitable for low-density particles High over flow rate High metal selectivity	High capital cost High maintenance and operating cost
Ion exchange	High removal efficiency High regeneration Rapid and efficient process	Highly sensitive to Ph Limited commercial use

1.3 Advanced Treatment Process

The conventional treatment methods frequently turn inefficient in the treatment process due to the complex nature of effluent and are not achieve a high removal rate. High chemicals consumption, expensive, and sludge formation are the few limitations of the methods. Hence, the improvement of efficiency, advanced treatment methods such as oxidation and membrane system are focused and followed for the removal of pollutants from the aqueous solution.

Oxidation Process

In the oxidation process, the organic compounds are oxidised by the hydroxy ions that are powerful for degradation. A few different types of oxidation processes such as Fenton, ozone, and photocatalytic are employed for the waste treatment process (Lofrano et al. 2013). The amount of free radical generation and reaction time is the most important factor for the degradation process and this method is often applicable in pre-treatment of effluent (Korpe and Rao 2021). Sivagami et al. (2018) reported, ozonation method to be more suitable than the Fenton oxidation for COD and TOC removal; COD removal is influenced by pH, hydrogen peroxide and ferrous ions concentration.

Electrocoagulation and Adsorption

Electrocoagulation process is advanced coagulation mechanisms that facilitates reduction of the settling period comparatively than the conventional coagulation process. Metal electrodes, such as aluminum and iron are in general employed for flocculation and precipitation in electrocoagulation. (Ghumra et al. 2021; Züleyha et al. 2021; Züleyha et al. 2021) reported the removal of COD at different Ph 5 and 3 as 83.3 and 83.5% respectively using the electrocoagulation method from initial concentration of 6000–9000 mg/l.

Adsorption is a promising, cost-effective, eco-friendly, and easily applicable techniques. The adsorbents used for chromium removals are activated carbon, graphite, serpentine and zeolite etc. Farahat and Sanad (2021) reported adsorption capacity of 138 mg/g for chromium removal at low pH, 25 °C, and 60 min of contact time.

2 Membrane System

The membrane process is gradually receiving importance as promising tool for treatment of industrial wastewater/ pollutants in an efficient way. The process necessitates moderate cost, low energy, and there is no need for any additives compared to the

Table 3 Classification of Pressure driven membrane process

Types of membranes	Pressure (Bar)	Pore size	Mechanism
Microfiltration	2	0.05–10 μm	Sieving
Ultrafiltration	1–10	2–100 nm	Sieving
Nanofiltration	10–25	2 nm	Solution diffusion
Reverse Osmosis	10–100	<2 nm	Solution diffusion

other treatment technologies. Several types of membranes made of ceramic, polymeric, and composite elements are in usage for the treatment process in wastewater industries.

2.1 Principle of Membrane System

Membrane separation is a physical phenomenon that separates a solute from the aqueous mixture on the basis of size of molecules. Pressure-driven membrane process removes different sizes of molecules and is classified in Table 3 according to the pore size of the membrane into microfiltration (MF), ultrafiltration (UF), reverse osmosis (RO), and nanofiltration (NF) membranes respectively (Hakami et al. 2020). Membrane pore size and distribution, surface charge, fluid flow, and an active layer are the most important influencing factors (Mestre et al. 2019). The separation mechanism of the membrane is generally governed by size exclusion, Donnan exclusion effect, and adsorption. In size exclusion, the pore size of the membrane is less than molecule size, which will retain the molecule, and for large pore size passes through the membrane (Mulder 1996). The adsorption mechanism is similar to the typical adsorption process via physical or chemical interactions. Donnan exclusion is mostly employed for dense membranes. The feed solution pH is above the iso electric point, membrane repulse cation and vice versa for lower pH (Abdullah et al. 2019; Mulder 1996).

2.2 MBR Configuration for TWW Treatment

The selection of membrane module is important for the commercial treatment of TWW and the different factors such as flow rate, cleaning ability, module replacement are considered during the module selection. There are four types of commercially available membrane modules, namely, plate and frame module, tubular module, spiral wound module, and hollow fiber modules (Ezugbe and Rathilal 2020).

Plate and frame module is one of the earliest modules consists of membrane and spacers interconnected with a metallic frame. The module is mostly suitable for wastewater containing high level of suspended solids and it is easy to clean (Ezugbe and Rathilal 2020). Yang et al. (2021) studied the efficiency of the PVDF and PES ultrafiltration membrane with permeation efficiency and fouling behaviour. Spiral Wound Module consists of more membranes and spacers around the perforated collection tube and widely applied in RO and NF operations. The configuration offers a high packing density leading to high membrane surface area. It is suitable for large-scale operations due to its easy replacement (Ezugbe and Rathilal 2020). Cassano et al. (2001) used commercial polysulphone membrane with 20KDa of Molecular weight cut-off and achieved 55% removal of fat. Stoller et al. (2013) used commercial NF spiral wound module and achieved COD as 102 mg/l and removed total suspended solids. Similarly, the removal rate of chromium and COD for RO membrane was 86 mg/l and 0.04 mg/l respectively. The threshold flux of the NF membrane was also reported. Tubular Module consists of an outer shell and an inner tube. Tubular membranes are adapted to treating feed with high solid contents (Ezugbe and Rathilal 2020). Mohammed and Sahu (2015) reported, that chromium removal using three different commercial membranes and RO membrane achieved 99.9% removal efficiency. Amine functionalized tubular composite membrane employed for the heavy metal removal. The removal percentage of chromium and arsenic was reported as 97.22% and 96.75%. Hollow Fiber Module houses a bundle of hollow fibers, whether closed or open-end, in a pressure vessel. A very notable advantage of this module type is its ability to house large membrane areas in a single module (Ezugbe and Rathilal, 2020). Mukherjee et al. (2019) conduct a scale up study of chromium removal on mixed matrix ultrafiltration membrane using modelling. Vo et al. (2021) reported the COD and TDS of equalization tank wastewater using PVDF hollow fiber membrane and achieved 87 $\pm$ 14% of COD where the fouling level was reduced by the sponge material.

2.3 *Factors Influencing Performance of MBRs*

Different types of membranes are utilised for separation processes in TWW treatment depending on steps of the TWW generated. Membrane type, and material, pore size, pre-treatment of feed water, and fouling control methods are responsible to influence the membrane performance (Rosman et al. 2018). The Beam house process effluent contains high suspended solids with a significant quantity of colloidal particles. In this situation, a microfiltration membrane is mostly suitable for separation. Similarly, the final stage operation is mostly organic and some metals are present in the feed. However, ultrafiltration and nanofiltration membrane is suitable for the operation to get high efficiency. Hakami et al. (2020)**.** Foulant significantly affects the membrane material because of the interaction between the membrane surface and the foulants**.** The cost of the membrane material is another important consideration for membrane selection. Du et al. (2020). The selection of membrane material depends on the sources of wastewater. Both organic and inorganic membranes are used for the treatment of beam house effluent and attain better efficiency. Pore size is another important parameter to be considered for membrane selection. The membrane pore is important for flux and removal efficiency. Membrane pore size is also one of the factors considered for better efficiency as well as membrane fouling. Microfiltration membrane typically used for elimination of turbidity, suspended solids, and pathogens. Nanofiltration membranes can affect the removal of organic matter, sulphates, turbidity, suspended solids and other pathogens. Common application is for removal of monovalent salts in reverse osmosis membrane (Ghumra et al., 2021). Pal et al. (2020b) investigated NF and graphene oxide membrane for COD, TDS, and chromium removal. Both membranes revealed varied flux and efficiency and indicated that membrane hydrophilicity and formation of nanochannel influences the performance. Stoller et al. (2013) suggested polyamide RO membrane provides better removal in terms of chromium than NF. Roy (2020) studied heavy metal rejection by different polymeric membranes with pore radii about 1 nm where the membrane offered better rejection for Cu (II), As(V), Cr (97.22) and sustained better stability for continuous operation. Arif et al. (2020) applied ultrafiltration membrane for removal and reduction of chromium. Karunanidhi et al. (2020) observed removal of BOD, COD, and dye removed from tannery effluent using keratin-polysulfone membrane. The keratin modified polysulfone membrane has less pore size than the polysulfone membrane with high flux. Kaplan-Bekaroglu and Gode (2016) reported 90% COD removal by ceramic ultrafiltration membrane with membrane pore size of 10 nm. Whereas, membranes with high pore sizes have high flux with low efficiency than other membranes. Bhattacharya et al. (2013) reported that a microfiltration membrane can reduce the fouling tendency of the membrane due to the presence of dissolved solids during pre-treatment providing suitable influent to reverse osmosis membrane in a combination of ceramic microfiltration and reverse osmosis membrane system. Roy Choudhury et al. (2018) found hydroxyethyl cellulose and CuO blend composite membrane showed better metal removal from the wastewater. The membrane showed better rejection in chromium due to strong adhesion between support and nanoparticle which enhance the pore size reduction. Elomari et al. (2016) reported clay membrane with different pore sizes showed good retention in turbidity and conductivity. Romero-Dondiz et al. (2015) reported the performance of different molecular weight cut-off polysulfone ultrafiltration membranes in vegetable tanning wastewater. Low molecular weight cutoff membrane provides high rejection than other compared membranes due to its size exclusion mechanism. Kiril Mert and Kestioglu (2014) reported removal of pollutant from tanning process by three different types of membrane with different molecular weight cut-off. Membrane showed better efficiency in sulfate and suspended solids removal when 200 MWCO membrane shows better performance than other membranes in the chromium recovery. Rambabu and Velu (2016) studied modified PES membrane for BOD, COD and TDS removal. $CaCl_2$ modification improved the performance than pure PES membrane. Similarly, chitosan modified PES membrane showed the significant removal in chromium separation from the wastewater. The membrane shows better rejection in salts.

2.4 Advantages and Challenges of Membrane Reactors

Membrane technology has several advantages over other separation processes which include no requirement of chemicals, low energy consumption, no phase change, and is suitable for continuous processes and easy adaptation with other systems (Mulder 1996). However, fouling is a major challenge due to the relatively higher amounts of organic matter and particulates in the feed wastewater. Membrane fouling is reduced flux, membrane life, increases energy like pressure, and also process cost (Ezugbe and Rathilal 2020). Suspended solids, microbes, and organic materials are a few components that are the reasons for fouling due to deposition on the membrane surface (Hakami et al. 2020). Fouling is characterized by colloidal, organic, inorganic, and biofouling. Membrane fouling depends on feed characteristics like pH and ionic strength, membrane characteristics like roughness, hydrophobicity, etc., and process conditions like cross-flow velocity, trans-membrane pressure, and temperature. The different techniques are used to avoid or reduce the fouling in the membrane. Turbulence promotors, ultra-sonication, backwashing are the techniques reduces the membrane fouling (Ezugbe and Rathilal 2020; Hakami et al. 2020).

3 Moving Bed Biofilm Reactor (MBBR)

Moving bed biofilm reactor (MBBR) is a biofilm reactor system, currently with wide attraction from researchers and environment scientists for treatment of complex and municipal wastewater due to its efficiency, cost effectiveness and easy operating facilities. Microbes are most suitable agents selected for bioremediation; however indigenous microbes due to its poor populations many times fail to attain the treatment level. In MBBR, microbes are provided with inert porous carrier media as substratum in suspension for microbial adhesion and growth (Odegaard et al. 1994). High microbial density, tolerance toward toxic/ hazardous chemicals, environmental stress and parameters are major attributes for high performance efficiency of MBBR.

3.1 Configuration of MBBR

Biofilms are intricate surface architecture made of well-structured communities of adherent microorganisms attached to inert/abiotic or living/biotic surface (Huang and Li 2014; Muhammad et al. 2020). The aggregated microorganisms may belong to same species or diverse species of bacteria, protozoa, archaea, algae, filamentous fungi, and yeast that strongly attach to each other and/or to surfaces and are held together by self-produced polymer matrix known as extracellular polysaccharide matrix (EPS). Production of EPS, interaction between microbial cells as well as surrounding media, and their intermolecular forces such as electrostatic/

van der Walls interaction, hydrophobicity etc. predominantly regulates adherence of microbes to the substratum (Carniello et al. 2018). Surface charge of microbes is another prime factor that affects biofilm production through adhesion of negatively charged microbes (due to presence of considerable amount of carboxyl, amino, and phosphate groups in cell wall) with positively charged substratum. EPS, the very significant component responsible for biofilm formation and performance is mainly composed of polysaccharides, secreted proteins, and extracellular DNAs (Costa et al. 2020). EPS influences protection of cells, transportation of nutrients and electron acceptors, solubilization of hydrophobic/recalcitrant elements and cell to cell signalling by Quorum Sensing Interference (QS). Bestowing the physicochemical and molecular properties of the microbes, biofilms get maturation followed by dispersal or detachment. The microbial position at substratum in two-dimensional Brownian motion facilitates easy separation of biofilms triggered by any shear effects or bacterial movement (Carniello et al. 2018) (Fig. 1).

The growth of microbes in the biocarriers in the MBBR creates several zones such as aerobic (in the biocarrier surface) and micro anoxic (interior biofilms) zones. These stratifications highly facilitate slow growth nitrifiers in the biofilm surface and anoxic denitrifies providing nitrification and partial denitrification of accumulated nitrite and nitrates in the influent (di Biase et al. 2019). The flexibility of MBBRs with its suspended carrier bed make it feasible to operate in batch mode, sequencing as well as sequential configuration for achieving high treatment efficiency (Di Iaconi et al. 2003; Odegaard et al. 1994; Sahariah and Chakraborty 2013). For a commercial laundry with daily flow of 0.6–1.0 m^3/d wastewater, a two staged MBBR treatment with Kaldane K5 carrier media provided more than 95% of organics and anionic/nonionic surfactants removals with attainment to its limit for discharged to receiving environment (Bering et al. 2018). Treatment of high organic strength wastewater,

Fig. 1 Lab scale MBBRs with Polyurethane cubes as biofilm carrier media

for example, Cheese industries, MBBR stands suitable treatment process for carbon, phosphorous and ammonia removals Tsitouras et al. (2021). Similarly, MBBRs are continuously employed for treatment of metals, dyes from various industries with higher rate of success (Leyva-Díaz et al. 2020; Li et al. 2015; Su et al. 2019). MBBR with modified low-density polyethylene–polypropylene carriers provided maximum elimination capacity of Congo red as high as 214.4 mg/L day (Sonwani et al. 2021). In a study for mixed azo dye (1200 mg/l) and chromium (300 mg/l) treatment from textile wastewater, MBBR with bacterial consortium of *Bacillus circulans, Bacillus circulans, Bacillus subtilis* and *Terribacillus gorriensis* accomplished more than 80% decolorization simultaneously with 56% degradation of chromium from the initial stages of treatment (Biju et al. 2022).

3.2 Influencing Factors in MBBRs

Being a bioreactor, MBBRs are also responsive to environmental parameters (pH, temperature etc.) and operational parameters (HRT, loadings etc.) to some extents. However, it is significantly resistance to minor changes to these parameters compared to other biological reactors. Major influencing parameter for MBBR performance involves its carrier media for biofilm growth and filling density of the carrier element in the reactor. Carrier media with higher surface space for biofilm growth with minimum weight and 30–40% fill of the reactor volume is recommended for better performance of MBBRs.

3.3 Advantages and Limitations of MBBRs

The advantages of biofilm reactor are more enhanced in case of MBBR due to of MBBR configuration with carrier material of high surface area for the growth of the microbes, that keep moving inside the reactor due to movement of external force or reactor fluid. Requirement of less land area, high sludge retention time, reduction of head loss in MBBR, no sludge recirculation, lower sensitivity to shock loading, no sludge bulking, and appropriateness for slow-growing microbes, are another a few positive attributes of MBBR recognised for its high utility for industrial wastewater treatment (Bachmann Pinto et al. 2018; Li et al. 2015, 2019a, b; Sahariah et al. 2018; Sodhi et al. 2021; Yadu et al. 2018).

Along with so much more advantages there are few limitations come across application of MBBRs such as necessity of manual monitoring as its functional attribute is microorganisms. Therefore, there is need of skilled technician and operators having rich knowledge in biological wastewater treatment to monitor reactor performance and avoid unwanted situations such as entry of insects that spoil carrier element in the reactor and direct escaping of the same.

4 Conclusion

High efficiency for pollutant removal is utmost association with MBRs and MBBRs. Pollutants of various category, organics, recalcitrant, heavy metals, inorganics are well manged in these reactors. Tannery wastewater is a complex wastewater with huge load of organics, large biomolecules responsible for high COD, dyes, heavy metals such as chromium, along with inorganics sulphides, nitrogen etc. The fact of these pollutant removals in MBR and MBBR are well established. Considering requirement and high consumption of water resources in tannery industry, these advanced but easy operation MBR and MBBR are recommended to achieve high effluent quality.

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Nanobioremediation: A Sustainable Approach for Wastewater Treatment

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Abstract Urbanization and industrial revolution has led to the pollution of the existing water bodies at an alarming rate. Heavy metals, pesticides, dye, oil spills and other hazardous chemicals are among the key refractory pollutants that cannot be removed effectively by the conventional wastewater treatment processes which are often expensive and have high energy requirements. Nanotechnology is employed in various fields more recently that include textiles, food, pharmaceutics, agriculture and even environment. Small dimension of the nanoparticles offers larger surface area for adsorption of the toxic pollutants that can be eventually removed. Such nanoparticles have exotic physicochemical and optoelectronic properties that determine their appropriate field of applications. Nanotechnology driven solutions for wastewater treatment is not only rapid and efficient but also economical. In view of the background, this chapter discusses in detail, the recent advances in the field of wastewater nanobioremediation through application of various surface active nanostructures. Photocatalytic degradation of pollutants in wastewater by nanostructured catalyst in presence of appropriate source of illumination has been reported in several studies. Some of the common nanocatalysts covered here include titanium dioxide (TiO_2), zinc oxide (ZnO), ferric oxide (Fe_2O_3), zinc sulfide (ZnS), magnetic nanoparticles (MNPs). Further, pressure-driven nanofiltration using different nanomaterials such as carbon, metal oxides are also elaborated. Eventually, nanosorbents mediated removal of several organic and inorganic pollutants from wastewater samples are also covered. Hence, further optimization and scale up of nanomaterial mediated wastewater treatment can help to implement the process for treating industrial effluents to ensure a safe environment.

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1 Introduction

Water is a vital part of the planet which is naturally recycled to maintain an adequate supply of clean water for human consumption (Bora and Dutta 2014). However, uncontrolled growth rate of the human population along with excessive industrialization has resulted in shortage of potable water in various parts of the world (Luikham et al. 2018). In addition, majority of water supply processes require a large amount of resources that are primarily involved in purification and treatment of wastewater to control the organic and inorganic content in such contaminated water samples (Chorawala and Mehta 2015).

Conventional wastewater treatment methods are cumbersome and typically consists of three stages namely, preliminary, primary and secondary (Ghosh 2020). Moreover, the treated samples are also subjected to ultraviolet (UV) disinfection before they could be discharged into the water bodies. In some instances, tertiary methods are also employed to remove trace amounts of contaminants which are extremely costly (Ghosh et al. 2021a, b). Hence, novel, cost-effective and improved wastewater treatment methods are required that are rapid and environment friendly as well (Qu et al. 2013). Nanotechnology has recently been demonstrated to provide such solutions wherein different nanomaterials such as titanium dioxide (TiO_2), zinc oxide (ZnO), polymer membranes, carbon nanotubes (CNTs), and magnetic nanoparticles (MNPs) were used for effective wastewater treatment (Ghosh and Webster 2021a, b; Ghosh et al. 2021c, d, e). Therefore, this chapter discusses in detail the use of nanomaterials for photocatalysis, nanofiltration as well as nanosorption of organic and inorganic pollutants from wastewater. Different kinds of nanomaterials are summarized in Table 1 that have effectively displayed removal of various pollutants such as dyes, heavy metals, organic and inorganic pollutants from wastewater samples. Further studies on improvements, optimization and risk evaluation of these nanomaterials for their application in the environment can provide an effective nano-based wastewater treatment method that can be useful on a large scale.

2 Photocatalysis

Metal and metal oxide nanoparticles with attractive physicochemical and optoelectronic properties are reported to show promising catalytic activity that can be exploited for degradation and removal of refractory pollutants (Ghosh et al. 2016a, b, c; Shende et al. 2017, 2018; Karmakar et al. 2020). In a recent study, Abd Elkodous et al. (2021) loaded carbon nanomaterials onto nanocomposite matrix that was utilized for photocatalytic treatment of wastewater. The matrix was made

Table 1 Nanomaterials used for removal of refractory pollutants for treatment of wastewater

Type of nanomaterial	Pollutant degraded	Maximum removal	References
Photocatalysis			
CNFST/C-dots 10%, CNFST/rGO 10%, and CNFST/SWCNTs 10% nanocomposites	Chloramine-T	65%	Abd Elkodous et al. (2021)
SeSnNPs	Methylene blue and malachite green	98% and > 95%	Saray et al. (2019)
TiO_2NPs	Cyanide	–	Ijadpanah-Saravy et al. (2014)
ZnS QDs	Naphthalene	95%	Rajabi et al. (2016)
ZnSe/PANI nanocomposite	Methylene blue, Cr(VI)	>50%	Shirmardi et al. (2018)
Nanofiltration			
Cellulose acetate NF membrane	Sulphate, sodium and calcium	–	Choi et al. (2002)
Commercial nanofilter	Potassium, chloride	–	Nataraj et al. (2006)
ES10, ES10C, LES90, LF10, NTR729HF, and NTR-7410	Various organic and inorganic pollutants	–	Thanuttamavong et al. (2002)
MPF34, NF90, and NF270	Arsenite, sulphate, and chloride	68%, 98%, and 84%	Andrade et al. (2017)
Polyacrylonitrile (PAN) membrane with polydopamine (PDA)/polyethyleneimine (PEI) layer and β-FeOOH nanorods	Methyl blue, Congo red, methyl orange and rhodamine B	100%, 100%, 69.9% and 77.3%	Lv et al. (2017)
Nanosorption			
GEPCD-MNPs	Congo red and Cr(VI)	–	Cai et al. (2017a)
GO/Chitosan–PVA nanopolymer composite	Congo red	88.17%	Das et al. (2020)
NiONPs	Rhodamine B dye	76%	Motahari et al. (2015)
Polyindole nanofiber	Cu(II)	121.95 mg	Cai et al. (2017b)
PVA coated PES-TiO_2 nanohybrid membrane	Organics, salts and ammonia–nitrogen	95.83%, 72.40% and 95.66%	Kusworo et al. (2021)

up of $Co_{0.5}Ni_{0.5}Fe_2O_4/SiO_2/TiO_2$ (CNFST) that was conjugated with C-dots, single walled-carbon nanotubes (SWCNTs), and graphene oxide (rGO) nanomaterials. X-ray diffraction (XRD) patterns of the nanocomposites then displayed anatase phase of the titanium dioxide (TiO_2) that was one of the primary components of the matrix along with presence of the three different carbon nanomaterials loaded onto the CNFST. Further, scanning electron microscopy (SEM) displayed uniform distribution of C-dots and rGO nanomaterials over the surface of CNFST while SWCNTs demonstrated sheet-like soots as well as uniform cross-linking with CNFST. Thereafter, transmission electron microscopy (TEM) results demonstrated spherical shaped particles with an average diameter of 90 $\pm$ 14 nm. Brunauer–Emmett–Teller (BET) surface area of the synthesized nanocomposites loaded with rGO and C-dots was smaller when compared to standard TiO_2 photocatalyst (P25) samples which was a result of increase in size of the nanoformulations as well as poor loading or increased agglomeration of the conjugated carbon nanomaterials. On the other hand, SWCNTs conjugation led to significant increase in the surface area. The pores in all the conjugated samples were multimodal and broad-shaped wherein the mesopores had a diameter ranging from 2 to 50 nm while the macropores had a diameter greater than 50 nm. Raman analysis further showed anatase TiO_2 as the dominant phase in the nanomaterial coated nanomatrix.

A comparative study of the three different carbon-loaded nanocomposites showed 28, 32.7, and 41.6% chloramine-T dye degradation by the CNFST/C-dots 10%, CNFST/rGO 10%, and CNFST/SWCNTs 10% nanocomposites, respectively after 40 min of ultraviolet (UV) irradiation. Moreover, subsequent increase in contact time to 90 min resulted in further increase in dye degradation ability with best removal activity observed using CNFST/SWCNTs 10% nanocomposites. In addition, the increase in the dosage concentration of the prepared nanoformulations resulted in subsequent increase in photocatalytic degradation of dye. Likewise, dye degradation ability of the CNFST/SWCNTs 10% nanocomposites was increased from 35 to 65% with concomitant decrease in the pH value of the system from 10.0 to 3.0, respectively. The zeta potential of the SWCNTs loaded nanocomposite was +15 mV which facilitated effective binding of the dye through electrostatic attraction for improved photocatalytic activity. The primary reactive oxygen species (ROS) mediated chloramine-T dye degradation was mainly attributed to the hydroxyl radical that was further scavenged on addition of isopropanol resulting in 40% decrease in dye degradation.

Organic pollutants were also reported to be degraded by tin selenide nanoparticles (SnSeNPs) that were prepared using co-precipitation method (Saray et al. 2019). XRD pattern analysis demonstrated orthorhombic phase of the particles. However, the crystallinity of the particles was dependent upon the Se/Sn ratio wherein a ratio of 1.0 resulted in formation of crystalline particles while particles made with a Se/Sn ratio of 1.3 did not exhibited a crystalline phase. Moreover, field emission scanning electron microscopy (FESEM) and TEM results demonstrated complete agglomeration of the particles when the Se/Sn ratio was 0.8 with presence of oxygen as impurity as confirmed by energy dispersive X-ray (EDX) spectroscopy results. Meanwhile, particles with Se/Sn ratio of 1.2 were pure and smaller in size. The photocatalytic activity of the prepared SnSeNPs was then evaluated in which methylene blue degradation was analysed in presence of visible light. NPs prepared using a Se/Sn ratio of 1.2 was able to effectively degrade 98% of methylene blue dye within 25 min of incubation. Furthermore, the reusability of the nanomaterials was also evaluated wherein similar dye reduction ability of the SnSeNPs was observed after four successive cycles. The phase of the NPs prepared using Se/Sn ratio of 1.2 was stable as observed in XRD patterns even after four cycles of dye degradation while particles with Se/Sn ratio of 0.8 completely changed its phase after four successive photocatalytic processes. Likewise, malachite green degradation ability was also monitored in presence of the SnSeNPs with varying ratios of Se and Sn, respectively. More than 95% of the dye was effectively degraded by NPs with Se/Sn ratio of 1.0 within 45 min of incubation. The textural properties of the NPs were also evaluated wherein a maximum specific surface area equivalent to 77.441 m^2/g was observed with Se/Sn ratio of 1.2 along with a pore diameter and volume of 2.43 nm and 0.124 cm^3/g, respectively. The energy of the valence bond of SnSeNPs with Se/Sn ratio of 1.2 was calculated to be 1.21 eV that was further used for investigating the electronic structure of the particles. Therefore on the basis of electronic structure, formation of hydroxyl radical using SeSnNPs was proposed to facilitate efficient dye degradation which could be applicable in wastewater treatment as well.

In another study, Ijadpanah-Saravy et al. (2014) reported fabrication of titanium dioxide nanoparticles (TiO_2NPs) by controlled hydrolysis of 3 M titanium tetrachloride. The nanomaterial demonstrated photocatalytic degradation of cyanide in wastewater samples. X-ray diffraction (XRD) patterns then displayed characteristic peaks of anatase and rutile form of TiO_2NPs with a crystalline size of 18 and 22 nm, respectively while scanning electron microscopic (SEM) images demonstrated spherical morphology of the particles with an average size of 20 nm. Thereafter, the photocatalytic properties of the NPs were determined wherein a 4:1 ratio of anatase to rutile form of the particles was optimal for maximum photocatalytic activity to degrade cyanide. Interestingly, pH 11 was optimal for cyanide degradation with maximum photocatalytic efficiency as well as lowest electrical energy consumption as compared to other catalysts.

Rajabi et al. (2016) also demonstrated effective photocatalytic removal of industrial pollutants using zinc sulphide quantum dots (ZnS QDs). Fast and efficient chemical precipitation technique was followed for preparation of ZnS QDs which exhibited a blue shift in UV–vis absorption peak at 235 nm that was attributed to the particle size decrease, band-gap energy increase as well as due to quantum size confinement effect. Additionally, the direct optical band gap value of the prepared nanomaterial was 4.09 eV. XRD patterns then demonstrated a cubic zinc blend crystalline structure of the ZnS QDs without presence of any impurities. TEM image analysis further revealed spherical shape of the QDs with an average dimension of around 1 nm. Thereafter, naphthalene was used for demonstrating pollutant degradation activity of the prepared ZnS QDs wherein only 25–31% degradation was obtained in absence of light. An optimal pH of 11 resulted in maximum degradation efficiency of naphthalene which was decreased with concomitant increase in initial concentration of the pollutant. Moreover, a rapid increase in degradation rate was observed for

90 min that was reduced for the next 45 min followed by equilibrium. A low initial concentration of 10 mg of ZnS QDs then demonstrated up to 95% naphthalene degradation efficiency that remained fairly constant up to four cycles highlighting its reusability. Further, a maximum degradation rate constant of 6.90×10^{-5} min^{-1} was attained when the initial concentration of naphthalene was 20 ppm. The mechanism of degradation was then proposed to involve photoexcitation that may have resulted in electron–hole pair formation on the surface of ZnS QDs semiconductor that may have then oxidized naphthalene to 2-formylcinnamaldehyde.

Shirmardi et al. (2018) also demonstrated improved photocatalytic activity of zinc selenide nanoparticles (ZnSeNPs) after addition of polyaniline (PANI) that acted as an organic semiconductor. The ZnSe/PANI nanocomposite was prepared using co-precipitation technique whose heterostructure form was confirmed by XRD patterns. In addition, TEM images showed a distinct ZnSeNPs core and PANI shell structure whereas high resolution transmission electron microscopy (HRTEM) images demonstrated interplanar distance of 0.33 nm between the zinc blend structure as evident from Fig. 1. Raman spectroscopy of the nanocomposite indicated a weaker ZnSe peak at 500 cm^{-1} as compared to pristine ZnSeNPs that was attributed to the presence of PANI shell in the composite. Raman spectrum of the nanocomposite also demonstrated two other peaks at 1105 and 420 cm^{-1} that corresponded with C-H vibration of the semi-quinonoid rings and out-of-plane ring-deformation of PANI, respectively. X-ray photon spectroscopy (XPS) based valence band (VB) spectral analysis then demonstrated a nanocomposite VB potential of 0.48 ± 0.05 eV while the pristine ZnSeNPs had a VB potential of approximately 1.13 eV. Further, methylene blue dye degradation ability of the prepared nanocomposite was analysed under visible light irradiation. More than 50% of the initial dye concentration was reduced by the ZnSe/PANI nanocomposite within 30 min whereas it took 120 min for the pristine ZnSeNPs for completing similar levels of reduction. Additionally, presence of PANI displayed an increase in photocurrent intensity along with a decrease in the photoconductivity resistance. In addition, ZnSe/PANI nanocomposite was also investigated for its inorganic pollutant removal activity wherein absorption peak of $K_2Cr_2O_7$ at 375 nm was decreased in presence of the nanocomposite with gradual increase in visible light irradiation time. Hence, Cr(VI) ions were reduced to Cr(III) efficiently by the nanocomposite as well.

3 Nanofiltration

Nanoparticles impregnated polymeric films are often used as superior membranes for nanofiltration of water in order to remove certain hazardous pollutants which are recalcitrant in nature (Ghosh et al. 2022a, b; Ghosh and Webster 2022). Nataraj et al. (2006) removed colour and contaminants from distillery effluent using a hybrid system of nanofiltration (NF) combined with reverse osmosis (RO). A commercial nanofilter as well as thin-film composite (TFC) polyamide RO membrane was used in this study wherein effective colour removal ability of the NF was attained when

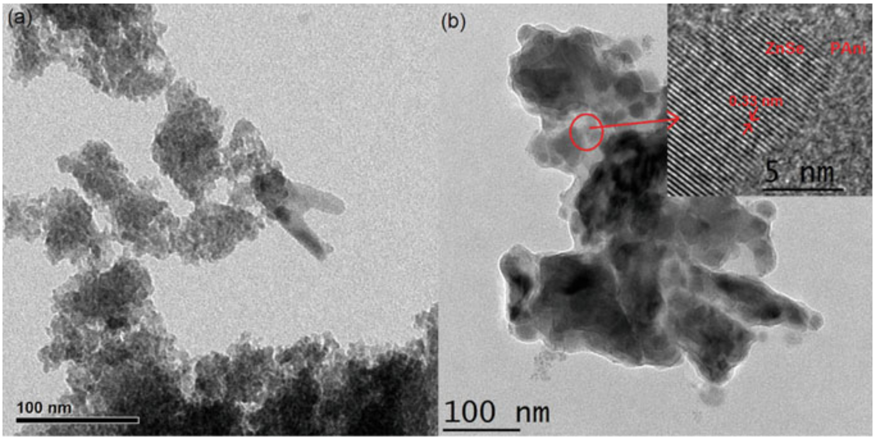

Fig. 1 TEM image of **a** pristine ZnSe NPs and **b** ZnSe/PANI nanocomposites. The inset shows an HRTEM image of ZnSe/PANI nanocomposites. Reprinted from Shirmardi A, Teridi MA, Azimi HR, Basirun WJ, Jamali-Sheini F, Yousefi R, 2018. Enhanced photocatalytic performance of ZnSe/PANI nanocomposites for degradation of organic and inorganic pollutants. Applied Surface Science 462:730–738. Copyright © 2018 Elsevier B.V

the size of the particles was in the colloidal range. Thus, in at an optimal pressure of 30–50 bar, the total dissolved solids (TDS) was reduced from 51,500 to 9050 ppm while conductivity and chloride concentration was reduced from 346 mS/cm and 4900 ppm to 15.06 mS/cm and 2650 ppm, respectively. High rejection efficiency against bivalent and trivalent ions was also exhibited by the NF membrane. Thereafter, evaluation of RO based pollutant removal was performed. The impact of feed pressure on the permeate properties was analysed in which the pressure increased linearly from 20 to 70 bar when distillery spent wash was used as feed. TDS and chemical oxygen demand (COD) rejection was altered from 83 to 99.06% and 95.6 to 98.96%, respectively when 50 bar pressure was applied. Additionally, high rejection percentages for potassium and chloride ions were observed.

Choi et al. (2002) used a cellulose acetate NF membrane for treatment of wastewater. The effective surface area of the hollow NF membrane used in this study was 11.7 m^2 along with 55% salt rejection property. A membrane bioreactor (MBR) system was set up to evaluate the efficacy of treatment which was operated for 71 days. The transmembrane pressure along with the relative productivity permeate after 20 days of incubation were 36 kPa and 1.0–1.2, respectively after which a drastic increase in relative productivity was observed. The change in sulphate, sodium and calcium ions concentration were similar to that of conductivity wherein no electrolytes were accumulated in the bioreactor due to charge effect of the membrane. It was hence proposed that the bioreactor could be operated under low suction pressure as the effect of osmotic pressure was insignificant along with high rejection of organic matter. The biodegradable membrane remained stable for 60 days in the bioreactor wherein nitrification and denitrification occurred simultaneously. In addition, atomic force microscope (AFM) images revealed a larger surface roughness on the cellulose acetate membrane after 40 days of bioreactor operation which was further increased till 71 days thus highlighting biodegradation of the membrane.

Thanuttamavong et al. (2002) also characterized NF rejection efficiency of organic and inorganic pollutants for treatment of wastewater. Six different commercial NF membranes namely ES10, ES10C, LES90, LF10, NTR729HF, and NTR-7410 were used in this study that were composed of aromatic polyamides, polyvinyl alcohols, and sulfonated polysulfones. The total dissolved organic and inorganic content of the polluted water sample were 1.8 mg/L and 3 mM, respectively. The pH and the turbidity of the water samples were in the range of 7.2–7.5 and 2.5–5.5 NTU, respectively. Long-term operations for a time period of 120 and 20 days were carried out with and without microfiltration pre-treatment, respectively. A transmembrane pressure of 0.15 MPa was maintained in both cases along with a standardized permeate flux temperature of 25 °C. A significant decline in permeate flux was observed for all the membranes during the first 10 days of operation. The permeate flux of the untreated loose membrane NTR7410 continuously declined and reached a steady state value of 0.28 m/d in 15 days of operation whereas the treated membranes showed a faster steady state attainment. Further, a stable rejection of all the components was observed throughout the NF operation which highlighted that membrane fouling does not interfere with the rejection mechanisms. Additionally, significant change in zeta potential values of the membrane surface was observed after long-term operation. The MWCO value for ES-10, ES-10C, LES-90 and LF-10 was 100 Da while it was 200 Da and more than 350 Da for NTR-729HF and NTR-7410, respectively. Moreover, organic matter with a size range of 300–1800 Da was found in the permeate of NTR7410 NF membrane. With regards to inorganic matter rejection, divalent ions such as Ca^{2+} and Mg^{2+} exhibited higher rejection as compared to monovalent ions such as Na^+ and Cl^- which was attributed to the charge effect of the membrane. Thereafter, the effective charge density of the NTR-729HF membrane was changed from −2 to −0.5 mol/L after long-term operation which suggested a decrease in the electrostatic property of the membrane after its utilization. The partitioning coefficient of the membrane for nitrate ion was also decreased from 5.0 to 2.6 after operation.

Likewise, Andrade et al. (2017) demonstrated gold mining effluent treatment using NF and compared with RO. Five different membranes were used in this study namely, TFC-HR and BW30 that were RO membranes while MPF34, NF90, and NF270 were the three NF membranes. The permeate flux of RO membranes were 7–12 folds lower than NF membranes that was attributed to higher resistance in the RO membrane. Moreover, a more intense membrane fouling was also observed in case of TFC-HR whereas MPF34 exhibited minimum fouling. The initial pollutant retention efficiency of all the membranes was considerably high. However, a high sulphate concentration in the mining effluent was proposed to interfere with the reuse of water as it could cause metal precipitation which could further result in membrane fouling. Among the RO membranes, TFC-HR demonstrated maximum pollutant retention efficiency with 75%, 99%, and 78% retention of arsenic, sulphate, and chloride ions, respectively. Similarly, NF90 membrane exhibited excellent arsenic, sulphate, and chloride retention of 68%, 98%, and 84%, respectively. Moreover, the effluent was pre-treated to increase its pH which resulted in decrease in conductivity as well as concentrations of calcium and magnesium. Concentration of arsenic was also reduced with subsequent increase in pH of the effluent from 2.2 to 5.0 that subsequently enhanced recovery rate from 27 to 70% along with higher resistance to membrane fouling. The permeate recovery rate (RR) was also studied in which the quality of the permeate remained almost similar when the RR value was up to 40% above which the removal efficiency started to gradually decrease. The concentration of polarization of NF membranes was then increased to 1.45 using a synthetic solution of $MgSO_4$ which resulted in increase of RR to 70% while decreasing the removal efficiency. Hence, it was concluded that such NF based effluent treatment could be optimal as well as feasible for obtaining industrial water samples from the same.

Lv et al. (2017) reported fabrication of a photocatalytic nanofiltration (NF) membrane that had self-cleaning property and thus, could be used for wastewater treatment as represented schematically in Fig. 2. Co-deposition method was carried out on an ultrafiltration polyacrylonitrile (PAN) membrane with polydopamine (PDA)/polyethyleneimine (PEI) to form an intermediate layer after which β-FeOOH nanorods were mineralized to further create a photocatalytic layer on the NF system. X-ray photoelectron spectrometer (XPS) results of the mineralized membrane then displayed additional binding energy peaks at Fe 2p along with a change in oxygen/carbon ratio from 0.32 to 1.22 which confirmed presence of FeOOH group on the membrane surface. SEM images also demonstrated uniform distribution of β-FeOOH nanorods over the surface of the membrane with a vertical orientation and a thickness of around 0.45 μm. The enhanced wettability of the membrane was analysed through calculation of the dynamic water contact angle that was decreased from 60° to 20° within 100 s. Such improved permeability of the mineralized membrane was attributed to the hydrophilicity of the β-FeOOH nanorods that were attached on the surface of the membrane. The surface charge properties of the membrane were then analysed wherein the isoelectric point of the membrane surface decreased from 6.3 to 5.6 after mineralization. In addition, the molecular weight cut-off (MWCO) value showed a steady decrease from 20,000 to 2000 Da with subsequent increase in deposition time from 1 to 6 h, respectively. Thereafter, the dye rejection performance of the stable mineralized NF membrane was studied in which 100% rejection of methyl blue and Congo red was attained at pH 3 and 7, respectively with a deposition time of 2 h. Likewise, 60.8 and 69.9% of methyl orange rejections were observed at pH 7 and 3, respectively. On the other hand, rhodamine B dye rejection was increased up to 77.3% when a positive surface charge was attained on the membrane. Thereafter, a continuous cross-flow membrane reactor was set up for observing the photocatalytic performance of the prepared membranes. The colour of the feed solution containing 20 mg/L of methyl blue and 30% H_2O_2 became colourless after 6 h of incubation. The self-cleaning property of the membrane was also evaluated by immersing the fouling membrane into an acidic H_2O_2 solution and in presence of visible light wherein the water flux was recovered to its original value. A high NF performance of 97.3% was achieved even after five successful photocatalytic cycles indicating its reusability and recyclability.

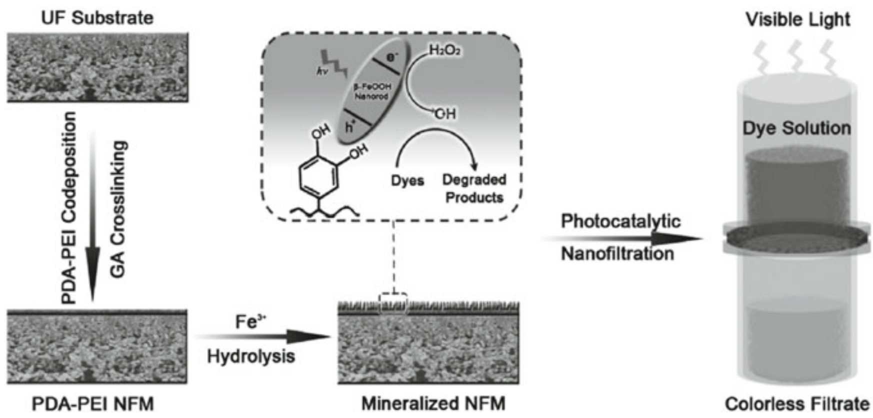

Fig. 2 Schematic representation of preparation process for the β–FeOOH mineralized NFM and its application in photocatalytic nanofiltration. Reprinted with permission from Lv Y, Zhang C, He A, Yang SJ, Wu GP, Darling SB, Xu ZK, 2017. Photocatalytic nanofiltration membranes with self-cleaning property for wastewater treatment. Advanced Functional Materials 27:1,700,251. Copyright © 2017 WILEY–VCH Verlag GmbH & Co. KGaA, Weinheim

4 Nanosorption

Nanocomposite based adsorbents are advantageous compared to conventional sorbents due to their superior ability for purification and convenience in manipulation of their activity by rational physico-chemical modification. Cai et al. (2017a) also reported formulation of a novel multi-layer magnetic adsorbent (GEPCD-MNPs) using ring opening polymerization process wherein a multi-layer cationic polymer was coated on the surface of magnetic nanoparticles (MNPs). These nano-adsorbents were then utilized for dyeing wastewater treatment. SEM images of the nano-adsorbents showed spherical morphology of the MNPs that had a smaller diameter as compared to pristine MNPs which suggested presence of the polymer on the active sites of MNPs. The contact angle of the nano-adsorbent was less than 90° when immersed in Congo red and Cr(VI) containing solutions thus indicating its hydrophilicity. The magnetic properties of the nano-adsorbent were also evaluated which provided a saturation magnetization value of 54.7 emu/g. The removal efficiency of Congo red increased with subsequent increase in the pH value of the system from 7.25 to 9.25 that was attributed to protonation of the MNPs and the functional groups of the polymer which may result in improved coordinate affinity with the dye. On the contrary, removal rate of Cr(VI) from the solution decreased with concomitant increase in pH value from 2.98 to 11.94 which was proposed to transform $Cr_2O_7^{2-}$ into CrO_2^- that has lower electrostatic interaction with the nano-adsorbent. The adsorption kinetics analyses for Congo red and Cr(VI) adsorption followed a pseudo-second order reaction while both the Langmuir and Freundlich adsorption isotherm models were properly fitted for the adsorption processes. Therefore, GEPCD-MNPs were proposed to be economically viable and effective nano-adsorbents that could be potentially be used in large-scale wastewater treatment processes with further optimization.

A hybrid hydrogel nanopolymer was prepared by Das et al. (2020) using graphene oxide (GO), chitosan and polyvinyl alcohol (PVA) that was investigated for its wastewater treatment efficacy. SEM images of the prepared GO reinforced chitosan-PVA hydrogel nanopolymer displayed homogenous distribution of GO over the matrix without any agglomeration. Fourier transform infrared (FTIR) spectroscopy analysis then displayed peaks at 3268.06 and 1377.33 cm^{-1} which corresponded with $–NH_2$ stretching vibrations of chitosan and $–CH_2$ deformation vibrations of PVA due to cross-linking, respectively. FTIR spectra of the prepared nanopolymer then displayed characteristic peaks corresponding with surface functional groups of chitosan, PVA and GO. A maximum positive surface charge on the nanopolymer membrane was observed at a pH value of 3.3 that was considered ideal for anionic dye adsorption. The swelling behaviour of the nanopolymer membrane was also evaluated wherein maximum swelling of 17.24 g/g was achieved after 90 min of immersion in water that further increased to 25.89 g/g at a pH value of 2.0. This change was attributed to ionization of the functional groups present on the membrane. Thereafter, dye adsorption studies were conducted using a biological oxygen demand (BOD) shaker at 140 rotations per minute (rpm) under room temperature. A maximum Congo red dye removal efficiency of 84.31% was obtained at an initial dye concentration of 10 mg/L. Additionally, the adsorption efficiency increased from 76.62 to 81.1% with subsequent increase in dosage of adsorbent from 1 to 6 g/L, respectively. An acidic pH of 2.0 was further demonstrated to provide maximum adsorption efficiency of 88.17% because of effective electrostatic interaction between the cationic nanopolymer membrane and anionic dye molecules. Adsorption isotherm studies predicted that Langmuir model was best fitted for explaining the dye adsorption while adsorption kinetics revealed pseudo second order reaction of the adsorption process.

In another study, Motahari et al. (2015) cost-effectively synthesized nickel oxide nanoparticles (NiONPs) for rhodamine B dye removal from wastewater samples. H_2acacen ligand was used for hydrothermal formation of nano-scale $Ni(OH)_2$ followed by calcination to obtain NiONPs. XRD patterns of the prepared NPs corresponded with the face-centered cubic as well as crystalline structure of nickel oxide. FESEM images then displayed monodispersed NiONPs while TEM micrographs showed spherical morphology of the particles with an average size of 10 nm. The BET surface area of the NiONPs was 176.56 m^2/g that were porous in nature with an average pore size of around 9.7 nm. Later on, dye adsorption studies were carried out in which 76% of rhodamine B dye at a concentration of 10 mg/L was efficiently removed within the initial 30 min of reaction. The reusability of the particles was also highlighted wherein no change in dye adsorption activity was observed after reusing the same particles. Adsorption isotherm studies then provided maximum dye adsorption capacity of 111 mg/g using Langmuir isotherm model whereas kinetic studies showed suitability of pseudo second order reaction for explaining the NiONPs mediated rhodamine B dye adsorption reaction. Optimal pH 7.0 then demonstrated efficient dye adsorption that was exothermic in nature as the dye adsorption capacity decreased with subsequent increase in the temperature of the system.

Cai et al. (2017b) also fabricated polyindole nanofibers using electrospinning that could act as nano-adsorbent for removal of heavy metal ions from wastewater. SEM and TEM images displayed spherical morphology of the nanofibers that had a smooth surface along with an average diameter range of 140–300 nm. The ranges of pore size, diameter and specific surface area were 0.217–0.250 $cm^3\ g^{-1}$, 55–68 nm and 64.43–86.41 $m^2\ g^{-1}$, respectively. Moreover, the specific surface area and pore volume decreased with concomitant decrease in the fibre diameter while the opposite was observed for the pore diameter. Thereafter, effect of pH on the metal adsorption capacity of the nanofibers was investigated wherein maximum Cu(II) adsorption was observed at an acidic pH value of 6.0 that was used for further experiments in this study. The maximum adsorption capacity of the nanofibers having a polyindole concentration of 1.6% was 121.95 mg/g within 15 min of incubation that was the most effective contact time as well. In addition, the Cu(II) adsorption capacity of the nanofiber was insignificantly affected in presence of low concentrations of other metal ions such as Na^+, K^+, Mg^{2+} and Ca^{2+} that highlighted the specific Cu(II) adsorption capacity of the nanofibers. Moreover, the adsorption isotherm studies then demonstrated Langmuir model to be a best fit for the Cu(II) adsorption using polyindole nanofibers. Additionally, the pseudo second order reaction was followed by the adsorption process. Moreover, the reusability of the nanofiber was also investigated wherein the adsorption efficiencies were 95%, 88.7% and 82% of the original adsorption capacity of the nanofibers after the third, fifth and seventh cycle, respectively. In addition, Cu(II) adsorption by the nanofibers was proposed to be mediated by chemisorption which consists of coordination between the metal ion and nitrogen containing functional groups of the membrane may result in chelation.

In another similar study, a nanohybrid membrane that was cross-linked with PVA and coated with polyether sulfone (PES)-TiO_2NPs was reported as an effective adsorbent in wastewater treatment (Kusworo et al. 2021). SEM images of the prepared membranes showed a smooth surface with minimal nodules while the sub-layer structures of the membrane were asymmetric with a dense skin layer along with a porous sub-layer made up of finger-like structures as evident from Fig. 3. XRD patterns of PVA coated PES-TiO_2 nanohybrid membrane further showed a wide band at 19–20° that corresponded with orthorhombic lattice along with the polycrystalline behaviour of PVA along with weak bands that highlighted the amorphous PES and TiO_2 crystalline peaks. In addition, the water contact angle of the 3.0 wt.% PVA complexed nanohybrid membrane was 27.67° that highlighted its hydrophilicity. Mechanical properties of the 3.0 wt.% PVA linked membrane was also studied that demonstrated a thickness of 90 μm with a tensile strength and elongation break of 6.4 ± 0.15 MPa and 1.6 ± 0.10%, respectively. Thereafter, addition of TiO_2NPs on the membrane was attributed to the increase and stabilization of the permeate flux at 80 $L/m^2/h$ as it could degrade the attached foulants. Hence, gradual increase in TiO_2 concentration resulted in improved COD, total dissolved solids (TDS), surface wettability as well as rejection percentages. Likewise, addition of PVA resulted in considerable increase in COD, TDS, and NH_3-N rejection by 244.80%, 56.70%, and 5.29%, respectively. Furthermore, organics, salts and ammonia–nitrogen rejection were also enhanced up to 75.00%, 51.61%, and 90.47% that was further improved by 95.83%, 72.40% and 95.66%, respectively when integrated with ozonation.

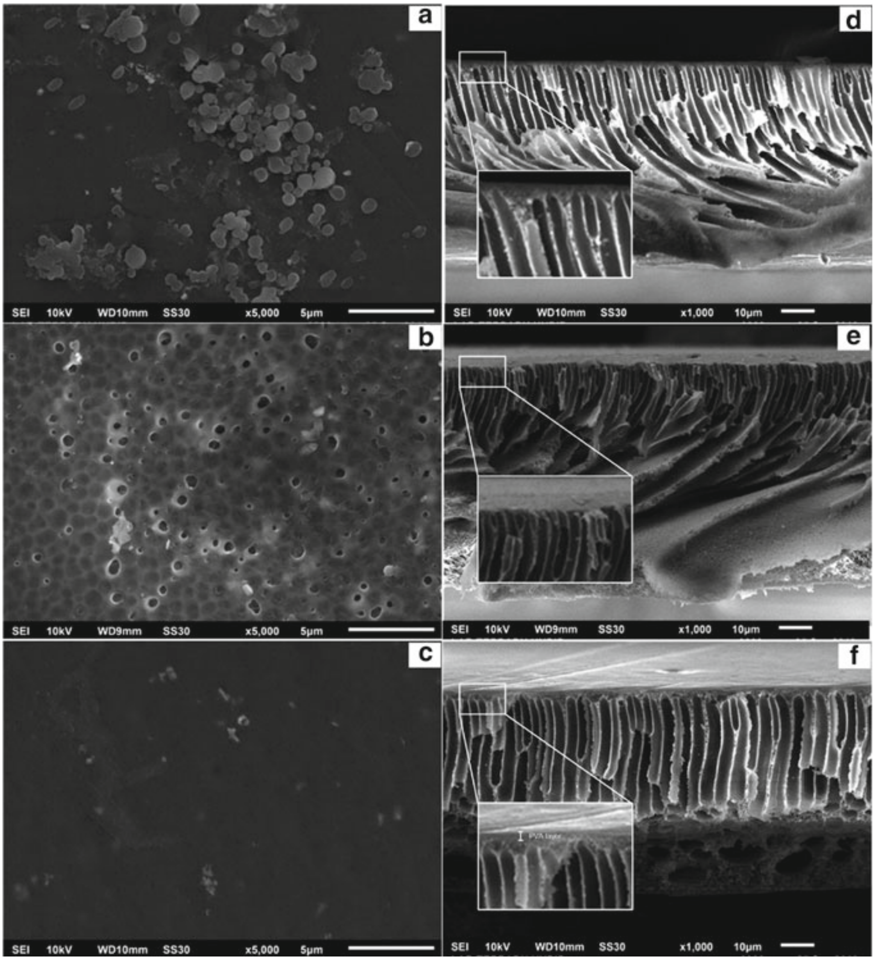

Fig. 3 Membrane surface morphology of **a** PES-TiO_2 1 wt-%, **b** PES-TiO_2/PVA 0.5 wt-%, **c** PES-TiO_2/PVA 3.0 wt-% and cross-section images of **d** PES-TiO_2 1 wt-%, **e** PES-TiO_2/PVA 0.5 wt-%, **f** PES-TiO_2/PVA 3.0 wt-%. Reprinted with permission from Kusworo TD, Kumoro AC, Utomo DP, Kusumah FM, Pratiwi MD, 2021. Performance of the crosslinked PVA coated PES-TiO_2 nano hybrid membrane for the treatment of pretreated natural rubber wastewater involving sequential adsorption–ozonation processes. Journal of Environmental Chemical Engineering 9:104,855. Copyright © 2020 Published by Elsevier Ltd

5 Conclusion and Future Perspectives

Surface active nanoparticles exhibit notable physical and chemical properties that are exploited in various biomedical applications (Ghosh et al. 2018). The attractive features of the nanoparticles have extended their applications for infection control, agriculture, textiles, and even environment (Adersh et al. 2015; Rokade et al. 2017; Jamdade et al. 2019; Bhagwat et al. 2018; Ghosh et al. 2016d). The nanostructures used so far for the treatment of wastewater for photocatalysis, nanofiltration or nanosorption are either fabricated by physical or chemical methods which often use hazardous chemicals and reaction conditions (Bloch et al. 2021; Ranpariya et al. 2021). However, biological route for synthesis of metal and metal oxide nanoparticles are environmentally benign, rapid and efficient (Shinde et al. 2018). Microbe synthesized nanoparticles are reported to have efficient photocatalytic activity that can be further integrated in the membranes for effective dye degradation and removal (Ghosh 2018). Similarly, various medicinal plants like *Gloriosa superba, Dioscorea bulbifera, Gnidia glauca, Plumbago zeylanica,* and others are reported to synthesize exotic gold, silver, copper, platinum, and palladium nanoparticles that can be further explored for wastewater treatment (Rokade et al. 2018; Ghosh et al. 2015a, b, c; Jamdade et al. 2019; Salunke et al. 2014). Composite nanoparticles either bimetallic or functionalized with metal removing or dye degrading enzymes, bioactive principles can be employed for multimodal wastewater treatment (Robkhob et al. 2020; Ghosh et al. 2015d; Kitture et al. 2015). In view of the background it can be concluded that development of nanotechnology assisted strategies for wastewater treatment can serve as a powerful tool to ensure clean environment.

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Bioremediation of Textile Dyes for Sustainable Environment—A Review

Rajalakshmi Sridharan and Veena Gayathri Krishnaswamy

Abstract Development of science has taken over our lives and made it mandatory to live with science. The synthetic technology takes more than it has given for our welfare. The process of meeting the demand of the consumers, industries supported the synthetic products to meet the same. Textile industry is one among them which uses synthetic dyes to dye fabrics. The end result of the process is the production of fabrics resistant to physical, chemical and biological stresses. The process not only meets the consumers demands it also consumes customers health by polluting the environment. The pre-treated or non-treated textile dyes released into the environment requires effective treatment process. Bioremediation, an eco-friendly method is a broad spectrum which includes—microbial remediation, enzymatic remediation, phyto-remediation, phyco-remediation and bioreactors -. The current review focuses on the microbial, enzymatic and bioreactor remediation of textile dye contaminated effluents. This is the need of the hour to restore the healthy and sustainable environment.

Keywords Textile dyes · Bioremediation · Enzymatic process · Bioreactors

1 Introduction

Industrialization, a significant factor in the growth of textile industries met the demands of the consumer. The handmade cloths have become mechanized and sold for lesser rate to reach wider consumers. The contribution of textile industries to the country's economy was nearly 1 trillion dollars which involved approximately 35 million workers. Textile industries started consuming fuel, electricity, water, and chemicals for production. Nearly three trillion gallons of fresh water were consumed to produce 60 billion Kg of fabrics (Desore and Narula 2018). Handling of pollutants released from the industries requires special attention. The mutagenic and the carcinogenic effects of the textile dye pollutants/effluents were reported as a case

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Table 1 Types of dyes and its applications

Dyes	Applications	References
Direct dyes	Cotton, paper, leather, nylon and blends	Cavaco-Paulo (1998)
Vat dyes	Cotton, linen and rayon	Liang et al. (2008)
Reactive dyes	Cellulosic, fabrics and fibre	Tanthapanichakoon et al. (2005)
Acid dyes	Silk, wool, leather, nylon, paper, food and cosmetics	Daneshvar et al. (2004)

study. The direct discharge of industrial effluents into the Amani Shah Ka Nallah drainage in Rajasthan polluted the surface and ground water making it unfit for agriculture and other purposes (Mathur et al. 2005).

Dyes are colouring agents that is majorly used in textile, pharmaceutical, paper, printing industries etc. In dyes the lights are absorbed in visible spectrum and they contain minimum a single chromophore group and has a structure of single and double bonds in alternate manner. The stabilizing force for the organic structure of the dye is given by electron resonance (Gowri et al. 2014). There are more than 10,000 different dyes were used in textile industries (Gupta et al. 2015). Table 1 gives the types of dyes and its applications.

Synthetic dyes were discovered by Willian Henry Perkin in 1856. These synthetic dyes are resistant to sweat, sunlight, chemicals etc. and are applied on fabrics for permanent colour. Textile industries uses the major quantity of synthetic dyes and which are 70% composed of azo dyes (Saratale et al. 2011; Shah 2020). Reactive azo dyes are dyes that have heterocyclic rings containing halogens substituent and forms covalent link between the functional groups in the dye and the cellulose (Xie et al. 2008).These reactive dyes undergo nucleophilic substitution as it is electronegative. This attack causes the fixation on fabric after reactive dye undergoes hydrolysis and are resistant to chemical, physical and environmental agents (Al-degs et al. 2000).

The general structural formula for reactive dyes

S…F…T…X

S- Solubilizing group, F- chromophore, T- binding group, X- reactive system (Gowri et al. 2014).The major group of dyes are composed of azo dyes containing (**–N = N-**) azo group which is a chromophore (Lavanya et al. 2014) (Fig. 1).

Different types are azo dyes are formed when there is substitution of azo group by benzene or naphthalene containing –Cl, $–CH_3$, $–NO_2$, $–NH_2$ and –OH and –COOH

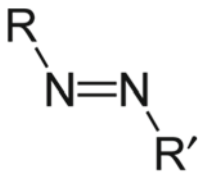

Fig. 1 Structure of azo dye

(Saratale et al. 2011).Based on the number of azo groups, they are classified as monoazo, di azo, triazo, tetrazo and polyazo dyes. Azo dyes are produced when primary amine is deazotised by HCl and $NaNO_2$ at very low temperature forms diazonium salt which is conjugated with aromatic compounds, the azo dye are formed (Lavanya et al. 2014). More than ten thousands of dyes are used in which fifty percentage are azo dyes (Leena and Selva Raj 2008). Since the azo dye comprises maximum percentage in the wastewater from tanneries, treatment of this water is required to reduce environmental impact. International Agency for Research on Cancer (IARC) has classified many of these amines as carcinogens to humans (Sax 1975). Since azo dyes have poor exhaustion properties, the applied initial dyes remains unbound to the fabric and could be found in the effluents. Removal of these dyes from the effluents is a difficult process because of the stability of dyes towards light, oxidizing agents and heat. These properties make them non-biodegradable (Rani et al. 2014).

Exposure to the textile pollution might cause ailments such as—headaches, lung infection, skin infection, nausea, congenital malformation, carcinogenetic, and mutagenic illness (cancer)–. The discharge of the effluent containing azo dyes into the ecosystem causes the formation of unpleasant smell. As dyes are exposed to sunlight, they are reduced and the reduced metabolites affects photosynthesis, concentration of dissolved oxygen, decreases the water quality and also affects aquatic organisms, fauna, flora and human beings. This discharge of azo dyes also adversely affects the Total Carbon Concentration (TOC), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (Saratale et al. 2009).The review provides a glimpse of different biological methods employed in the removal/treatment of textile effluents released from the textile industries.

2 Treatment of Wastewater

Conventional methods for treatment of azo dye contaminated effluents are difficult and less effective. Azo dyes are now widely treated by physical, chemical, photo catalytic and biological methods (Lade et al. 2015). Dye treatment produces more toxic metabolites which pollutes the land and water. To avoid further problems, it is necessary to find an effective, less time consuming and cost effective treatment process. Physical and chemical methods include flocculation, ozonation, bleaching, membrane filtration, adsorption by activated carbon and irradiation are the most commonly used methods for the treatment of dyes in wastewater. Since the dye contains a complicated molecular structure these conventional process such as oxidation by ozonation, activated carbon, coagulation, ultra filtration treatment can be done. Though these treatments are expensive they aren't efficient and also they produce abundant secondary waste products which require further treatment to be eradicated (Robinson et al. 2001) (Table 2).

Table 2 Merits and demerits of physical and chemical methods of dye degradation

Physical and chemical methods	Merits	Demerits
Membrane seperation	Decolorization of chemical class dyes	Sludge generation
Coagulation/flocculation	Simple, cost effective	Sludge production, handling and disposal problem
Oxidation	Quick process and effective	Expensive
Ion exchange	Effective	Economic constraints
Ozonation	COD and color removal is complete, there are no toxic metabolites produced	Expensive, half life is short
Fenton reagent	Decolourises both soluble and insoluble dyes	Sludge formation
Photo chemical	Absence of sludge production and no odours are produced	Production of secondary pollutants
Electrochemical destruction	Absence of sludge production and Chemical consumption is less	Electricity requirement is high
Electro kinetic coagulation	Economical, dye removal is high	Unsuitable for acid dyes, generation of sludge
Adsorption by Activated carbon	Effective process	Unsuitable for disperse and vat dyes
Irradiation	Effective for lab scale and less volume of effluent	DO requirement is high

Bioremediation methods have drawn many scientists as they degrade the azo dyes in an efficient and effective manner which is cost effective and eco-friendly. The microorganisms adapt to the toxic environment and they become resistant to the toxicity of the dyes and transform the toxic dyes to non toxic or less toxic compounds (Saratale et al. 2011; Shah 2021). Decolourisation by microorganisms are carried either by aerobic or anaerobic methods. In aerobic conditions, the microorganism takes long time for chemostatic growth in the presence of oxygen and then decolousises the azo dyes. But in case of anaerobic microorganisms, they are not specific for the azo compound and hence, this method is used universally (Pearce et al. 2003). Wide varieties of microorganisms exist that are capable of decolorizing dyes of various classes. Usage of single isolate isn't effective because single isolates can only decolorize particular class of dyes. Therefore in the biological treatment consortium is used to decolorize the dyes present in the effluent. Mixed cultures were effective when compared to individual strain. Since the process is inexpensive and the end products are mineralized with less toxicity, there are numerous studies done on biological treatment of industrial textile wastewater (Mohana et al. 2008; Chang et al. 2001). Microorganisms also biosorb the azo dyes present in the effluent. Biosorption is the characteristics of certain biomolecule produced by microorganisms that bind specifically to the ions or molecules present in the textile effluent. Microorganisms produces enzymes that degrades the azo dyes and forms metabolites that are less toxic (Revankar et al. 2007).

Biological degradation is usually done by using fungi, bacteria, algae and yeast and some are listed below in Table 3.

The below Table 4 shows the advantages and disadvantages of biological treatment.

Table 3 Microorganisms used in degradation of dyes

Organisms	Dyes	References
Bacteria *Bacillus sps*	Methyl red Methyl orange Congo red Erichrome black T	Kalyani et al. (2008)
E.coli *P.fluorescence*	Reactive orange –MER Reactive blue-M58 Reactive yellow-M46 Reactive black-B	Saranraj et al. (2014)
B.odyssey *B.thuringiensis* *B.subtilis* *Alcaligenes sps* *Nocardiopsis alba*	Acid red	Saranraj et al. (2014)
P.extremorientalis	Congo red	Neifar et al. (2016)
P.aeruginosa	Acid blue 113	Rani et al. (2016)
Fungi *Trametes versicolor*	Acid orange 7 Acid blue 74 Reactive red 2 Reactive black 5	Ramírez-Montoya et al. (2015)

Table 4 Advantages and disadvantages of biological treatments (Robinson et al. 2001)

Biological methods	Advantages	Disadvantages
Biodegradation	Economical, dependent on metabolism of the microbes	Nutrition requirement is continuous, slow, continuous monitoring
Biosorption	Inexpensive	Large scale treatment has not been studied yet, disposal is trickier
Enzymatic	Effective	Expensive
Phytoremediation	Eco-friendly	High maintenance and slow

3 Aerobic Treatment

Depending on the requirement of oxygen bacteria can be divided into aerobic bacteria, anaerobic bacteria and facultative bacteria. In the aerobic biological treatment, aerobic and facultative bacteria are used for the treatment at an aerobic environment (Wang et al. 2011). The process is classified into Activated sludge process and Biofilm formation.

4 Activated Sludge Process

In activated sludge process a floc is used which comprises of numerous organisms which are capable of degrading and absorbing the organics and therefore it is called as Activated sludge. Activated sludge process is an effective method for the degradation and this process is used in oxidation ditch and sequential batch reactor.

In this Sequential batch reactor process, there are five steps involved they are inflow, reaction, sedimentation, outflow and standby. This method of sequential batch reactor activated sludge process is commonly used because it shows high COD removal and decolorization. In the biofilm process, numerous organisms are allowed to grow and attach to an object surface and the wastewater is let to flow on the surface. The contact between the wastewater and the object surface with the biofilm attached will result in degradation and purification. The main biofilm processes are biological contact oxidation, rotating biological contactor and biological fluidized bed.

5 Anaerobic Treatment

In anaerobic treatment anaerobic microorganisms are used. There is high concentration and low concentration treatment in anaerobic treatment process. There are many studies done on anaerobic treatment of textile wastewater but a combination of aerobic-anaerobic treatment gives better results for dye degradation (Wang et al. 2011).

6 Enzymatic Methods

Microorganisms degrade azo dyes by the enzymes, so the decolourisation and mineralization of the dyes occurs in specific conditions. The enzymes that degrade the dyes have wide substrate specificity (Pandey et al. 2007; Esposito and Durán 2000; Mester and Tien 2000). The major enzymes involved in the azo dye degradation are azoreductase, laccase, lignin peroxidise, manganese peroxidise and hydroxylases (Rani et al. 2016).

7 Azoreductase

The reaction carried by azoreducatse is catalysed in presence of NADH and FADH (Robinson et al. 2001). It carries reductive azo dye degradation. In aerobic reaction, oxygen inhibits reduction of azo bond and NADH consumption is dominated and they interfere with the transfer of electrons from NADH to azo dyes (Sandhya et al. 2008; Elisangela et al. 2009) and azo dye reduction in extracellular environment. It has also been studied that redox mediator compounds with low molecular weight which acts as electron shuttles for NADH dependent azoreductase. Microorganism that produces azo reducatses are mostly Bacteria and Fungi. *Lactobacillus casei* (Seesuriyachan et al. 2007)*, Bacillus odyssey, B.thuringiensis, B.subtilis, B.cereus, Alcaligenes sp, Nocardia alba* (Saranraj et al. 2014).

Laccases are enzymes containing four atoms of copper, type 1 paramagnetic copper are responsible for blue colour and the substrate oxidation. Type 2 copper and two 3rd type copper reduces the oxygen molecule to two water molecules. Phenolic compounds are oxidised by laccases and forms non toxic aromatic amines (Ramírez-Montoya et al. 2015).The azo dye containing phenolic group is oxidised by laccase enzyme which generates phenoxy radical and oxidises to carbonium ion (Camarero et al. 2005). Bacteria that produces Laccase enzymes are *Pseudomonas desmolyticum* (Kalme et al. 2007), *Bacillus sps* (Dawkar et al. 2008), *Coriolus versicolor, Paraconiothyrium variabile,* and *Tremetes versicolor* (Asadgol et al. 2014).

Peroxidises are enzyme groups with hemoproteins groups that carries the reaction in Hydrogen peroxide presence. The lignin and manganese peroxidise have similar mechanism which works in hydrogen peroxide presence. Lignin peroxidise were first isolated from *P.chrysosporium* which oxidises the non- phenolic compounds. Whereas Mn^{2+} to Mn^{3+} by Manganese peroxidise because Mn^{3+} is responsible for oxidation of phenolic compounds (Glenn et al. 1983).

8 Bioreactors in Textile Wastewater Treatment

Bioreactors are ex situ bioremediation process to treat the industrial wastewater. To treat the industrial effluents there are many types of bioreactor configuration to achieve maximum degradation. The process usually is aerobic or anaerobic and sometimes combination of both. Decolorization and degradation of the textile effluents are done using biological source in the bioreactors. There are numerous research papers has been published on sequential, combined and even integrated aerobic-anaerobic treatment in bioreactors to degrade the dyes. Different configuration of bioreactors

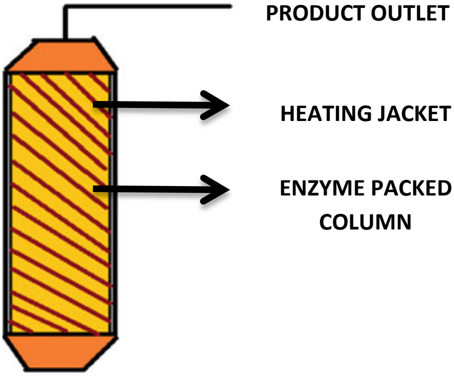

Fig. 2 Packed bed bioreactor

for the dye degradation has been used such as packed bed bioreactor, Sequential Batch reactor, fluidized bed reactor, Trickiling filter and Moving bed biofilm reactor. Bioreactors are mainly used as to retain the biomass for a longer period of time for effective degradation of dyes in the effluent (Ramachandran et al. 2013). Packed bed bioreactors are mainly used when fungi are used in the treatment of dye degradation in reactors. To degrade synthetic dyes, *Irpex lacteus,* a white rot fungi has been used in a packed bed reactor which had a solid support of polyurethane foam (PUF) and pine wood. The synthetic dye, Remezol Brilliant Blue R was degraded by the white rot fungi in the packed bed reactor containing the support (Kasinath et al. 2003). In some packed bed bioreactors activated carbon used for the treatment also gave effective results in degradation of synthetic dyes (Mesquita et al. 2012). Packed bed bioreactors can be continuous flow or fed-batch. Most of the studies on degradation of textile dyes were done using a continuous flow packed bed bioreactor. Congo red dye has been decolorized in a continuous flow packed bed bioreactor that was packed with rice hull, *Schizophullum* sp. F17 was used in the process (Li and Jia 2008). The Fig. 2 shows packed bed bioreactor.

Fluidized bed bioreactors have been used in the treatment of wastewater. In a fluidized bed reactor, a fluid (or gas) is passed through a solid that is suspended by the velocity of the fluid flow. The velocity of the fluid should be as such that it suspends the solid but doesn't wash off the solid from the reactor. For the wastewater treatment, to increase the degradation capacity, fenton process is combined with the fluidized bed and is called as fluidized bed fenton process. There are many such researches published on the usage of FBF process for the treatment of industrial effluent. Effective results were reported on the degradation of dyes using Fluidized bed fenton process in which SiO_2 were used as carriers which gave a complete removal of dye in a short period of time (Wang et al. 2011). The sequential batch reactor contains a cycle of reaction such as fill, react, settle and draw. The time period of these cycles will differ with the type of set up and the type of reaction in the reactor. Sandhya et al. (2005) designed a microaerophilic-aerobic sequential batch reactor to treat simulated wastewater containing mixture of dyes and the final concentration of dye in the synthetic wastewater was 56 mg/L. There were two reactors R1 and R2 which was microaerophilic and aerobic reactor respectively. There was no decolourization observed under shaking condition, only COD removal was observed but under static condition, degradation of the dye was observed. Therefore the bioreactor was switched between static and shaking conditions to achieve COD, BOD removal and decolourization of the dyes. During static condition 78.9% of decolourizationwas obtained and during static-shaking condition 31.8% of decolourization was reported. Baskar and Sukumaran (2015) used a sequencing batch reactor for treating wastewater produced by meat processing industry. Initially the BOD was 330 mg/L. The SBR contained an air diffuser and had a volume of 1200 L. There was a drastic decrease in the COD and BOD levels in 9 days. From 50 mg/L the BOD reduced to 10 mg/L and from 80 mg/L the COD reduced to 30 mg/L in nine days. Therefore SBR was proved to reduce the BOD and COD and also the turbidity of the wastewater was decreased to 90%, making the wastewater harmless to the environment.

There are mainly two technologies used for the treatment of wastewater they are activated sludge process and trickling filters. Compilation of these two technologies, a new type of treatment called Moving Bed Biofilm Reactor was developed in Norway by Norwegian University of Science and Technology in co-operation with Anox Kaldnes AS, a Norwegian Company. The biomass present in MBBR is usually in two forms, one is suspended and the other is attached to carrier material. In the year of 1989, the first MBBR was installed. In a MBBR, carrier materials are used which supports the growth of biofilm. Since the carrier is of lighter density than water, they have a movement along with the stream of water inside the reactor. For aerobic MBBR, the movement of the carrier element is given by air diffuser from the bottom of the reactor. In case of anaerobic treatment, a mechanical stirrer is used for the movement of the carrier element in the reactor. Francis and Sosamony (2016) studied on Moving bed bio-film reactor to treat textile wastewater that was pre-treated in a fluidized bed fenton process. The moving bed bio-film reactor had a height of 50 cm, internal size of 15 X 15, a total volume of 11.25 L. They used Poly Vinyl Chloride (PVC), the inlet pipe of a washing machine as carrier material for formation of biofilm. The sludge was collected from Augastan textiles and *Micobacterium marnilacus* was isolated from the sludge. The synthetic wastewater contained Chemistar turq blue (100 mg/L) as the dye, which was treated in the fenton process to bring the COD from 780 mg/L to 336 mg/L. The pre-treated textile wastewater was then treated in the moving bed, the maximum COD removal was seen in 2.5 days, at a pH of 7.33 and a carrier filling ratio of 67%. COD removal was estimated to be 87.22% and BOD removal of 80% was achieved using moving bed biofilm reactor. Koupaie et al. (2011) treated synthetic wastewater containing Acid Red18 (AR18) 40 mg/L in a conventional Sequential batch reactor and three Moving bed bio-film reactor containing three different carrier materials in each. The filling ratio was 50%. All the reactors measured 50 cm of height, 14 cm inner diameter, 9.8 L total volume and 5 L working volume. The sludge was collected from Zargandeh municipal wastewater treatment plant, Tehran (Iran). The HRT was set for 2 days. The dye concentration was gradually increased from 40 mg/L to 1000 mg/L. COD removal in sequential batch reactor, moving bed bio-film reactor 1, moving bed bio-film reactor 2 and moving bed bio-film reactor 3 were 96.1%, 97.7%, 97.6% and 97.5% respectively. Usage of different carrier materials didn't show any influence on the COD removal. The decolorization of acid red 18 dye wasn't observed and was concluded that the bio-film attachment wasn't enough for the dye removal. Delnavaz et al. (2008) used three moving bed bio-film reactors measuring 70 cm of height, 10 cm of inner diameter, 0.4 cm wall thickness and 60 cm of working volume with Light Expanded Clay Aggregate (LECA) as carriers of 50% filling ratio. They set a HRT of 8, 24, 48, 72 h to degrade the amine compounds such as aniline, para-diaminobenzene, and para-amino phenol. Efficient results were obtained after three days of operation. 90% of COD removal was seen for influent COD that was 750 mg/L, 87% of COD removal in influent COD of 100 mg/L and 90% for 2000 mg/L for para-diaminobenzene, para-aminophenol and aniline respectively. The Fig. 8 below shows typical moving bed biofilm reactor design. Koupaie et al. (2011) used anaerobic sequencing batch reactor/moving bed sequencing batch biofilm reactor to study the kinetics of decolourization and biodegradation of Acid Red 18 azo dye. The reactors measured a diameter of 14 cm, height of 50 cm and effective volume of 5.5 L. Polyethylene biofilm carriers at a filling ratio of 50% were used as support in the moving bed biofilm reactor. There were three anaerobic sequential batch reactor to which different concentration of dyes were added, 100, 500 and 1000 mg/L. in the operation period of 90 days, 83% of COD removal was seen. Therefore anaerobic sequencing batch reactor along with moving bed gave an effective result for wastewater treatment. Jafari et al. (2013) built an anaerobic fluidized bed reactor and aerobic moving bed bioreactor to treat synthetic wastewater using Bee-Cell 2000 as carriers in the moving bed which had a height of 90 cm, inner diameter of 10 cm, 60 cm of working volume and 1.5 L of the carriers. The operation period was 255 days at a HRT of 48, 40, 32, 24, 18 h. The sludge was collected from aerobic digesters of municipal wastewater treatment plant. The effluent COD of the fluidized bed was 935 mg/L and the moving bed biofilm reactor reduced the COD level from 935 mg/L to 350 mg/L. The removal of COD was 97.5% in the moving bed biofilm reactor.

Synthetic wastewater containing different concentrations of Reactive orange 16 dye in 25, 50, 75 and 100 mg/L was treated in a combination of ozonation and moving bed biofilm reactor. Two moving bed bioreactors were used in which one had ozonated synthetic wastewater and the other with non ozonated synthetic wastewater in a 200 mL cylindrical glass. Kaldnes K1 was used as support material in the reactors, the filling ratio was 40%. The activated sludge was collected from municipal sewage treatment plant and the HRT was set for 6 h. The operation continued for 60 days. About 94% of the influent COD was achieved in the moving bed. It was concluded that the moving bed treating non ozonated synthetic wastewater didn't show much degradation of dye and COD. Therefore moving bed biofilm reactor can only enhance the removal of COD and dye in a pre-treated wastewater.

A pilot scale moving bed bioreactors which contained three 15 L reactors with mechanical stirrer and air diffuser was designed by Park et al. (2007). All the reactors contained Polyurethane-Activated carbon (PU-AC) foam as carrier material with a 20% filling ratio in each reactor. The first two reactors were anaerobic and the last reactor was aerobic reactor. The pilot scale reactor was initially operated without the carriers and then compared the performance with the carrier material in it. The carriers were incubated with the microorganisms for seven days for better attachment of the microorganisms to the foam. Removal of COD was seen in the first day of operation, HRT was set as 44 h in all the reactors. The COD removal was high with high packing material. They concluded that A20 moving bed bioreactor was a better treatment method than other conventional process. Treatment of synthetic wastewater in a moving bed biofilm reactor followed by a membrane separation process. The moving bed biofilm reactor contained aerobic and anaerobic section. The total volume was 70 L in which 60 L was the effective volume. The length was 70 cm, width was 25 cm and depth was 40 cm. The cylindrical carriers were used and the filling ratio was 35%. The synthetic wastewater contained an average of 500 mg/L of COD, after the treatment 85% of the COD was removed. The synthetic wastewater was added with Brilliant Red X-3B dye which was reduced in the anaerobic moving bed bioreactor. Highest of COD removal was seen in the aerobic moving bed bioreactor (Dong et al. 2014).

9 Treatment of Textile Azo Dyes Using Combination of Bioreactors

Moving bed biofilm reactor plays an important role to enhance the pre-treated water and to result in high quality final effluent. Therefore, the combination of reactors is used for the maximum degradation. A system of anaerobic fluidized bed reactor and aerobic moving bed biofilm reactor was used by Jafari et al. (2013). The effluent of anaerobic fluidized bed reactor was fed to an aerobic moving bed biofilm reactor was operated at different hydraulic retention time. Maximum COD removal was obtained in anaerobic fluidized bed reactor, aerobic moving biofilm reactor just polished the results of the pre-treated synthetic wastewater. Francis and Sosamony (2016) worked on a combination of fluidized bed fenton process and moving bed biofilm reactor. Comparison study of moving bed biofilm reactor and conventional sequential batch reactor was studied by Koupaie et al. (2011).They have concluded COD, dye concentration and turbidity results of both the reactors did not show much difference.

10 Conclusion

The treatment of the textile effluent is the need of the hour. The efficient and eco-friendly process (Bioremediation) converts the complex dye compounds into simpler structure which could be lesser toxic than parent compound. The current review summarizes the methods of bioremediation used in the treatment of textile dye contaminated effluents. The method of selection of bioremediation depends on the requirement and the site of treatment.

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Microbial Contamination of Environmental Waters and Wastewater: Detection Methods and Treatment Technologies

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Abstract The majority of waterborne pathogens of public health concern do not originate in natural water reservoirs but are instead introduced by point sources of pollution and non-point sources of pollution. Pathogens introduced in the environment by anthropogenic activities are diverse and even if present in low concentrations have serious public health implications. Due to their diversity and low concentrations, there are technical challenges to their accurate quantification, which is particularly true for pathogenic viruses. To overcome the limitations, or as a result of the limitations, water monitoring programs use indicators of fecal contamination as a surrogate for pathogen occurrence. Most methods to detect human viruses in aquatic environments follow these steps: Concentration, nucleic acid extraction, amplification of the genomic segment (or segments) chosen, and detection/quantification of the amplified genomic segment. Wastewater treatment technologies are the most important step to prevent public health burden and to safely reuse waste. Due to the difference in health conditions of people living in different countries, the pathogen content is notably different and therefore the appropriate treatment options are also different and should be diverse. There are warnings that climate change is likely to exacerbate health risks related to deficiencies in water supply, sanitation and hygiene in many regions of the world. These risks can be minimized though a continuum surveillance system using the One Health Approach. This book chapter discusses the pathways

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and routes of transmission of waterborne pathogens in water bodies, water treatment technologies and their role to control the spread of pathogens, as well as the challenges to detect waterborne pathogens in the environment.

Keywords Emerging diseases · Emerging pathogens · Fecal indicators · Human viruses · Pathogen removal · Wastewater treatment · Waterborne pathogens

1 Introduction

Sewage pollution is a major human health concern due to the known risk of exposure to human waste and the public and regulatory will to reduce sewage pollution. Pathogens are microorganisms that cause diseases. Pathogens that are transmitted through water are called waterborne pathogens. When present in water bodies, pathogens are capable of infecting humans by skin contact during bathing and swimming, consumption of contaminated fish and shellfish, and ingestion of contaminated water (Gonçalves et al. 2018). The nature and abundance of waterborne microbial communities and their composition are highly dependent on location and time, as well as various environmental and anthropogenic factors. Most waterborne pathogens of public health concern do not originate in natural water reservoirs but are instead introduced by point sources of pollution such as sewage discharges and non-point sources of pollution such as agricultural runoff, precipitation, and direct pollution by livestock and wildlife effluents (Fig. 1). Stormwater runoff generated during rainfall and snow events contains pollutants that include pathogens. In the USA, polluted stormwater runoff is a leading cause of contamination of water bodies, both chemical and biological (USEPA 2002). The issue is not present only in the USA, but is instead a global problem as surface water is polluted with pathogens in most countries. A study in China analyzed samples from 16 surface waters and in all of them waterborne pathogens were detected (Jin et al. 2018). A study conducted in Finland analyzed 139 surface waters from 7 lakes and 15 rivers, and 51 of them were contaminated with pathogens (Hörman et al. 2004). Similar studies with a wide range of pathogens have been made worldwide and all report contamination of pathogens in surface waters (Baker et al. 2021; Kongprajug et al. 2021).

The main source of pathogens entering waters that can be used for recreation and fishing is fecal contamination. Diseases caused by waterborne pathogens are a major global burden, estimated to cause more than 2.2 million deaths each year. The most common diseases include diarrhea, gastrointestinal diseases, and systemic diseases. Of the 2.2 million deaths per year, about 1.4 million are children. It is estimated that waterborne diseases have a global economic cost of nearly 12 billion US dollars per year. Interestingly, the number of outbreaks underestimates the true incidence of waterborne infections. Nonetheless, the number of waterborne diseases has declined dramatically since the 1900s as a result of the widespread implementation of waste treatment systems. Water treatment systems, both for drinking water and wastewater,

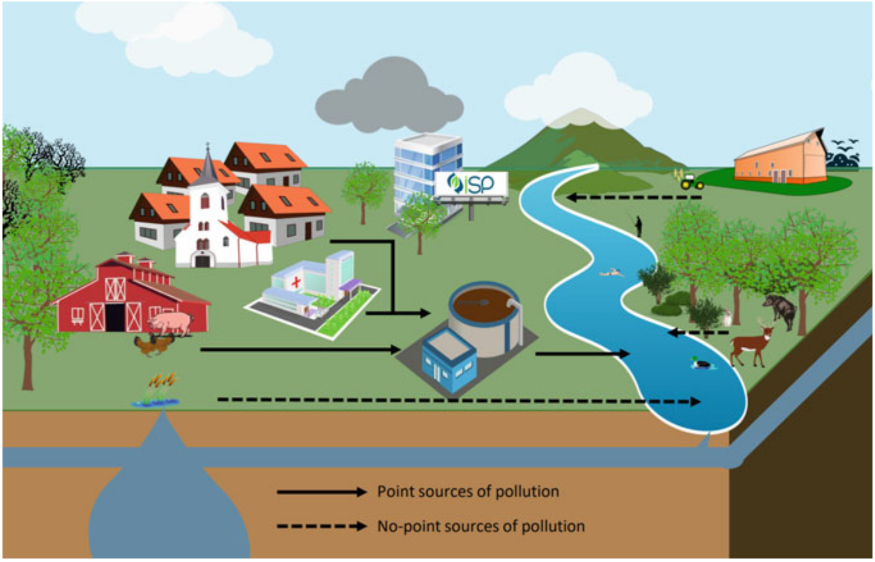

Fig. 1 Illustration of the main routes of contamination of water bodies with waterborne pathogens, such as sewage discharges and non-point sources of pollution such as agricultural runoff, precipitation, and direct pollution of streams and rivers by livestock and wildlife. Examples of activities that pose risk to humans and animals by using contaminated waters are also illustrated (recreational activities and consumption of fish)

have been and continue to be key players in the reduction of waterborne infections (Mbanga et al. 2020; Shah 2020).

Over time, bacteria, viruses, protozoa, and helminths have evolved various mechanisms that facilitate their rapid responses to environmental changes. These rapid responses may be key factors in their infectivity and pathogenicity under climate variability. The major groups of waterborne pathogens present in water bodies include bacteria, viruses, protozoa, and helminths. In this book chapter, we will focus mainly on two groups: Bacteria and Viruses.

Climate change and climate variability has the potential to increase even further the burden of climate-sensitive diseases, in particular waterborne and foodborne, through direct impacts such as in the case of extreme events (flood and sea level rise). Indirect impacts, such as temperature ad humidity, influence the process of pathogen growth and survival. Other indirect impacts include agriculture, water resource management, conflicts, displacements, among others. There are warnings that climate change is likely to exacerbate health risks related to deficiencies in water supply, sanitation and hygiene in many regions of the world. (Cissé 2019; Shah 2021).

The risks of climate change can be minimized though a continuum surveillance system using the One Health Approach. This approach connects human, animal, and environmental health by implementing programs, policies, legislation, and research in which multiple sectors communicate and cooperate to achieve better public health.

Future actions to control the emergence and re-emergence of infectious diseases need to consider the impacts of climate change and integrated interventions. The present book chapter will discuss the pathways and routes of transmission of waterborne pathogens in water bodies, water treatment technologies and their role to control the spread of pathogens, as well as the challenges to detect waterborne viruses in the environment.

2 Fecal Indicator Bacteria in Water Bodies and Emerging Waterborne Pathogens

The detection of pathogens in contaminated water by fecal sources presents some challenges, including the high cost of pathogen detection and the non-continuous transport of pathogens in water bodies. In addition, the pathogens present in water are diverse and usually present in low concentrations, and thus, their detection poses some technical difficulties. To overcome the limitations, or as a result of the limitations, water monitoring programs use indicators of fecal contamination as a surrogate for pathogen occurrence (Gonçalves 2018; Korajkic et al. 2018).

Fecal indicator bacteria (FIB), particularly *E. coli* and *enterococci*, are considered good indicators of risk to human health in freshwater and untreated sewage. An important reason for their wide acceptance as fecal indicators is because they are present in high concentrations in the feces of mammals. Nevertheless, concentrations of fecal indicator bacteria might not hold a good correlation with other pathogens once they enter the aquatic environment due to a range of factors, such as dilution, water flow and survival rates in different environments (Ahmed et al. 2018; Balasubramanian et al. 2016; Gonçalves 2018; Gonçalves et al. 2018). Results regarding the use of faecal indicator bacteria and microbial source tracking (MST) markers to determine the presence of pathogens in aquatic environments are not unanimous. Korajkic et al. (2018) reviewed 73 publications that were generated over a 40-year period studying the relationship between one or more faecal indicator bacteria with one or a group of pathogens. Nearly half of the review publications did not include statistical analysis and the rest were evenly split into those who observed statistically significant correlations and those who did not. The study concluded that FIB and MST markers might be suitable as indicators of faecal pollution, but their relationship with waterborne pathogens is not clear. Several factors might influence the relationship, such as the frequency of detection, variable shedding rates, differential fate and transport characteristics. In the case of waterborne viruses, the relationships are even less understood (Gonçalves et al. 2018a). The efficiency of FIB is even less optimal where the dominant sources of faecal pollution in water are low levels of diffuse pollution from a range of non-human and human sources (Evans et al. 2019), including leaking from sewer pipes and land runoff from wildlife and agricultural sources. On the other hand, point sources originate from municipal wastewater treatment plants (WWTPs) and generally result in high levels of FIB that are identified more quickly than with non-point sources. The FIB originated from non-point sources exhibit lower concentrations but might be persistent and include a mixture of recent and aged faecal inputs, making difficult to track the source of pollution (Teixeira et al. 2020).

The detection of fecal coliforms was one of the first methods proposed to assess water quality in 1966, although in this period, it was though that fecal coliforms can only survive and replicate while in the homeostatic intestinal environment of an host (Geldreich and Clarke 1966). Nowadays, it is known that fecal coliforms are not exclusively found in the intestinal environment of hosts and research has been done suggesting that they can growth or persist in water bodies (Teixeira et al. 2020).

3 Antimicrobial Resistance Genes as Emerging Environmental Contaminants

Antibiotics are small molecules that can either inhibit or kill bacteria and are an essential therapy for bacterial infections. However, some bacteria grow and survive despite antibiotic administration. This property, known as antimicrobial resistance, decreases available treatment options in clinical settings, resulting in increased morbidity and mortality (Boolchandani et al. 2019).

Antibiotic resistance has been historically regarded as a clinical concern and considered to be exclusively related to the excessive use and misuse of antibiotics (Cacace et al. 2019). In recent years, the fate of antimicrobial resistance genes (ARGs) released to wastewaters has received increasing interest and there is a worldwide consensus that raw municipal wastewater, treated effluent and wastewater sludge are reservoirs of ARGs and crucial hotspots for the evolution and spread of antibiotic resistance (Liu et al. 2018). Antibiotics entering water and wastewater are insufficiently removed and/or inactivated in treatment plants, causing a significant fraction being released directly into the environment in effluent waters. A part of these are retained in the sludge, which accumulates these compounds (Núñez-Delgado et al. 2019).

Direct contact between pathogenic bacteria and environmental ARG carriers, as well as, the continuous selective pressure enforced by traces of antibiotics in wastewaters make WWTPs an ideal hub for horizontal transfer of ARGs between microorganisms. There are two main mechanisms that render wastewaters critical hotspots for the evolution and spread of antimicrobial resistance: Selective pressure and horizontal gene transfer (Fig. 2). Thus, the presence of antibiotics in wastewater affects the natural selection of bacteria present by favouring one group of organisms over another. Antibiotics cause a selective pressure by killing susceptible bacteria, while allowing antibiotic-resistant bacteria to survive and multiply and proliferate (Zhang et al. 2021).

In addition to the selective pressure, wastewaters are also hotspots for horizontal gene transfer, which enable the spread of ARGs between different bacterial species. Horizontal gene transfer is the movement of genetic information between organisms. Several resistance genes evolved long ago in natural environments without influence,

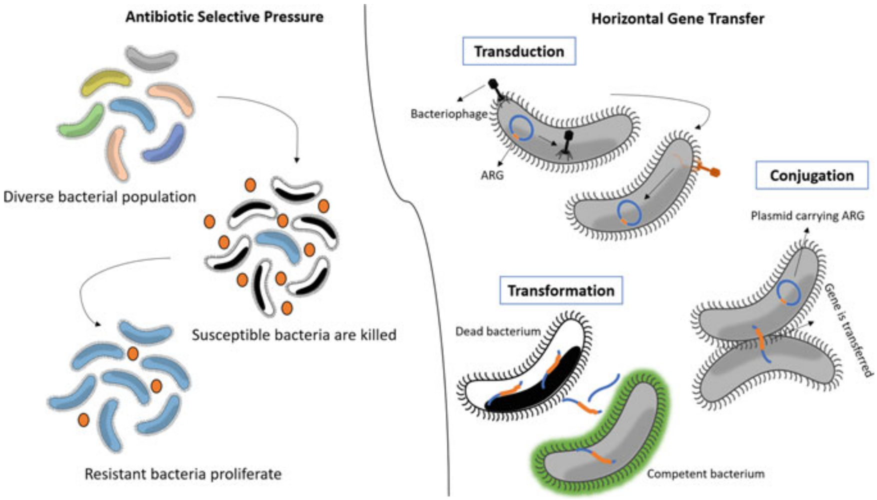

Fig. 2 Mechanisms that aid the spread and evolution of ARGs in wastewaters. There are two main mechanisms: Selective pressure and horizontal gene transfer. Horizontal gene transfer can occur by transduction, conjugation, and transformation

but these genes are now rapidly spreading to and among human pathogens. As seen in Fig. 2, there are three genetic mechanisms for horizontal gene transfer: transduction, conjugation and transformation. Transduction of ARGs occurs when a bacteriophage accidentally packages host ARG gene(s) within it capsid, either in place of the viral genome or with the viral genome. The ARG or ARGs are then incorporated into the genome of the next bacteria being infected by the bacteriophage (Harper et al. 2021). As opposed to transduction and transformation, conjugation requires direct cell-to-cell contact via cell surface pilus or adhesions. Conjugation offers a better protection from the environment and a more efficient way to transfer genes from a donor to a recipient bacterium. Once connected, the two bacteria will directly contact and form a coupling bridge by which DNA is transferred from the donor to the recipient. Transformation is the process in which extracellular free fragments of DNA are incorporated by certain bacteria. For this to occur, several conditions need to be in place, such as the availability of free DNA fragments and competent recipient bacteria. In addition, the translocated DNA must be integrated into the recipient genome or encircled as plasmid DNA (Thomas and Nielsen 2005).

With the rapid spread of ARGs among pathogenic bacteria and the consequent increase in human and animal disease caused by these pathogens, ARGs have been considered as emerging environmental contaminants by the World Health Organization (World Health Organization 2014). Due to the global occurrence of environmental ARGs (Xia et al. 2017) and their potential acquisition by clinical pathogens in environmental settings, they are an ongoing global concern (Hatosy and Martiny 2015). Studies have demonstrated that raw wastewater contains significantly higher amounts of ARGs than treated wastewater. However, its discharge to the receiving water bodies significantly increases ARG quantities in the environment (Jäger et al. 2018). ARG-carrying mobile genetic elements, such as conjugative plasmids, integrative and conjugative elements, transposons and integrons, were also reported to be present downstream of WWTPs (Marano and Cytryn 2017).

Bacterial communities found in wastewater are complex but show high resemblance at high taxonomic ranks. Raw wastewater is dominated by members of the phyla *Proteobacteria*, *Actinobacteria* and *Firmicutes*, and classes such as *Bacilli*, *Clostridia*, *Bacteroid*es, *Alpha*-, *Beta*- and *Gammaproteobacteria*. Human commensal microorganisms are the main bacterial representatives in WWTP influent water. These groups include bacteria frequently reported as potential ARG carriers, such as *Enterobacteria*, *Enterococci*, *Staphylococci* and *Pseudomonas* (Narciso-da-Rocha and Manaia 2017). Among the screened ARGs and genetic elements, intI1 is the most abundant in both effluents and water environments and it has been proposed as a potential marker of anthropogenic pollution (Gillings et al. 2015). The Infectious Disease Society of America (IDSA) has identified six bacterial species, the ESKAPE pathogens, as especially dangerous due to their patterns of antibiotic resistance: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp* (Boucher et al. 2009).

Several different approaches have been used to describe the genetic backgrounds and hosts of ARGs with different levels of success, including cultivation and isolation (Paiva et al. 2017), high-throughput sequencing (Guo et al. 2017) and Emulsion, Paired Isolation and Concatenation PCR (epicPCR) (Hultman et al. 2018). The isolation of pure cultures is still considered the most important method to study antimicrobial resistance. Antibiotic susceptibility testing of pure isolates is relatively inexpensive and provides important information on resistant patterns needed for clinical microbiology. Harmonized databases of clinical breakpoints, such as EUCAST, are used worldwide to monitor antibiotic resistance with common epidemiological cut-off values for resistance (ECOFFs). Despite its importance for clinical breakpoints, ECOFFs cannot be directly applied to wastewaters since it requires a large number of environmental isolates. Only a fraction of environmental bacteria can be grown under laboratory conditions. Thus, culturing, and antimicrobial susceptibility testing has limitations for a comprehensive study of resistance in wastewaters. The combination of culture methods, susceptibility testing and molecular biology tools allows the identification of previously unknown resistance determinants that may be either intrinsic or acquired by mutations and horizontal gene transfer. Whole-genome sequencing is a powerful tool to study the genetic environment of ARGs and enables the detection of genes located on mobile genetic elements, capable of horizontal gene transfer.

Metagenomic analyses of urban wastewater have been suggested as an ethically acceptable and feasible approach for continuous global surveillance and prediction of antimicrobial resistance (Hendriksen et al. 2019). Metagenomics overcomes the need for prior knowledge of resistance gene diversity, and it is not restricted to a few chosen genes. Through the sequencing of the total DNA extracted from the microbial community, the whole resistome can be predicted (Lakin et al. 2017). On the other hand, the annotation of ARGs relies on known genes in antibiotic resistance databases, such as Resfams, MEGARes and CARD. The big advantage is that ARG databases are regularly updated with novel gene variants and sequences can be re-analysed with the updated databases (Wallace et al. 2017).

Metagenomic analyses have been used in several environments to study antibiotic resistance (Fresia et al. 2019; Hendriksen et al. 2019). However, due to the low number of ARGs in comparison to other functional genes, deep sequencing is needed, which significantly increases sequencing costs. The produced short-reads provide only limited information and, by assembling short reads to longer overlapping regions (contigs), information about phylogeny and genetic location of genes can be inferred. Long-read sequencing technologies (also known as third-generation technologies), such as Pacific Biosciences and Oxford Nanopore, were recently developed and differ from second-generation sequencing (NGS) (e.g. Illumina) in their ability to generate long reads that can span long genomic regions, thus providing an opportunity to link the ARGs with their flanking regions at a much lower cost. The higher per-read error rate of long-read sequencing, when compared with the well-established NGS technologies, such as Illumina, is a limitation that has been recently addressed by specially designed bioinformatic algorithms for error correction and high accuracy de novo assembly (van der Helm et al. 2017). Recent studies on the resistome from wastewaters have shown that these bioinformatic tools are reliable and that Nanopore sequencing is largely consistent with that based on Illumina sequencing platforms (Che et al. 2019).Nevertheless, the study and understanding of environmental and public health implications of the resistome in wastewaters needs to be further explored.

4 Human Enteric Viruses in Receiving Waters. Challenges for Detection and Quantification

Monitoring of human enteric viruses in the aquatic environment began in the 1940s with the goal of monitoring microbial water quality and identifying major sources of water pollution. Wastewater treatment technologies have improved significantly in the last decade, however, waterborne diseases continue to have public health and socioeconomic implications and viruses are the main cause of waterborne infections.

A wide range of enteric viruses were reported to be the major agents of waterborne outbreaks. Illness can occur upon exposure to few viral particles, therefore methods to detect viruses from environmental samples should be sensitive enough to detect very low concentrations (Zhang et al. 2019). The available diagnostic techniques have limited the detection of enteric viruses from environmental samples and consequently these pathogens have not been frequently identified as agents of waterborne diseases. Most methods to detect human viruses in aquatic environments follow these steps: (1) Concentration of the targeted virus from the water sample into a suitable volume; (2) RNA or DNA extraction from the targeted organism; (3) Amplification of the genomic segment (or segments) chosen; (4) Detection and/or quantification of the amplified genomic segment.

4.1 Concentration Methods for Viruses in Aquatic Environments

Due to the low concentrations of human viruses in natural waters, the implementation of a concentration step is crucial for a successful detection (Gonçalves et al. 2018b). This procedure should be simple and fast, have a high recovery rate, be suitable for a wide range of enteric viruses, produce a small amount of concentrate, and be inexpensive. The efficiency of the concentration method is highly dependent on the quality of the water sampled. A wide range of methods for concentrating viruses from environmental water samples have been tested with varying degrees of success. Most methods exploit the physicochemical properties of viruses. Concentration techniques include ultracentrifugation, ultrafiltration, adsorption and elution-based methods, and viral precipitation (Symonds and Breitbart 2015). Each of the available methods has important advantages and limitations.

Ultracentrifugation uses forces ranging from 100,000 × g to 235,000 × g to concentrate viruses from a given sample. It is mostly used as a secondary concentration method to adsorption-elution because it is difficult to process large volumes of water samples. However, it has been successfully used as a direct concentration method for viruses from wastewater and heavily polluted recreational waters (Zheng et al. 2022).

Methods based on ultrafiltration, such as vortex filtration or tangential flow filtration, use size exclusion to concentrate viruses. Water and ions smaller than the pores of the filter pass through the membrane, while microorganisms that are larger are concentrated in the retentate. Due to the generic size exclusion principle of ultrafiltration, this filtration technique can be used to concentrate several waterborne pathogens, including viruses, bacteria and protozoa (Liu et al. 2012; Zheng et al. 2022).

Precipitation methods are typically used as a secondary step. In organic flocculation, buffered beef extract is used to precipitate viruses from concentrated samples by lowering the pH to 3.5. The precipitate formed is centrifuged and the pellet suspended in sodium phosphate. A well validated technique using polyethylene glycol (PEG) has been used on several viruses and various environmental waters (Hmaïed et al. 2016). PEG precipitation consists of precipitation of viral particles by adding 0.5 M NaCl and 7% PEG to beef extract with constant stirring for 2 h at 4 °C, followed by a centrifugation step and resuspension of the pellet in Tris-buffered saline. Despite its use, beef extract has been reported to have inhibitory effects in PCR assays and widely discarded. Skimmed milk flocculation was successfully used as a one-step protocol to concentrate viruses from coastal waters. The viruses adsorb to the pre-flocculated milk proteins. The flocs sediment by gravity and the precipitate is dissolved in PBS and centrifuged. Skimmed milk flocculation was tested for human adenovirus (HAdV) in seawater samples, with recoveries ranging from 42 to 52%. The protocol requires agitation of samples for 8–10 h to successfully ensure adsorption of viruses by skimmed milk floccules, followed by another 8–10 h of sedimentation (Calgua et al. 2008). Recently, skimmed milk flocculation has also been efficiently used for simultaneous concentration of Human Adenovirus (HAdV), Bacteriophage MS2 (MS2), Rotavirus (RoV) and Bovine Viral Diarrhea Virus (BVDV) as well as various bacteria and protozoa

(Gonzales-Gustavson et al. 2017). The method is reproducible, reliable, and inexpensive, but has the disadvantage of requiring more than 16 h to process the samples. A combination of filtration through nitrocellulose membranes with skimmed milk flocculation was successfully used to concentrate HAdV and NoV from seawater samples. In the same study, glass wool filters were used in combination with skimmed milk flocculation for freshwater samples. The processing times were significantly reduced (Wyn-Jones et al. 2011).

A wide range of filters and filtration methods are traditionally used to concentrate large volumes of water (up to thousands of liters), such as electropositive and electronegative cartridge filters, glass fiber filters, glass wool filters, vortex flow filtration, tangential flow filtration and acid flocculation (Wyn-Jones et al. 2011).

Adsorption-elution based methods are the most common procedure in environmental virology and are based on adsorption of the viral particle to a surface followed by elution. This approach is effective to recover viruses from water samples, but it is highly dependent on water quality conditions such as pH, ionic strength and organic content. There are two main filter types to adsorb virus: negatively charged filters and positively charged filters. Electronegative filters rely on the manipulation of the water sample to cause a positive surface charge of a viral particle. Enteric viruses are negatively charged in waters and will only adsorb to a negatively charged membrane under acid conditions or in the presence of Mg^{2+}, or other multivalent cations. Compared to electropositive filters, electronegative filters show higher virus recoveries in marine waters and waters of high turbidity. The major weakness of electronegative filters is the need for preconditioning the water sample or filter, prior to filtration, as the sample must be pH adjusted. It is also a complex and time-consuming procedure (Haramoto et al. 2018).

Electropositive filters are based on the innate negative charge of virus particles in environmental waters, and consequently they do not require preconditioning of the water before filtration. Viruses that are more electronegative adsorb better to positively charged surfaces, which has an impact on the elution efficiency. To elute viruses from the filters, solutions with various amino acids and complex proteinaceous solutions are commonly used. The most used solution consists of beef extract at a pH of 9.0–9.5 and a concentration of 1.5–3.0% of 0.05–0.1 M glycine. 1MDS Zetapor Virosporb (CUNO, Meriden, CN) is the most used electropositive filter and it was designed by the U.S. Environmental Protection Agency to recover viruses from drinking water (U.S. Environmental Protection Agency 1996). NanoCeram filters (Argonide, Sanford, FL) are a good and cheaper alternative, as they have been efficiently used to concentrate various enteric viruses from tap and river water samples. Most electropositive filters are easy to use and are suitable for field use. However they are easily clogged and have low recovery rates for viruses in marine water due to the presence of salt and the alkalinity of seawater, which cause low adsorption of the viruses to the filter. Glass wool coated with mineral oil has hydrophobic and electropositive sites on its surface and have been used as an adsorption material for viral concentration. The suspension of viruses flows through the pore space of the glass wool membrane and the fiber surface attracts and retains negatively charged viruses at a neutral pH and without addition of cations. The efficiency of concentration by glass wool depends on the type of viruses, water pH and water matrix. Glass powder, such as borosilicate glass beads (with a diameter of 100 ± 200 μm), are a good adsorbent for viruses. Due to the formation of a fluidized bed, the filter matrix cannot be clogged as with glass wool materials. In both glass wool materials and glass powder, the recoveries widely depend on the sample type. Magnetic beads coated with antibodies or other molecules can be used to concentrate viruses. Viruses bind to the beads and are magnetically separated from the sample. Viruses are eluted using an appropriate buffer (Haramoto et al. 2018).

4.2 Quantification Methods for Viruses in Water Environments

Available detection techniques have limited the detection of enteric viruses from environmental samples and, consequently, these pathogens have frequently not been identified as agents of waterborne diseases. Culture based methods can determine the concentration of infectious viruses, however, these methods have a high associated costs, require intensive laboratory work, give late results and fail to detect many waterborne viruses, as for example NoV that cannot yet be cultivated (Symonds and Breitbart 2015).

Transmission electron microscopy (TEM) is widely used to detect enteric viruses in public health laboratories, but this method is subjective, requires specialized personnel, is laborious and time consuming, and exhibits a low sensitivity. For these reasons it has been largely dismissed for water samples. Immunological tests, such as enzyme immunoassay (EIA), radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), have been used to detect enteric viruses and many have commercially available kits tested for the main enteric viruses. Immunological tests are widely used in clinical samples, but due to their poor analytical sensitivity, these tests are not good enough for environmental samples. The detection of enteric viruses in surface waters is most frequently based on molecular technologies, due to their highest sensitivity, specificity, and reduced processing times. However, they have the disadvantage that infectivity cannot be confirmed.

Molecular detection of viruses requires the extraction of genomic nucleic acids (either DNA or RNA) followed by virus-specific DNA or RNA target detection using several different approaches. Extraction of viral DNA or RNA can be done by a wide range of commercially available kits that are reliable, offer high reproducibility and are easy to use. Proteinase K treatment followed by phenol chloroform extraction and ethanol precipitation, sonication and heat treatment are also widely used. Automated methods for nucleic acids extraction have been developed in recent years and have been successfully applied to analyze viruses in water samples (Wang et al. 2020).

The first molecular technique used to detect viruses was PCR, invented by Kary Mullis et al. in 1986 (Mullis et al. 1986). PCR detection is based on the amplification of a virus-specific section of viral genome using a pair of short oligonucleotides (primers) that guide the PCR polymerase to amplify the target sequence of the viral genome. In the case of RNA viruses, reverse transcription (RT) of viral RNA to complementary DNA (cDNA) is required before the PCR. PCR products are most commonly visualized by agarose gel electrophoresis and results can be further confirmed by hybridization of the PCR product using an internal oligonucleotide probe or by sequencing the DNA product (Kojima et al. 2002). Semi-nested or nested PCR use a second internal primer or primer set, which allows to improve the sensitivity and specificity. It can also be used as a confirmation step.

In the nineties, real-time PCR (qPCR) was developed by Heid and collaborators (Heid et al. 1996) and it is nowadays widely used in research and diagnostics. qPCR shares the same principle of target amplification as PCR, but it combines primer amplification with detection of the amplified product in a single reaction mix in real-time. By using fluorescent labelled primers or an oligonucleotide probe, in addition to the primer set, the signal is detected via intercalating fluorescent dyes that bind to the amplified PCR products. In the same way as PCR, only DNA can be amplified.

In order to amplify RNA, a reverse transcription of the RNA to cDNA needs to be performed first, and the process is called RT-qPCR. Recent qPCR approaches allow very specific detection of one or more targets in one reaction. One of its best properties is that qPCR allows the quantification of the target template initially present in the sample. qPCR is the most widely used molecular biology technique due to its robustness, speed, miniaturization of the reactions, and excellent automation possibilities. It is well established in scientific research, industrial development and it has become an essential tool of service companies, quality control applications and diagnostics. qPCR, as well as PCR and many enzymatic reactions, is prone to inhibition. Environmental samples often contain inhibitory compounds that in extreme situations can lead to false negative results. By using low sample volumes or by diluting the samples this effect can be minimized.

Another important variant of PCR is digital PCR (dPCR). In dPCR, a single bulk PCR reaction is partitioned into thousands or in some cases millions of nanoliter or picolitre reactions. Partitioning of the reaction was only possible by recent advances in nanofluidics and emulsion chemistries (droplet dPCR or ddPCR). The partitioning of the sample struggles to assure that each individual partition only contains one target molecule or none. Quantification is obtained by counting partitions with amplified products and those without amplified products. However, since some partitions may have multiple amplified products, a Poisson correction coefficient is applied to the positive partitions for compensation. The advantages of dPCR in relation to qPCR are the precision of nucleic acids quantification, no need for standard curve, high reproducibility, and lower susceptibility for environmental PCR inhibition (very small reaction volume). The costs of dPCR are high but the trend is to decrease. More and more equipment are available in the market, which indicates that dPCR can become popular as a tool to monitor water quality.

Isothermal methods, which require a single constant temperature for amplification, can also be used for sensitive detection of nucleic acids. While in PCR reactions, temperature changes are required, isothermal methods, such as nucleic acid sequence-based amplification (NASBA) and loop mediated isothermal amplification (LAMP) perform amplification without temperature changes and thus require less complicated and cheaper instruments. Some of these methods involve different enzymes than DNA polymerase or in some cases several enzymes. The popularity of NASBA and LAMP has been increasing as alternatives to PCR method (Becherer et al. 2020).

Next Generation Sequencing (NGS) offers the sequencing of a massive quantity of short DNA fragments in a single sequencing reaction that produces base pair reads covering an enormous amount of information in just a few days. NGS can be used to confirm positive PCR results or as a powerful detection tool. This technique overcomes the limitation of target-based methods, such as PCR, qPCR and isothermal methods, which require prior knowledge of the sequences of at least part of the viral genomes. Additionally, NGS provides an exceptional insight into viral diversity by viral metagenomics studies. Most NGS platforms are still very costly, but there are a lot of service providers available. The processing and interpretation of the results obtained by NGS rely heavily on bioinformatic tools, which requires skilled personnel (Vinkšel et al. 2021). The development of sensitive detection methods for enteric viruses is a challenging task. The standardization and validation of protocols is an imperative requirement to implement molecular techniques in either clinical or environmental fields (Cassedy et al. 2021).

5 Wastewater Treatment Technologies to Minimize the Spread of Waterborne Pathogens

In wastewater treatment Plants (WWTPs) pathogens are removed from wastewater and concentrated in the form of sewage sludge during the primary treatment, common in most WWTP of any size. Preliminary and primary treatment are aimed at removing larger materials and solids and includes sorting units and primary settlers where coagulants or polymers may be applied to enhance sedimentation. In medium and large-size WWTP, the biological process during secondary treatment converts approximately 40% of the available organic matter into CO_2 and H_2O and the rest accumulates and is used for the growth of biological sludge, where viable forms of pathogens can accumulate. The removal of soluble and particulate organic matter occurs during secondary treatment, typically in Activated Sludge (AS) reactors and their variants, including biofilm and membrane systems. Several variants of AS technology can achieve high removal efficiencies of nitrogen and phosphorus, which are the main focus of tertiary units. Additional treatment may be applied at the WWTP (tertiary treatment) before water is discharged; depending on the final use of water, disinfection and membrane processes are sometimes applied to further reduce the concentration of pathogens. Bio trickling filters, pond and membrane bioreactors

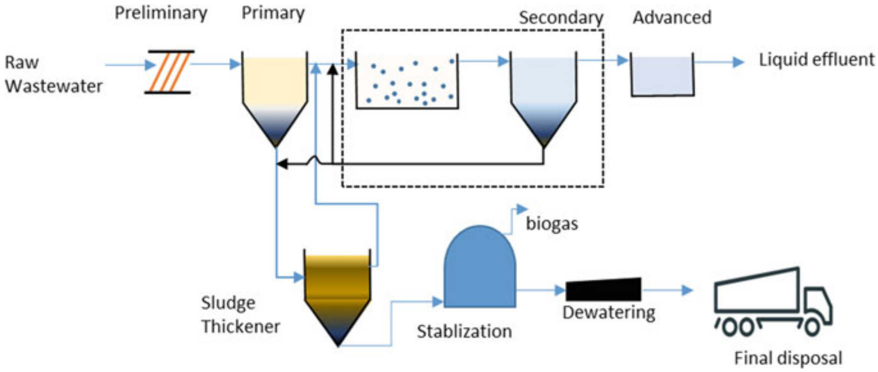

Fig. 3 Main wastewater treatment steps commonly found in wastewater treatment plants

(MBRs) are widely applied tertiary treatment technologies. In facilities in which the removal of pathogens or specific pollutants are required, advanced treatment processes are applied and include UV radiation, ozonisation, activated carbon, chlorination among others. Further details of pathogens an virus removal reported in recent literature are provide for each treatment step, as well as the removal of antimicrobial resistant bacteria (ARB) and ARG, for which detection methods are currently being developed and standardized (Miłobedzka et al. 2022). Figure 3 summarizes the main treatment steps in common WWTPs.

5.1 *Preliminary Treatment*

Preliminary treatment is used to remove floating organic material (called scum) and coarse solids dragged in the sewer system that would interfere with mechanical equipment, reducing the suspended solids load for subsequent treatment processes. This step comprises gravity sedimentation of settleable solids in the screened wastewater and scums, and may involve the use of coagulants to enhance the process. Preliminary treatment has a limited effect on the removal of pathogen from the liquid stream, as removal by settling is not expected. However, pathogens may be removed when attached to particles, typically reaching removals from 0 to 1 Log_{10} units of coliforms, norovirus and other fecal indicators. Chemically enhanced primary treatment and advanced primary treatment may increase removals during primary sedimentation from 1 to 2 Log_{10} for virus, bacteria, and protozoa and 1–3 Log_{10} for helminth eggs. Screenings, grit, and sludge will contain high concentrations of pathogens and must be safely treated and/or disposed to protect public health.

5.2 Secondary Treatment

Secondary treatment typically comprises aerobic or anaerobic processes for biological removal of carbon and nitrogen. Other technologies include systems usually applied for decentralized solutions, such as septic tanks, pond systems, wetlands, and sand filters.

Activated sludge/MBR: The most common technology is Activated Sludge System, which in its most basic configuration comprises an aerated reactor followed by a secondary settler conventional Activated Sludge (CAS). Advanced configurations include the substitution of the secondary settler by MBRs for the enhancement of biomass separation or process modifications to achieve nitrification–denitrification and enhanced biological phosphorus removal (EBPR). Together, primary settling followed by CAS or MBR have a certain disinfection capacity due to the natural predation, decay or adsorption onto the biological flocs of pathogens, subsequently removed by the solid's separation processes. Secondary treatment usually increases in about 1 Log_{10} unit the reduction achieved during primary sedimentation, with removals of coliforms and indicators in CAS ranging from 1.0 to 1.6 Log_{10} units (Lucena et al. 2004). Similarly, Alcalde et al. (2012) reported removals ranging 1.6 to 1.8 Log_{10} units for *E. coli*, *Fecal enterococci*, somatic bacteriophages, and spores of sulphite-reducing clostridia. Furthermore, MBR systems can substantially increase pathogen removal during secondary treatment (Alcalde et al. 2012; Lucena et al. 2004). In this sense, De Luca et al. (2013) reported 2.7 and 1.7 Log_{10} higher reductions of somatic coliphages and F-RNA specific bacteriophages compared with conventional activated sludge process (De Luca et al. 2013), whereas Zhang and Farahbakhsh (2007) reported a complete removal of fecal coliforms and up to 5.8 logs removal of coliphages was observed in a pilot MBR system (Zhang and Farahbakhsh 2007). The use of aerobic granular sludge also showed higher removals than CAS systems, with increases from 1.3 to 2.1 Log_{10} removals for F-specific RNA bacteriophages and 1.1–2.3 Log_{10} removals for E. coli, Enterococci, and Thermotolerant coliforms (Barrios-Hernández et al. 2020).

Other technologies: Other technologies for secondary treatment include the use of anaerobic processes or trickling filters (TF). Although their total removal is not achieved, a reduction of 2.34 and 1.36 Log_{10} in the concentration of E. coli was produced along the water lines, respectively, in a systems applying trickling filters (TF) followed by secondary settlers (Marín et al. 2015).

For anaerobic technologies, although significant contributions to coliforms removal are not expected, Tandukar et al. (2007) reported total and fecal coliform removals of 4 and 3.7 Log_{10} units, respectively, in a upflow anerobic sludge blanket reactor followed by down-flow hanging sponge unit (Tandukar et al. 2007). In addition, decentralized systems such as septic tanks, sand filters, wetlands and pond systems have shown potential to remove substantial quantities of pathogens, with HRT being the most significant factor that can be optimized. Particularly, high removals in filtration units were favoured by fine grain sizes of filter media, uniform water distribution methods and low hydraulic loading rates (Wang et al. 2021a, b).

5.3 Tertiary and Advanced Systems

In a CAS systems followed by nitrifying rotating biological contactors (RBCs), sand filtration and chlorination/dechlorination, Zang and Farahbakhsh (2007) recorded up to 5.7 Log_{10} removal of coliforms and 5.5 Log_{10} of coliphages were observed in the conventional treatment process with advanced tertiary treatment (Zhang and Farahbakhsh 2007). The addition of chemical coagulants appeared to improve the efficacy of primary and secondary treatment for microorganism removal. Other technologies of tertiary/advanced treatment may include electro-Fenton (EF) with continuous hydroxyl radical generation, which can achieve up to 4 Log_{10} units in coliforms/*E. coli* inactivation (Wang et al. 2021a, b). Similarly, a water reclamation system treating effluent of a CAS WWTP in Spain, and comprising coagulation-flocculation, settling, sand filtration and chlorination achieved additional reductions of 3.4 2.5, 0.9 and 0.6 Log_{10} units, respectively (Alcalde et al. 2012).

ARG and ARB, antibiotic resistant *E. coli* and several ARG providing resistance can be removed from discharged water in WWTP with primary and secondary treatment or secondary treatment followed by disinfection; 2 Log_{10} removal was found for E. coli and 1.5–2 Log_{10} for other resistant bacteria (Proia et al. 2018b). However, most of these resistant bacteria and resistance genes are concentrated in sewage sludge and can potentially re-enter water bodies if reused for land application or not properly managed.

Techniques of virus surveillance in wastewater are expensive and due to limitations in their cultivation methods, only a few enteric viruses are regulated by standards for virus control. During the COVID-19 pandemic, environmental risks of viruses gained more attention and several studies focused on the development of indicators of enteric viruses in wastewater samples as their relative abundance is expected to be higher than in other aquatic environments. In this regard, the concentration of norovirus and coliphages was determined in reclaimed water from two WWTP in Spain, revealing a correlation between the concentration of each of them only at high concentrations (Truchado et al. 2021). On the contrary, JC and BK polyomaviruses were good indicators of total enteric viruses and the Log_{10} reduction of their concentration during wastewater treatment was lower than that of faecal indicator bacteria (Tandukar et al. 2021) indicating the necessity of further application of advanced and tertiary processes.

5.4 Sewage Sludge Treatment and Disposal

The use of sludge for land application is common throughout the world. Some countries prohibit its use for this purpose and some others require sludge to be treated and converted into a biosolid, which depending on its physic and chemical characteristics and pathogen content, that could be applied to soil. In this regard, the Standards for the Use or Disposal of Sewage Sludge (2003) published by the United States Environmental Protection Agency (40 CFR Part 503) (Environmental Protection Agency 2003) and the Proposal for a Directive of the European parliament and of the Council on the spreading of sludge on land (2003) are a reference and limits the concentrations of faecal coliforms, *Salmonella* sp, enteric viruses, *Clostridium perfringens* and viable helminth ova in biosolids to be used for land application. Depending on the concentration of those, biosolids are classified and the lowest concentration is required for allowing its use for fertilization. Those biosolids that do not meet requirements cannot be used for land application and are commonly incinerated. In addition, the presence of regulated indicators does not correlate with the presence of other pathogens (Fijalkowski et al. 2017), such as those carrying ARGs and thus antibiotic resistant. Despite regulated concentrations of some pathogen, detailed studies on the effect of pathogens transmissibility by land application are scarce as well as its release to water bodies by drainage. In this regard, long-term studies on trial fields revealed no association between the relative abundance of potentially pathogenic bacteria and the seasonal application of stabilized (under thermophilic conditions) biosolids; however, an increased relative abundance of *sul1* and *tetW* was significantly related to biosolid application (Stiborova et al. 2021).

Sludge treatment in medium and large WWTP is often performed by anaerobic digestion of the sewage sludge to recover energy in the form of biogas. Many pathogens are inactivated during anaerobic digestion, particularly at the thermophilic range (~3 Log_{10} reduction) and at long retention times (Zhao and Liu 2019) but the mechanisms of pathogenic inactivation are not well reported (Li et al. 2022). Thermophilic AD can achieve the requirements for land application; however, recent studies on pathogens not covered by regulations revealed the presence of several parasites such as *Cryptosporidium* spp. *Entamoeba* spp. *Giardia duodenalis* (Benito et al. 2020) and some controversy is found regarding ARG removal at different temperatures during AD; because some pathogens are heat-resistant and the abundance of total ARG was higher at 55ºC than al lower temperatures (Huang et al. 2019).

Virus infectivity has been reported to decrease by more than 90% during anaerobic digestion of sludge. However, biosolids obtained from AD contain higher levels of somatic coliphages than traditional bacterial indicators which are more persistent in solids and may be a complementary indicator of pathogenic viruses (Martín-Díaz et al. 2020) since at high concentration of coliphages and enteric viruses a positive correlation could be established (Truchado et al. 2021).

Thermal pre-treatments of sludge are popular for increasing pathogenic reduction when AD does not meet quality criteria, due to the high temperatures employed (up to 160–180 ºC) and the fact that some of the energy required can be recovered as additional biogas. Although the process has been traditionally employed as a pretreatment to increase energy recovery, recent studies show an increased potential of pathogens, ARB and ARG inactivation when performed as a post-treatment because bacterial regrowth during AD of solid fraction is prevented by digesting only the liquid hydrolysate (Cai et al. 2021). Anyhow, temperature during AD and thermal treatments is the dominant factor for the inactivation of pathogens in sewage sludge management systems (Li et al. 2022). Thermal hydrolysis achieved a very high removal of *E. coli* and coliphages, in contrast to the sonication process and also a positive correlation between coliphages and enteric viruses; according to the reported ratios between them, a tentative concentration of $< 10^4$ PFU/g DW was proposed as quality limit for land application (Levantesi et al. 2015).

6 Conclusion

The World Health Organization (WHO) estimates that, in 2012, 12.6 million deaths in the world were attributable to environmental issues, which represents 23% of all deaths. Among the environmental factors, food and water contaminations are of particular relevance in the transmission of diseases (WHO 2018). Waterborne diseases include many different types of infections that are transmitted through water. These include pathogens from a range of taxa (viruses, bacteria, protozoa and helminths).

Waterborne pathogens are a public health problem worldwide and are still a major cause of serious illness and mortality. Pathogen indicators need to be constantly improved as many emerging pathogens cause waterborne diseases and outbreaks.

Increasing demands are being placed on the water treatment industry to reduce the risk of disease from both chemicals and microorganisms. To meet these demands, we need a better understanding of microbial resistance at the molecular level, and we need to develop faster methods to evaluate treatment efficacy.

Climate change and climate variability has the potential to increase even further the burden of climate-sensitive diseases. The risks can be minimized though a continuum surveillance system using the One Health Approach. This approach connects human, animal, and environmental health by implementing programs, policies, legislation, and research in which multiple sectors communicate and cooperate to achieve better public health.

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Use of Genetic Engineering Approach in Bioremediation of Wastewater

Jutishna Bora, Saqueib Imam, Vardan Vaibhav, and Sumira Malik

Abstract The principles of genetic engineering led to unlocking the door to many possibilities, expanding its application into several fields of life sciences. Recently scientists have attempted to combine the disciplines of genetic engineering and bioremediation as a possible solution to free the environment of possible organic contaminants such as oil, solvents, and herbicides, as well as cycling out toxic heavy chemicals, which are further used by the microbes for carrying out their metabolic cycles and as their source of food and energy. This paper addresses the various microbes and genetic tools involved in bioremediation. Potential novel ways are highlighted that are being developed using gene editing tools to better the waste management system. Further, the key metabolic pathways are discussed, by which microbes cycle the harmful toxic pollutants from the environment into their system. The various actively used Gene editing tools for bioremediation of wastewater to get specific microbes included are TALEN, ZFNs, and CRISPR Cas9.

Keywords Bioremediation · Genetic engineering · Genetically Modified Microorganisms (GMO) · CRISPR/Cas 9 · ZFN · TALEN · Biosensor · Bioaccumulation

1 Introduction

With the increase in industrialisation over the years, the rate of pollution has increased drastically worldwide. Industrial instruments and batch processes have led to the release of toxic and carcinogenic substances in the air, land and water, such as pharmaceutical waste, by-products from petroleum refining, and dyes—polymers

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and even explosives. Extensive use of pesticides and herbicides by farmers to curb the growth of weeds and unwanted microorganisms on their crops has also contributed to polluting a more significant part of the environment since these pesticides and herbicides use various nitrogen-containing chemicals and other compounds which cannot be degraded or removed easily. WHO has reported that by the year 2021, 84% of the population all over India will have been living in areas with polluted air, which has exceeded India's overall own quality of air, with an average presence of 63 microns of particulate matter (PM) per cubic meter of air. Another survey has shown that, in the year 2020, India alone reported an average of 51.90% of land filled with various pollutants, damaging crops and human life altogether.

Further, reports by WHO have revealed that the average life expectancy of Indian life has also decreased by 5.2 years, indicating significant endangerment to human life in the upcoming years if the rate of pollution keeps increasing at this steady rate. Such an increased accumulation in the environment can render the soil, air and water bodies unfit for further use and cause health hazards to humans and animals. Hence, the demand for recycling and degradation/removal of these pollutants from the environment has gained a higher ground. Scientists have attempted to combine various life sciences disciplines with different technological advances, to develop an efficient and suitable technique for curing the environment from such harmful pollutants.

Conventional remediation techniques for the degradation/removal of pollutants were costly since they involved using specific instruments and chemicals, which not only contributed to causing additional environmental damage but were also not economically viable. Hence an alternative technique was needed for remediation of pollutants, which was inexpensive, caused minimum damage, was less time consuming, required minimum labour and was a long-term investment overall. That is when biological microorganisms were considered a possible replacement for the conventional techniques for bioremediation. These microorganisms were extensively studied under simulated environmental conditions with specific pollutants, which served as the substrate for the growth of microorganisms. It was observed that these microorganisms could degrade/bioaccumulate these pollutants via their various metabolic pathways, which was also found to be different for different strains of microorganisms. Microorganisms offer the remediation of pollutants via a natural process. Hence it became not only the ideal choice for farmers and industrialists, but also further research and experiments were conducted to modify their genome genetically, enzymes they produced, their structure or inserted with genes from other species to produce specific proteins, enhancing their productivity, meanwhile maintaining their biomass to a minimum concentration. The by-products released by them were also less harmful (Fig. 1).

Thus, when genetic engineering principles were applied to the field of microbiology, along with the use of nanotechnology to produce genetically modified organisms (GMO), it was observed that they provided enhanced efficiency with which remediation could be carried out compared to the conventional techniques. Microbes were naturally found to degrade certain compounds, regarded as pollutants in the environment, but the main problem was that these naturally occurring microbes

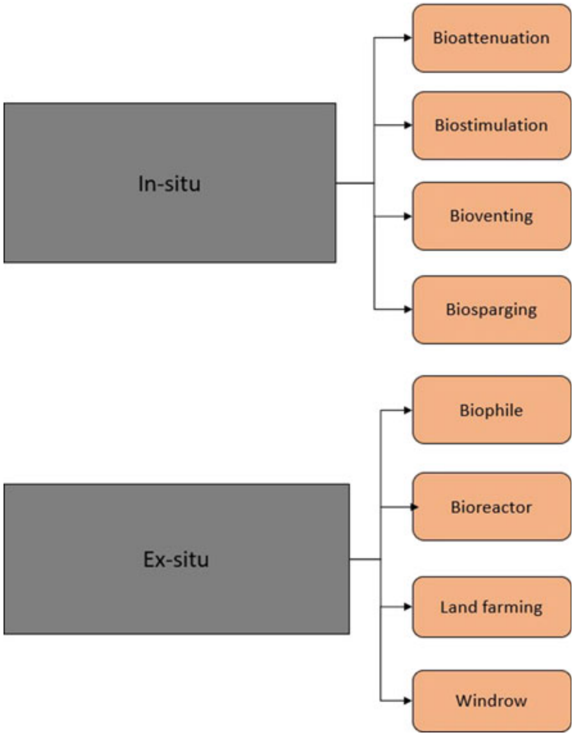

Fig. 1 The above figure depicts the various in-situ and ex-situ bioremediation techniques carried out by microbes and conventional instruments (Dixit and Malaviya 2015; Chibuike and Obiora 2014; Bhattacharya et al. 2015; Burgess et al. 2001)

provided a prolonged rate of degradation, which meant a very time-consuming process. Some microbes could not survive in a variety of extreme environmental conditions. That is when genetic engineering came into play, where we could manipulate an organism's genome to Impart additional abilities into it. This included manipulating the organism's critical genes involved in bioremediation or modifying the metabolic pathways. Such developed GMOs were successfully operated in the field to achieve the destruction of organic and inorganic waste to some extent. Genetic engineering helps unravel an organism's genome through which we can identify the physical and chemical mechanisms involved in transforming pathways for specific compounds. This knowledge can help us design a synthetic organism of our own from scratch.

Some of the principles of genetic engineering involved in the production of genetically modified organisms include.

- Bacterial engineering- Includes the optimisation of biocatalyst involved in the metabolic processes in a bacterial strain or the involvement of protein engineering to enhance particular enzyme activity.
- Genetically engineered fungi for Mycoremediation- Recent advances in molecular biology have made it possible to engineer a genetically advanced and improved

version of the original fungal strain for enhanced mycoremediation. Mycoremediation simply means the employment of fungal strains for remediation of toxic products from the environment. They were successful in the removal of harmful PCBs (Polychlorinated biphenyls) from the environment.

- Genetically engineered plants for phytoremediation- Even though the genetically modified microorganism offers enhanced activity over their naturally occurring counterparts, they might not perform as efficiently as under lab conditions. Biodegradation might not even happen since some of these engineered microorganisms have a poor survival rate in the contaminated soil. Hence, phytoremediation was considered the next best option since it was easier to manage as the autotrophs require very little nutrient input to work. Plants also offered protection against water and wind erosion; hence contaminants did not spread enough. They are renewable and hence an ideal option after microorganisms. Genetic engineering is used to overexpress the genes involved in metabolism, uptake or transport of specific pollutants since usual; unmodified plants are very slow towards biodegradation.

In this paper, we will discuss the coupling of the simple natural processes carried out by the microorganism with the modern-day principles of genetic engineering to develop a synthetic genetically modified microorganism for bioremediation of the environment, occurring safely without contributing additionally to polluting the environment.

2 Type of Contaminants Found in Everyday Waste Products

There are numerous amounts of contaminants and waste material being dumped into the environment by large-scale industries every day. Some of these contaminants include the widely used herbicides and pesticides to control crop damage by farmers and the agriculture industries (Dixit and Malaviya 2015). In doing so, the use of harmful chemicals has increased environmental pollution, leading to disastrous effects such as loss of soil viability, poor crop production and quality, and loss of flora and fauna (Dixit and Malaviya 2015; Chibuike and Obiora 2014). The different types of contaminants found in the environment include.

- Heavy metals- These are the most harmful contaminants found in the environment. Heavy metals accumulate in the environment, primarily because of burning fossil fuels, everyday mining, and extensive chemicals in the form of pesticides for crop growth. A microbial process cannot degrade heavy metals since they are not part of the microbial metabolism, so instead, these metals are transformed from their current oxygen state to the other. Mechanisms followed by microbes to cycle metals are biosorption, bioleaching, biomineralisation and enzyme-catalysed transformation. The most common mechanism involves the methylation of the heavy metals, making them volatile and easy to oxidise now. Heavy metals can accumulate in our body via the food we eat, initially grown in the soil with toxic metal ions. Such accumulation can lead to health problems such as destroying vital organs and glands such as the heart, brain, kidney and even liver (Chibuike and Obiora 2014).

- PVC- PVC and its related products, generated during the regularly conducted industrial processes, have contributed immensely to polluting the environment. Such a massive surge has been since there has been an increase in the demand for plastics for daily usage, where PVC finds its practical use in making drainage pipes, water pipes, window frames and even medical devices. Chlorine is the basic building block of PVC, which is a very toxic gas when exposed. The burning of PVC and its related products releases chlorine and dioxins into the environment. Chlorine is said to destroy the ozone layer of the atmosphere. It is the most concerning the reason for global warming, whereas dioxins are created wherever chlorine or chlorine-based compounds are formed, and it is one of the most toxic chemicals to be produced, contributing to health hazards such as cancer, mental health problems (is a neurotoxin), damage to the immune system and even reproductive problems (Chibuike and Obiora 2014; Avrilescu 2005).
- Radioactive metals- These are metals with an unstable nucleus emitting harmful radiation in the environment, which can even penetrate the human skin, damage the underlying organs, and cause-specific life-threatening mutations. Such emission includes alpha, beta and gamma rays. Hence, such metals are highly toxic to human health, causing various health hazards (Karaouzas et al. 2021). One of the prime Examples of radioactive poisoning in human history is the bombing of Hiroshima in 1945, where an estimated 90,000 to 120,000 people were killed, and many are still suffering the effects to date! (Singh 2017).
- Herbicides- They primarily contaminate the groundwater and are toxic to the plants. Damage to the plant species by herbicides is very selective; for example, it has been found that some herbicides are said to seep into the soil and inhibit photosynthesis; 2,4-d herbicide is said to kill the broadleaved plants but has minimal effect on the grasses. Increased use of 2,4-D herbicides can make broadleaved plants extinct and increase grasses. The most lethal herbicide now banned or being phased out in the European Union is Paraquat, which has increased the risk of neurological diseases in the human body, such as Alzheimer's and Parkinson's disease. Other harmful herbicides include Methomyl, 1,3-Dichloropropene, and Pyrethroid insecticides which are either banned or used and have been limited (Singh 2017).
- Petroleum Hydrocarbons- It is the most toxic contaminant for the existing aquatic life. Industries dumping their wastes, transportation or pipeline failure can lead to the accumulation of crude petroleum and its related products in the water, damaging the water quality and making it unfit for further use. Consumption of such untreated water can lead to health problems such as headaches, dizziness, nausea, nerve disorders such as the development of seizures, irregular heart rhythms, and the immune system is also drastically affected (Singh 2017; Guo et al. 2019).

3 Role of Microbes in Bioremediation

Microbes, as the name suggests, are microscopic living organisms with a unique metabolic system to carry out their day-to-day activities and play an essential role as part of nature's food chain. Their habitats range from soil on the earth to the air we breathe and are even present in the water we drink (Singh 2017). Some microbes also habitat the human body, such as Staphylococcus epidermidis (which lives on the skin and inside the nose) and obligate anaerobes (found in the abdomen and colon), to name a few. Some microbes can be single-celled such as bacteria (Rigét et al. 2010; Yi et al. 2011; Skerker 2008).

Previously used conventional techniques for bioremediation were soon becoming obsolete for the sole reason of being inefficient and uneconomic (Yi et al. 2011; Skerker 2008). One of these methods included the addition of a reagent to an aqueous solution to remove heavy metals. Such reagents increase the pH of that solution, which further converts the heavy metals into insoluble ones, which have to be next removed via precipitation (Skerker 2008; Gavrilescu et al. 2019). The problem with such methods was that the aggregates formed in the end were difficult to remove and expose. In a way, heavy metals removed from the aqueous solution were now disposed into the land as solid waste, so such methods proved unethical and uneconomical.

On the other hand, after years of rigorous research and recent breakthroughs in the field of life sciences, particularly the combined field of microbiology and genetic engineering, scientists found a possible and efficient way of manipulating some of the naturally found microbes to save the environment. In the simple process of bioremediation, microbes cycle the environmental polluting toxic organic and chemical waste, through their metabolic pathways, as their source of food, for generating energy to carry out daily tasks for their survival (Yi et al. 2011; Skerker 2008; Gavrilescu et al. 2019; Smaranda et al. 2016). In this way, with the employment of microbes in the areas of possible organic contamination, we can degrade these wastes into less or no toxic forms, potentially cleaning the environment of such harmful contaminants posing a threat to the life of humans as well as the environment.

Microorganisms have come up as a critical alternative to cleaning those polluted areas, which are out of bounds for regular human interaction for that very same purpose because their most important feature includes their survival in a variety of extreme conditions of the environment, which makes them suitable for cleaning the environment (Skerker 2008).

There are two factors in the ecosystem that work together to control the rate of degradation by microbes, which are-

- Abiotic components- This includes all the non-living components of the biosphere that affect the growth and survival of living things, such as temperature, pH, humidity, sunlight, oxygen and minerals (including the heavy metal waste in the soil) (Smaranda et al. 2016).
- Biotic components- These include all the living being present in the ecosystem, which is carefully sub-divided to show their significance and survival of the

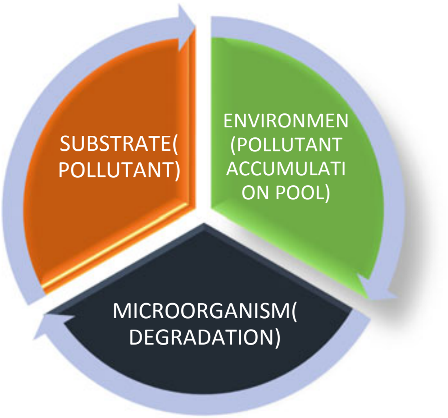

Fig. 2 Above figure shows the simple cyclic relation between the pollutant, the environment and the microorganism for bioremediation (Karaouzas et al. 2021; Singh 2017)

ecosystem as a whole. These components include- producers, consumers, decomposers and detritivores (which include our microbes) (Smaranda et al. 2016) (Fig. 2).

4 Genetic Engineering of Microorganisms

The advantage it gives over the conventional methods is the ability to manipulate and edit the genome of a microorganism in such a way to produce a modified version of the original species, with much better metabolic rates, controlled growth, and showing resistance to extreme temperatures (Kaniecki et al. 2018). Microorganisms are engineered and designed according to the need to show maximum catalytic ability towards bioremediation, with a minimum cell mass production, so they do not hamper the working conditions of any other plant or its related biological process (Kaniecki et al. 2018; El Fantroussi and Agathos 2005). They are thus supplemented with additional genetic properties by insertion of parts of the genome from various other organisms showing capabilities for the biodegradation of specific pollutants, which the naturally occurring microorganisms cannot degrade or, even if they can, is not efficient enough to be applied on a large scale (Kaniecki et al. 2018; El Fantroussi and Agathos 2005; Madhavi and Mohini 2012). The combination of different life sciences disciplines to create a hybrid microorganism using various genes of different species owing to different metabolic abilities, influencing the biodegradation and bioprocessing of pollutants produced daily, which cannot be easily degraded or removed by the conventional methods (Boopathy 2000).

5 Steps Followed During Gene Editing of Microorganisms

Creating a genetically modified organism to clean up the environment depends on many factors critical to its development (Paranthaman and Karthikeyan 2015). Selecting strains which show specific responses towards the presence of a particular pollutant depends on the-

- Pollutant type and its composition.
- Species to which the microorganism belongs, showing response to the particular pollutant.
- Gene editing is done keeping in mind the physiochemical conditions of the environment harbouring the harmful pollutants to ensure the species' survival in bioremediation.

Now, keeping the above factors in mind, we design our microbes based on four basic approaches, which ensure not only a safe, efficient bioremediation process but also maintain a minimum microbial growth altogether, thereby minimising any further counter pollution by our own employed Genetically modified microorganism (GMM) (Paranthaman and Karthikeyan 2015; Jaysankar et al. 2008; Shah et al. 2013; Kukumar and Nirmala 2016). These three basic approaches which modify the organism's genome and capabilities include.

(1) Modification of enzyme specificity and affinity- The enzymes present in some of the specific strains of microorganisms are said to show biodegradable properties towards a wide variety of pollutants. These pollutants are recognised as the substrate by the different microbial enzymes, which at optimum temperature, pH and ionic concentration, help transform these harmful pollutants into harmless by-products of their metabolism. Examples of the enzymes include- cytochrome P450s, laccases (benzenediol oxidoreductases), hydrolases, dehalogenases, proteases and lipases extracted from microbial strains, which are naturally involved in bioremediation (Jaysankar et al. 2008; Shah et al. 2013; Kukumar and Nirmala 2016). These microbial strains include P*seudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium.* Some pollutants degraded by the enzymes produced by above mentioned microbial strains include- polymers, PAH, dyes, detergents and pesticides (Kehinde and Isaac 2016). Now such enzymes offer biodegradable properties towards selected pollutants but have a relatively slow conversion rate, coupled with an increased microbial biomass production (Kehinde and Isaac 2016; Phulpoto et al. 2016). Hence, to tackle this problem, genetic engineering principles come in handy. Gene editing tools are applied for the modification of enzymes. For example, we can chemically modify lipases by attaching aldehydes, polyethene glycols and imido-esters, thereby altering their conformation and hydrophobicity (Kehinde and Isaac 2016; Phulpoto et al. 2016). These alterations bring with them enhanced microbial activity, stability and selectivity. Hence, we can easily manipulate to produce a synthetic enzyme from a suitable microbial strain

to impart capabilities of efficient degradation of different types of pollutants that are engineered depending on their operational environmental conditions.

(2) Metabolic Pathway construction and regulation- Microorganisms use their complex metabolic pathways to cycle the elements from the external environment into their system to obtain energy and carry out their everyday activities. Now, with the advancement of science and technology, we can design, manipulate and regulate the metabolic pathway of a suitable microorganism, such that it can use the pollutants from the external environment as its nitrogen and carbon source for obtaining energy assisting in bioremediation (Kukumar and Nirmala 2016; Phulpoto et al. 2016; Hesham et al. 2012; Aranda et al. 2010). The main aim for the development of a genetically modified organism was to introduce such modifications so that the modified organism can exhibit a desired catabolic pathway, depending on selectivity and specificity, derived from a combination of determinants having a similar pathway, complementary to the pathway segments required to be integrated into the GMM. For example- the degradation of 2-nitrobenzoate is done by a new metabolic pathway by the bacterial strain *Arthrobacter sp.* SPG. 2-nitrobenzoate is a nitro-aromatic compound considered a harmful pollutant capable of causing high environmental toxicity and a significant threat since they harbour mutagenic activity (Aranda et al. 2010; Sarang et al. 2013). Hence, when strains of *Arthrobacter sp.* SPG was isolated and simulated in sterile and non-sterile environments, and they showed promising results of degrading more than 90% of 2-nitrobenzoate within 10–12 days (Kadirvelu et al. 2002). The metabolites produced on degradation were Salicylate and catechol produced from the native oxidative pathway, which is a first and only followed by the SPG strain. The native pathway followed for the degradation of nitroaromatic compounds was reductive, and the metabolites produced at the end of the reaction were also different from those produced by the SPG strain, i.e., the reductive pathway produced pyruvate and acetaldehyde as end-products (Riggle and Kumamoto 2000). Henceforth, due to the discovery of a more efficient pathway for degradation of nitroaromatic compounds, scientists started taking a keen interest in extracting the genes responsible for taking this new metabolic pathway for manipulation and designing of a genetically modified organism showing the combined capabilities of efficient degradation provided by the SPG strain of Arthrobacter. The surviving capabilities are derived from a different strain to create an organism capable of surviving in extreme environmental conditions, showing minimum unwanted microbial biomass enlargement and efficient working capacity (Riggle and Kumamoto 2000; Fomina and Gadd 2014; Mulligan et al. 2001). Another example of a modified pathway for degradation of 2-nitrobenzoate (2-NBA) was followed by the bacterial strain KU-7 belonging to *Pseudomonas fluorescens.* It was discovered that these strains could efficiently accumulate 2-NBA from polluted areas, serving as the sole source of carbon, nitrogen and energy sources for this bacterial strain (Texier et al. 1993; Perpetuo et al. 2011). KU-7 followed the reductive pathway for the degradation of 2-NBA, producing intermediate 3-hydroxyanthranalite, which was different from the intermediates anthranilate or catechol produced from

Table 1 Above table shows the list of microorganisms whose genes are modified by gene editing tools for bioremediation

Pollutant	Degradation by microorganism	Modification	References
Toluene/benzoate	*P. putida KT2442*	Metabolic Pathway	Panke et al. (1998)
Polychlorinated biphenyl (PCB)	*Pseudomonas sp. LB400*	Specificity for the substrate	Panke et al. (1998)
Mono/dichlorobenzoats	*Pseudomonas sp. B13*	Metabolic Pathway	Panke et al. (1998)
Trichloroethylene, toluene	*E. coli FM5/Pky287*	Regulating Pathway	Panke et al. (1998)
4-ethyl benzoate	*P. putida*	Metabolic Pathway	Panke et al. (1998)
PCB, benzene, toluene	*E. coli JM109 (Pshf1003)*	Specificity for the substrate	Panke et al. (1998)
Chloro-, methyl benzoate	*Pseudomonas sp. FR1*	Metabolic Pathway	Panke et al. (1998)

the reductive-degradative pathway followed by other bacteria. Hence, this indicated that an alternate pathway was followed by the KU-7 cells, which was different and more efficient, requiring less energy expenditure, i.e., oxidation of only 2 NADPH was required for completing the reaction. The intermediate 3-hydroxyanthranalite produced upon degradation was also less harmful and less toxic and thus could be easily removed via physical methods (Jaysankar et al. 2008; Shah et al. 2013; Texier et al. 1993; Perpetuo et al. 2011).

(3) Bio-affinity biosensor- Applications of such genetically modified biosensors include chemical sensing for detecting the specific chemical in a suspected environment and reducing or degrading the toxic products on its detection. Biosensors are also used to analyse various samples' chemicals by modifying the biosensors showing different selectivity traits. Some of the widely used genetically modified biosensors used for identifying and analysing specific pollutants from the environment include- *R. metropolis* used for successfully identifying 2,4-Dinitrophenol and *Staphylococcus* plasmid pI258 for the identification of arsenic in the polluted pool of water (Gavrilescu et al. 2019; Madhavi and Mohini 2012; Jaysankar et al. 2008) (Table 1).

6 Gene Editing Tools Employed for Waste Management

With the growing advances in biological science and engineering principles, it has become possible to modify and produce our microbes suited for safely disposing of everyday harmful waste via bioremediation. Various gene editing tools have been employed and are widely used for generating microorganisms specifically for managing waste, with the added advantage of displaying maximum catalytic ability with minimum cell mass, thereby not hampering the working of any other plant or its related biological process (Boopathy 2000; Jaysankar et al. 2008; Shah et al. 2013). Each gene editing tool below includes a nuclease that can be engineered and customised according to our need to recognise, bind and cleave the specific sequences in the foreign genome.

6.1 CRISPR/Cas 9

CRISPR/Cas 9 protein, also known as the (CLUSTERED REGULARLY INTERSPACED PALINDROMIC REPEATS), is a specialised molecular scissor discovered and developed by Nobel prize-winning scientists Emmanuelle Charpentier and Jennifer Doudna. This protein has a precise targeting mechanism, which has been shown to edit any type of genome of any given size. Due to its efficient and versatile gene editing capabilities, it has shown promising results in the field of life sciences, helping in producing drugs, thereby providing a much better therapeutic approach towards the treatment of many neurological and immunological diseases such as SCID (Severe combined immunodeficiency disorder), Alzheimer's disease (Jaysankar et al. 2008; Shah et al. 2013; Kukumar and Nirmala 2016).

Cas9 is a DNA endonuclease, usually found in bacteria Streptococcus pyogenes, which is guided by a particular type of RNA molecule called the gRNA. CRISPR, on the other hand, is a base repeat of about 30–40 bp, separated by a spacer sequence that is said to complement the sequence of the genome being edited, i.e., the foreign DNA sequences (Jaysankar et al. 2008; Shah et al. 2013; Kukumar and Nirmala 2016).

Nowadays, CRISPR is being applied to increase and modify the genetic metabolic pathway of that microorganism already known for degrading environmental wastes such as hydrocarbons, plastics, Pesticides, heavy metals etc. The edited microorganisms formed are called Genetically Modified organisms (GMO) (Perpetuo et al. 2011; Roane et al. 2001).

The CRISPR/Cas 9 protein consists of two components-

- a single guide RNA (gRNA).
- Cas 9 protein.

Both the components work in close sync to edit the target genome. Before CRISPR/Cas 9 protein is delivered to the patient's body via a suitably designed vector, the gene sequences targeted by the protein must be identified first. CRISPR is highly suited for editing Archeal and bacterial genomes (El Fantroussi and Agathos 2005; Madhavi and Mohini 2012; Boopathy 2000).

Mechanism of Action of CRISPR/Cas 9

We first identify and select the gene of interest by DNA sequencing methods. For this, we use high-end gene sequencing tools such as microarray and High throughput sequencing (HTS) to get the gene's correct, accurate base sequence to be edited. Next, we transcribe the CRISPR repeats giving us the crRNA (Fantroussi and Agathos 2005; Madhavi and Mohini 2012; Boopathy 2000). The gRNA (or the guide RNA) is next obtained by combining the transcribed crRNA and Cas9 protein, giving us the Ribonucleoprotein called the crRNP or the gRNA. The RNA controls the target specificity since it contains a 20-base long protospacer complementary to the target sequence of DNA.

The gRNA works by identifying the specifically targeted sequence of DNA in the foreign genome and thus guiding the Cas9 protein to come and bind to the targeted site on the DNA, introducing double-stranded breaks (DSB) (Boopathy 2000). Now, these breaks are repaired naturally by the host's system in two ways-

- error-prone nonhomologous end joining (NHEJ)- causes the Knockout of gene function.
- High-fidelity homology-directed repair (HDR)- causes the Knockdown of gene expression.

Before these repairs are done, we can efficiently insert another gene or even delete our target sequence according to the required specific modification. Hence, after insertion/deletion, the nick in the ds DNA finally gets sealed through repairing mentioned above pathways. Prokaryotes usually apply the HDR method to repair their dsDNA breaks, thereby creating precise modifications in their genome (Boopathy 2000).

Let us, for example, there is a bacterial strain *Bacillus cereus A*, which has been found to have great application for the bioremediation of diesel oil. Its optimum pH range was 6.5, and the optimum working temperature was found to be 45 °C. Hence, this bacterial strain having a very narrow working range of pH and temperature made it difficult to apply to areas of environment with extremely low pH or low-temperature ranges. Hence, to make this strain suitable to work in various pH and temperature ranges, we would use the CRISPR/Cas9 technology to edit its genome. We do this by identifying the gene we have to edit with DNA sequencing tools; after that, we design our gRNA having the protospacer sequence complementary to the gene of interest. Then we will insert another DNA sequence at the targeted site, which was obtained from a bacterial strain of its related family member, such that the new genetically modified strain of *Bacillus cereus A* obtained can work in a wide range of pH and temperature changes.

6.2 TALEN (Find an Advantage Over CRISPR)

TALENs is a naturally occurring protein which has been found to hold powerful gene editing capabilities. It is originally derived from the plant pathogenic bacteria, *Xanthomonas spp,* which can efficiently alter the gene expression in the host plant cell it attaches. TALEN is an acronym which stands for Transcription activator-like effector nucleases. Unlike CRISPR/Cas9, which has protein and RNA components, TALENS are protein based (Kadirvelu et al. 2002); Riggle and Kumamoto 2000). They are chimeric protein molecules consisting of DNA-binding modules called TAL. TAL comprises 33–35 amino acids, differing only at the 12 and 13 positions, respectively, representing the DNA contact sites called Repeat variable residues (DVDs) (Riggle and Kumamoto 2000; Fomina and Gadd 2014). DVDs dictate the DNA binding specificity of each TALEN molecule; hence engineering the TAL repeats harbouring the DVDs enable us to introduce the necessary modification required for gene alteration, thereby giving us the flexibility to target different sequence. It has the sensitivity of recognising a single base pair of the DNA, at a time, in the host cell. Hence, TALENs is a five times more efficient gene editing tool than CRISPR/Cas9. It is made up of two functional domains-

- A DNA-recognition Transcription activator-like effector (TAL) domain
- A nuclease domain

TALENs are created by the fusion between DNA-recognition Transcription activator-like effector (TAL) with DNA cleaving domains, i.e., the nuclease domain. TAL effectors bind to the DNA sequences, activate its gene expression, and even alter it. TAL effectors having the DVDs are engineered in such a way to show high affinity to a predetermined target DNA sequence in the host (Riggle and Kumamoto 2000; Fomina and Gadd 2014) (Table 2).

Table 2 The table, as mentioned earlier, describes the different combinations of DVDs in the TAL of TALENS and its target nucleotide in a DNA sequence

Target nucleotide	RVD (Repeat variable Di-residues)	References
Adenine	Asparagine-isoleucine	Boopathy (2000), Jaysankar et al. (2008), Fomina and Gadd (2014)
Guanine	Asparagine-Asparagine	Boopathy (2000), Jaysankar et al. (2008), Fomina and Gadd (2014)
Cytosine	Histidine-aspartate	Boopathy (2000), Jaysankar et al. (2008), Fomina and Gadd (2014)
Thymine	Asparagine-glycine	Boopathy (2000), Jaysankar et al. (2008), Fomina and Gadd (2014)

Mechanism of Action of TALENs

The mechanism of action of TALENs for editing a genome is similar to the CRISPR/cas9 method, where a DBS (Double-stranded break) is introduced in the genome, and the cells respond to the break by repairing it via the two previously mentioned mechanisms of DNA repair, i.e., error-prone nonhomologous end joining (NHEJ) and high-fidelity homology-directed repair (HDR). When the TALENs introduced the break at the targeted site, the engineered TAL part having the right combination of DVDs targets the nucleotide and modifies the host cell's genome. Now, the DBS breaks are ligated via any of the two repair mechanisms, and we finally get our genetically modified organism (GMO) (Riggle and Kumamoto 2000; Fomina and Gadd 2014).

6.3 ZFNs

ZFNs are a much more commonly used endonuclease for gene editing. It is a synthetic protein, which means they are genetically engineered in a lab. Hence they can only target and manipulate the specific targeted part of the organism's genome, which needs editing, thereby giving us accurate results and minimum chance of error. Hence, ZFNs are "Highly Specific Genomic Scissors" (Smaranda et al. 2016; Kaniecki et al. 2018; Mulligan et al. 2001); Mu. The construction of ZFNs depends on the species of the organism whose genome is being edited and the type of modification that needs to be done in its genome, i.e., insertion or deletion (Smaranda et al. 2016; Kaniecki et al. 2018).

ZFNs stand for Zinc Finger Nucleases and are also classified as DNA binding proteins, similar to TALENs. They are made up of two components which are-

- DNA binding domain is made of two modules (Zinc Finger Protein).
- DNA cleaving endonuclease domain, obtained from a Bacteria.

The DNA binding domain or the Zinc finger domains are the part of ZFNs engineered in the lab to specify the DNA sequence it needs to bind in the host organism. The zinc finger domain is a minimal protein motif with multiple finger-like projections in their 3-D structure, making tandem contacts with their targeted DNA sequences and binding zinc in its structure. This domain consists of two modules, where each module recognises a unique hexamer of a 6 bp long DNA sequence in the host cell. Both these modules, when fused as a single domain in the ZFNs, form a finger-like structure called the Zinc Finger Protein (ZFP), and each ZFP shows a unique specificity of up to 24 bp long DNA sequences in the genome (Smaranda et al. 2016; Kaniecki et al. 2018).

The DNA cleaving endonuclease usually fuses with the ZFP while constructing ZFNs is *the Folk endonuclease enzyme*. *Folk endonuclease* enzyme is obtained via recombinant DNA technology, where the restriction endonuclease gene from the bacterial strain *Flavobacterium okeanokoites* IFO 12,536 is first introduced into the host cell, where the gene expresses itself completely under suitable conditions. Then the endonuclease is isolated from the host cell, purified and finally fused with the ZFP to give the ZFNs gene editing synthetic protein molecule (Kaniecki et al. 2018).

The *Fo*lk domain needs to dimerise to effectively introduce a Double Stranded Break (DBS) in the host genome while gene editing. The dimerisation of the *Fo*lk domain helps increase the specificity factor of the ZFNs, thereby reducing off-target attacks on the host's genome.

Mechanism of Action of ZFNs

ZFNs also work via the exact general mechanism as all the other gene editing tools, i.e., by the introduction of the Double-Stranded Breaks (DBS) in the structure of the host's genome, which enables us to either insert our gene of interest at that specific position or delete the specific targeted site, in order to get the modified version of the original genome with enhanced capabilities and survival rates. The introduction of a DBS break in the genomic DNA stimulates the natural DNA repair mechanism of the cell, which includes- the nonhomologous end joining (NHEJ) followed in all the phases of the cell cycle or the high-fidelity homology-directed repair (HDR) followed exclusively during the G2 and the S phase of the cell cycle (Boopathy 2000; Mulligan et al. 2001). Both of these repair-mechanism work efficiently to accurately seal the single-stranded overhangs at the end of DBS breaks, thereby completing the process of gene editing. We must ensure that the repair mechanism is completed accurately because an imprecise repair can also lead to the loss of nucleotides, including our gene of interest (Fig. 3).

7 Types of Genetically Modified Microorganisms (GMM) and Techniques they Apply for Bioremediation

With the previously mentioned gene editing tools, scientists and researchers have created a wide array of genetically modified organisms and developed novel techniques targeted explicitly for bioremediation and effective waste management, with minimum side effects or addition of any unwanted pollutant in the environment (Kadirvelu et al. 2002; Mulligan et al. 2001). The primary reason for a shift towards adaptation of microorganism and biotechnology principles to carry out waste management was expensive alternative and conventional strategies for remediation. They showed destructive side-effects since these techniques involved the use of many harmful chemicals which, when accumulated in the environment, contributed additionally to polluting the environment, i.e., conventional methods were less efficient and not ideal (Mulligan et al. 2001). Hence, with the discovery of the possibility for quickly editing any genome, more complex models on the metabolism and working of microorganisms were being created, with certain modifications. These changes

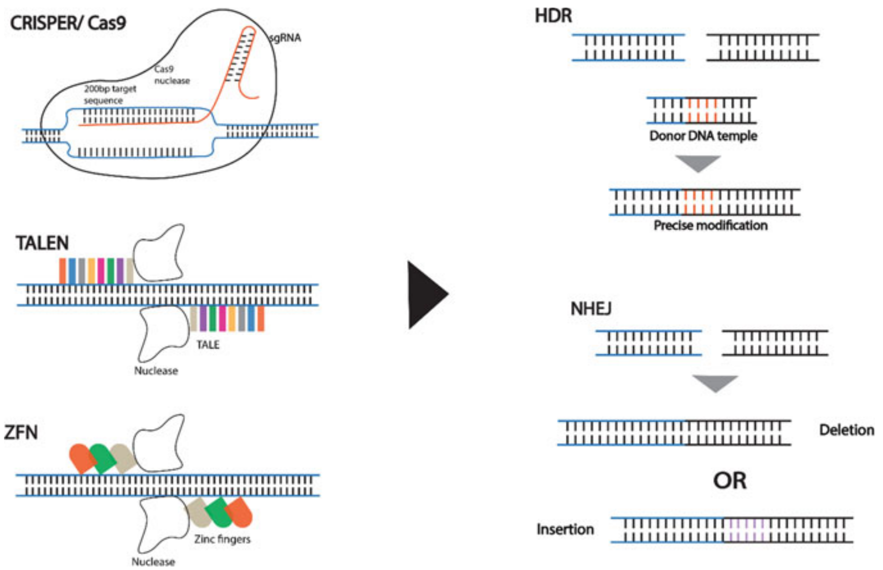

Fig. 3 Above figure depicts the three genome editing nucleases (ZFNs, TALENs and CRISPR/Cas9) that modify the genome by inducing DSBs (Double-stranded breaks) at the targeted specific site requiring modification. Now, these DSBs are repaired by the two cell repair pathways: NHEJ and HDR. DSB repair by the NHEJ pathway leads to indels (Insertion or Deletion) in the genome. Hence, when two DSBs in the genome carry out an insertion, deletion of the intervening sequences is created, leading to the repair by the NHEJ pathway. In the HDR pathway, a donor DNA template is required. When there is a donor repaired HDR DNA template, the gene responsible for the HDR pathway induces a DSB at the desired locus (Kaniecki et al. 2018)

were introduced to enhance the uptake rate of contaminants, increase the degradation rate of certain pollutants, the survival rate in non-ideal conditions, and reduce the oxygen demand of microorganisms, which made them an ideal choice for treatment of the daily generated waste products. Contaminated soil, water and even air could be purified using genetically modified organisms (GMOs). There are different types of genetically modified organisms belonging to different species of organisms, used for detecting, degrading and cyclising pollutants (Dixit and Malaviya 2015; Avrilescu 2005; Gavrilescu et al. 2019).

7.1 *Genetically Modified Biosensors for Pollutant Detection*

Biosensors have been used by biologists for a very long time for tagging and identifying certain chemicals, biological molecules and even a cell. Their application has expanded to a wide array of industrial fields, finding their main application in the food industry, where they help in quantifying the presence of alcohol, carbohydrates and acids during the ongoing fermentation processes, as well as the pharmaceutical industry, where they help in indicating drug production or presence of any unwanted molecule of chemical or biological origin (Kaniecki et al. 2018; El Fantroussi and Agathos 2005; Madhavi and Mohini 2012). Recent studies have expanded their use in marine applications, where the level of eutrophication of the water bodies could be detected by applying nitrite and nitrate sensors.

A biosensor is a device applied for measurement of any reaction of either biological or chemical origin, which works by the generation of signals, having the intensity proportional to the concentration of the analyte in the reaction, where the analyte in the reaction mixture is the substance which we are interested in for detection (Texier et al. 1993; Roane et al. 2001). There are three different types of biosensors, based on their target and mechanism-

- A biocatalytic group comprises enzymes.
- Bio-affinity group, including antibodies and nucleic acids.
- Microbe-based containing microorganisms.

We will be more focused on the Microbe-based biosensors, which are exclusively used for detecting certain pollutants for bioremediation (Texier et al. 1993; Roane et al. 2001). Using biosensors, an environmentalist can analyse and assess the type of pollutant present in the given environment and design and engineer any suitable microorganism to degrade that pollutant (Mejáre and Bülow 2001; Wasilkowski et al. 2012).

Usually, the original microbes currently in use as biosensors show some limitations when working under certain environmental conditions. They either do not remain viable long enough to show accurate signals or degrade due to unfavourable environmental conditions. Hence, this is where genetic engineering comes in handy (Mejáre and Bülow 2001; Wasilkowski et al. 2012). We can use any of the genes mentioned above editing tools to modify the genome of the microbes, which are already being used as biosensors, to impart capabilities to them, such that they can not only survive but also perform efficiently under a wide variety of environmental conditions, i.e., in extreme pH, temperature, acidic or essential environment.

In order to create a genetically modified organism, we usually use the CRISPR/Cas9 technology due to its advantage over other gene editing tools being cheap, simple and easy to apply by researchers as it can evaluate genetic interaction and their related genotypic and phenotypic expression when replaced by another gene of interest. Gene modification for creating a biosensor is done by inserting an "indicator gene" into the organism's genome (Mulligan et al. 2001; Mejáre and Bülow 2001; Wasilkowski et al. 2012; Roane et al. 2001). The organisms usually selected to serve as biosensors are bacterium since it has sensitivity detection of <0.2 mM. Although, some strains of yeast, such as Yeast SPT1 and SPT2, can also be used as biosensors. The presence of a pollutant and the environmental conditions make it suitable for the indicator gene to be expressed, and the resulting protein represents the detectible signal analysed and assessed.

Indicator Genes Involved in Genetic Modification of Biosensors

Luciferase is the most frequently used indicator gene for creating a genetically modified microorganism. It belongs to the class of oxidative enzymes isolated from those species of animal which produce bioluminescence. Hence, when luciferase is inserted in the genome of a selected microorganism, it works with the promoter gene that activates it in the presence of a specific pollutant, thereby generating a signal and indicating its presence.

The second indicator gene for pollutant detection is a green fluorescent protein (GFP) (Paranthaman and Karthikeyan 2015; Jaysankar et al. 2008). This protein is naturally extracted from jellyfish, a marine life animal. GFP expresses a green fluorescence signal when exposed to the ray of U-V light, which ionises it. It is made of 238 amino acids, out of which amino acids present at positions 65–67 (i.e. Ser–dehydroTyr–Gly) are responsible for reacting and producing visible green fluorescence under the U-V light. The gene expressing this protein can be inserted in several microorganisms, alongside many different promoter sequences, which activates this gene in the presence of different specific contaminants (Paranthaman and Karthikeyan 2015; Jaysankar et al. 2008).

The third type of indicator gene inserted to modify the microorganism's genome for detection genetically is the lac-z gene. This is the most widely used indicator/reporter gene for microbial and animal modelling systems (Paranthaman and Karthikeyan 2015; Jaysankar et al. 2008). This gene is also inserted alongside different 5' regulatory elements of many genes, which are known to express when exposed to different types of pollutants, specific to their activation (Paranthaman and Karthikeyan 2015; Jaysankar et al. 2008).

Example of Biosensors

Some of the widely used biosensors for pollutant detection include.

(1) *Thauera butanivorans*- This bacterium is used as a biosensor for detecting 1-butanol which poses a significant threat to aquatic life. 1-butanol is said to be easily biodegraded and can lead to oxygen depletion in the water bodies, ultimately harming aquatic life. This biosensor is created in E coli using the σ factor and promoter sequence present in this species. The promoter sequence (P_{BMO}) was attached at the 5' end of the tetracycline resistance fusion gene called *TETA-GFP*, a fusion of GFP protein and tet^r gene. The σ factor (*BMOR*), on the other hand, was expressed under the influence of the BMOR promoter (P_{BMOR}) (Jaysankar et al. 2008). Now, both the P_{BMO} and P_{BMOR} promoters are activated upon the binding of 1-butanol. Under increased concentrations of butanol, the σ factor (*BMOR*) caused an increase in the expression of TETA-GFP, causing an amplification in the signal generated by the GFP. This amplified signal is recorded to sense the quantity and presence of butanol in the environment (Jaysankar et al. 2008).

(2) *R. erthropolis*- This strain belongs to the class of gram-positive bacteria belonging to the genus Rhodococcus. Environmentalists and industrialists are widely using this strain to successfully identify 2,4-Dinitrophenol, which is said to pose a harmful contaminant. *R. erthropolis* is a whole-cell biosensor which feeds on the substrate 2,4-Dinitrophenol, as its sole nitrogen source. The microorganism response to the presence of that pollutant is in the form of increased biomass production (Shah et al. 2013). However, this strain for use as a biosensor has become almost obsolete. However, the genes responsible for identifying and detecting 2,4-Dinitrophenol, i.e., *npdG* and *npdI* genes, are genetically inserted into another host to create a genetically modified organism to attain a much greater sensitivity and efficiency towards detection and its degradation. An example of such GEM includes B (Shah et al. 2013). cepacia BRI6001L. 2,4-Dinitrophenol is a harmful air pollutant, which can lead to skin lesions on human skin, Cardiovascular disorders, and even cause chronic effects on the Central Nervous System (CNS) (Shah et al. 2013) (Table 3).

Table 3 An overview of all the microorganisms potentially found as useful biosensors with target detection in the environment

Target for detection	Microorganism	Limit of detection	References
Bioavailable Copper	*P. fluorescens DF57 with a Tn5 lux AB promoter probe transposon*	0.3 ppm	Park et al. (2013)
Bioavailable Naphthalene	*P. putida with NAH7m plasmid and an insertional gene fusion between sal promoter and the lux AB genes*	50–500 nM	Park et al. (2013)
Ni^{+} and Co^{+2}	*Ralstonia eutropha AE2515*	0.1 μM for Ni + ^{2} and 9 μM for Co^{+2}	Park et al. (2013)
Bioavailable Phosphorus	*Synechococcus PCC 7942 reporter strain*	0.03 μM	Park et al. (2013)
Halogenated organic acids	*Recombinant E.coli had DL-2-haloacid dehalogenase encoding the gene and lux CDABE genes*	> 100 mg/l	Park et al. (2013)
Tributyltin	*Bioluminescent recombinant E.colir:: lux AB strain*	0.02 μM in synthetic glucose medium and 1.5 μM	Park et al. (2013)
Hg^{+2}	*E.coli HMS174 was harbouring mer-lux plasmid pRB27 or pRB28*	0.2 ng/g	Park et al. (2013)

7.2 Bioaccumulation for Heavy Metal Removal Using Genetically Modified Organisms

In essential words, Bioaccumulation is the simple process of accumulating foreign substances by metabolically active living cells. These foreign substances include harmful environmental pollutants such as pesticides, heavy metals, PCBs, PAH (polycyclic aromatic hydrocarbons), and herbicides; which are absorbed directly by the cells of microorganisms at a rate faster than the rate at which the substance is lost or eliminated from its system by catabolism and excretion (Kehinde and Isaac 2016; Phulpoto et al. 2016).

Heavy metals are the most toxic and harmful contaminants found in the environment. It is the cause of various chronic neurological diseases in the human body, as well as the cause of death of many organisms (plants, land and water included) which thrive on the water and soil contaminated by heavy metals. Heavy metal removal by conventional technique is complicated, expensive, and a slow process; hence it is not deemed economically viable. The alternative and the best option for remediation of heavy metals was Bioaccumulation by genetically engineered microbes.

Bioaccumulation by the microorganisms comes in very useful for the removal of heavy metal ions from the water bodies and land, which, if left unchecked, would harm not only the aquatic life but also the humans who consume the crops growing on these fields or the water and the fishes floating in that water (Phulpoto et al. 2016). In this essential process, microbial biomass makes use of certain specific proteins that helps in creating a differential gradient, allowing the uptake of the metallic ions from the water into its intercellular space. Now, these metal ions are utilised in various cellular processes such as enzyme catalysis, signalling processes and stabilisation of charges in many biomolecules. With the increasing living standards, and the need to reduce the reliance on carbon emission products, protecting the environment from various pollutants has become a priority worldwide. The demand for metal resources increased by an average of 32% in 2021. Hence an efficient system for curbing the effects of this growing demand on the environment was needed. Hence, researchers innovated with the idea of using the strains of microorganisms that actively uptake metal ions as part of their natural metabolic system and genetically engineering it to increase the efficiency and selectivity of uptake while maintaining a lower microbial biomass growth (Phulpoto et al. 2016). Bioaccumulation has been thought to change the dynamic of industries disposing of waste by providing an alternative, such that this genetically engineered microorganism can not only remove but also recover those heavy metal ions from the waste products (Kehinde and Isaac 2016; Phulpoto et al. 2016; Aranda et al. 2010). Hence, these metal ions can then be reduced, purified and refined in the various downstream processing to obtain the metal in its pure form, thereby diminishing the need for disposal (Phulpoto et al. 2016; Aranda et al. 2010).

First of all, we should know that the processes of biosorption and Bioaccumulation are very different. Biosorption is the process of adsorption of the molecules on the surface of the microorganism using physical interactions such as electrostatic forces or chemical interactions such as Van der Waal's forces, whereas, Bioaccumulation is the metabolically active process of uptake of molecules around it into their intercellular space by creation of a translocation pathway from the external to the internal environment by the microorganism (Hesham et al. 2012; Aranda et al. 2010; Sarang et al. 2013). Once accumulated in the intercellular space, these molecules are sequestered or broken down by different proteins and peptide ligands.

Advantage of GEM (Genetically Engineered Microorganisms) Over the Wild-Type Strains

Using GEM (Genetically engineered microorganisms) for Bioaccumulation over the wild-type strains provides the advantage of being more robust than the latter. GEM help bioaccumulates heavy metal ions, pesticides, herbicides, and PAH, beyond the minimum inhibitory concentrations of wild-type strains. Since we know the wild type or the edited strain of the microorganism applied for Bioaccumulation uses the protein machinery which is controlled via the regulatory elements of the genome which has evolved to show the capability to bioaccumulate, i.e., this capability was not present in the primitive or the traditional form of this strain (Hesham et al. 2012; Aranda et al. 2010; Sarang et al. 2013). Hence, when their genome is genetically edited, we can insert additionally vital regulatory elements from different organisms to better the uptake and sequestering system. These regulatory elements inserted can be the promoter, ribosome binding sites, terminator or even a new protein-producing gene. The choice of insertion is a researcher's choice system to target the specificity of the chemical that needs to be absorbed into the system. Thus, GEM provides easy manipulation over the wild-type strain, offering superior control over the import-storage system of the pollutants surrounding the GEM (Aranda et al. 2010; Sarang et al. 2013). GEM is created by transferring the gene, mainly encoding this import-storage system from the wild-type strain to a different host microorganism. This is then simulated to test its growth and efficiency in the wastewater having single or multiple effluents with various pollutants for targeted and specific Bioaccumulation (Hesham et al. 2012; Aranda et al. 2010; Sarang et al. 2013).

Working of a Genetically Engineered Microorganism for Bioaccumulation (Import-Storage System)

Genetic engineering focuses on improving the uptake rate of pollutants by microorganisms into their cytoplasm. Such microorganisms are prepared via recombinant DNA technology, where a recombinant microorganism is prepared by genetically engineering a synthetic import-storage system for Bioaccumulation and cycling of the heavy metal ions and other chemicals from the environment. For creating such a system, genes are selected from different organisms based on their specificity for importing and storing certain chemicals, studied in simulations, and then combined in a different host to give a Genetically modified microorganism (Phulpoto et al. 2016; Hesham et al. 2012; Aranda et al. 2010; Sarang et al. 2013).

We start by selecting the importers for the import-storage system. Selection is based on the efficiency with which those microorganisms having the appropriate importers can uptake the chemical substances around them (Hesham et al. 2012; Aranda et al. 2010; Sarang et al. 2013). For example, for creating a GEM involved in the Bioaccumulation of Heavy metals from a polluted waterbody, we developed our import-storage system specific to uptake such harmful heavy metals.

Importers

Importers fall under three classes: the channels, secondary carrier protein-producing genes and active transporters.

- Channels- These are described as α-helical proteins that help in the passive diffusion of heavy metal ions from its surrounding via the creation of a concentration gradient across the inner membrane. These are energy-independent proteins, helping reduce the overall energy requirement by the microorganism for Bioaccumulation. Example-to increases the bioaccumulation rate of As^{3+} and Hg, and we use channels such as the homotetramer glycerol facilitators (GlpF) obtained from *Escherichia coli, Corynebacterium diphtheria* or *Streptomyces coelicolor* (Kadirvelu et al. 2002). These channels have zero-energy requirements and are the ideal choice for use as Bioaccumulation. However, in many cases, when the concentration of heavy metal surrounding the GEM is very high, Bioaccumulation must be carried out against the equilibrium concentration. To tackle this problem, porins are used, which have β-barrels that form translocation pathways working across the outer membrane of the GEM (Sarang et al. 2013; Kadirvelu et al. 2002). One such porin widely used for the uptake of Ni ion is the FrpB4 channel obtained from *Helicobacter pylori.* We overexpress the divalent cation, selecting porins to increase Ni's uptake concentration.
- Secondary carriers- These carrier proteins also facilitate the active transport of ions across the membrane. These carriers are again divided into three classes of-uniporters, symporters and antiporters. Out of these three, symporters are used for performing Bioaccumulation. Example- For uptake of Ni, Hg and Co, Nixa from H. pylori and its homologs from *Staphylococcus, Novosphingobium aromaticivorans, and Rhodopseudomonas palustris* have been used. These symporters belong to the NiCoT family, which falls under the receptor superfamily called the transporter-opsin-G-protein Receptor (Jaysankar et al. 2008; Sarang et al. 2013; Kadirvelu et al. 2002). Symporters are driven by the PMF (Proton Motive Force), where protons that generate the charge difference are used as co-substrates during the targeted metal ion uptake. Hence, it can perform efficient uptake of metal ions from wastewater.
- Primary Active Transporters- These transporters belong to a group of multicomponent protein complexes consisting of: the transmembrane component for allowing the translocation across the membrane, an ATPase component (for phosphoanhydride bond hydrolysis) which is an energy coupling component required in the

cytoplasm to thrust the translocation of substrates from the external environment, and a periplasmic solute binding component (Sarang et al. 2013; Kadirvelu et al. 2002). These importers can also carry the ions against the equilibrium concentration gradient using the energy derived from the hydrolysis of high-energy compounds such as ATP and GTP. These transporters primarily help bioaccumulate ions such as Cd and Cu. Primary active transporters such as MntA, and cdtB found in *Lactobacillus Plantarum* and TcHMA3 found in the plant *Thlaspi caerulescens.* are selected for the uptake of Cd, whereas for uptake of Cu involves the transporter Copa from *Enterobacter hirae.* Both of the mentioned primary active transporters belong to the P-type ATPase superfamily and hence utilise the ATP reserves of the cells to carry out the import of these ions (Jaysankar et al. 2008; Kadirvelu et al. 2002).

Now, after the appropriate selection of the importer for the uptake of the heavy metal ion from the wastewater, we have to design the proper storage system for the GEM to store and sequester the heavy metal ions inside its system.

Genetically Engineered Metal-Binding Entities as Storage System

For designing a storage system, scientists have stressed developing synthetic cytoplasmic metal-binding entities for the proper and efficient sequestering of the Heavy metal ions. These metal-binding entities can be either the protein ligands or the enzymes that produce peptides and other protein polymers for binding to the heavy metal ions (Smaranda et al. 2016; Kaniecki et al. 2018). These entities provide efficient binding sites for heavy metal ions, which importers earlier transported into the cytoplasm.

- Genetically encoded metal-binding proteins- The genes usually selected for genetically coding these metal-binding proteins belong to the largest protein-producing group. The protein is produced in the MT, a polyphyletic superfamily of the MBPs. The MT protein used for the storage system is found in every species, be it the prokaryotes, archaea or eukaryotes. They have a very high content of Cysteine residue in their amino acid chains, which is a necessary component for properly coordinated sequestration of the heavy metal ion by multiple MT proteins. MT protein can provide a binding site to metal ions such as Zinc (Zn), Cadmium (Cd), Copper (Cu), Mercury (Hg), Arsenic (As), and Nickel (Ni) and cobalt (Co) (Kaniecki et al. 2018; El Fantroussi and Agathos 2005). Usually, the overexpression of MT can reduce its storage capacity; to overcome this limitation, MT protein is fused with a soluble fusion partner such as the maltose-binding protein or the glutathione-S-transferase. To avoid fusion or Cysteine-rich MTs to prevent overexpression, histidine-rich Metal-Binding proteins are used. Such histidine-rich proteins function as a natural storage system for heavy metal ions (Kaniecki et al. 2018; El Fantroussi and Agathos 2005). An example of histidine-rich MT protein-Hpn obtained from *H.pylori-* is a characteristic Heavy metal storage protein that allows binding sites for Nickel.

Table 4 Above the table is a list of the microbes and their gene modification to uptake heavy metals

Heavy metals	Microbes	Modified gene expression	References
Mercury	*Achromobacter* sp AO22	Mercury reductase expressing mer gene	Ng et al. (2009)
Cadmium, Lead, Mercury, Zinc	*Pseudomonas fluorescens* OS8; *Escherichia coli*MC1061; *Bacillus subtilis*BR151; *Staphylococcus aureus* RN4220	MerR/CadC/ZntR/Pmer/PcadA/PzntA	Ng et al. (2009)
Chromium (VI)	*Methylococcus capsulatus* (Bath)	CrR genes for Cr (VI) reductase activity	Ng et al. (2009)
Cadmium	*B. subtilis* BR151 (pTOO24)	Luminescent Cadmium sensors	Ng et al. (2009)
Arsenic	*Sphingomonas desiccabilis* and *Bacillus Idriensis* strain	Overexpression of arsM gene	Ng et al. (2009)

- The enzyme produced Metal-Binding peptides and polymers- In this type of storage system, a gene selected from a specific species is said to produce an enzyme that is involved in mediating the production of small polymers and peptides from raw materials already present in the cytosol of the microorganism's cells (Kaniecki et al. 2018; El Fantroussi and Agathos 2005). Such polymers include phytochelatin, an oligomer of glutathione (GSH) (El Fantroussi and Agathos 2005). This polymer is produced in two steps: in the first step, there is a ligation between the L-forms of cysteine and glutamate to give γ-glutamylcysteine (γEC), which is finally followed by another ligation between the L-form of glycine and γEC in the second step. These steps are mediated by ATP-Dependent enzymes- ligase GshI and ligase GshII, respectively (Kaniecki et al. 2018; El Fantroussi and Agathos 2005). A total of eleven such fused γECs can be added in a sequential order to the GSH chain using the enzyme phytochelatin synthase (or PCS), commonly found in plants showing resistance to heavy metals (Kaniecki et al. 2018; El Fantroussi and Agathos 2005; Aranda et al. 2010). Although PCS is not very efficient in carrying out Bioaccumulation alone, its binding capacity is increased by the overexpression of the enzymes cysE, GshI and GshII, respectively, via genetic engineering principles. Another notable example is the production of polyphosphate (polyP) ester by the enzyme polyphosphate kinase found in *Klebsiella pneumonia*. PolyP is responsible for bioaccumulating Hg, As^{3+}, Cu, and Ni (Jaysankar et al. 2008; El Fantroussi and Agathos 2005; Aranda et al. 2010) (Table 4).

8 Challenges Faced During Gene Editing of a Microbe

Even though the concept of applying genetic engineering for the development of genetically modified plants (GMP) and microorganisms (GMM) sounds easy, it does bring many challenges. These challenges include the problems faced while the isolation and selection of proper species for gene editing and the political challenges faced after the formation and application of these GMM. Some of these prominent challenges include-

- Bioethical issues- Sometimes, the changes introduced during genome manipulation are not always advantageous and effective. Due to the insertion or deletion of a particular gene or genes, the germline or the species might mutate, or an undesirable change has been introduced unintentionally or even die and might lead to extinction. Furthermore, in order for a successful and broad application of the various gene editing tools for the creation and safe use of GMM and GMP in all areas, the worldwide legislation needs the first-hand opinion of the social and life workers, the policymakers of each state, as well as, the stakeholders of the sectors that whether the creation of GMM/GMP is an economically viable and safe option or not (Perpetuo et al. 2011; Rajendran et al. 2003; Mejáre and Bülow 2001).
- Genetic contamination- The introduction of GMM/GMP in the environment also leads to the genetic contamination of the naturally occurring species of organism in the environment. This may include the plants growing or the microorganisms involved in making the soil fertile (Mejáre and Bülow 2001; Wasilkowski et al. 2012; Demnerova et al. 2005). If a species in the environment that is sexually compatible with the GMM/GMP is introduced, it might lead to interbreeding among them. This will result in the disappearance of the novel traits introduced in the GMM, and the genes might get transferred into the native occurring species. This could be advantageous because specific tolerance abilities might develop in the native species, and efficiency for carrying out their natural day-to-day processes might also increase. However, introducing new genes might disturb their relationship with their current habitat and eventually destroy the ecosystem (Wasilkowski et al. 2012; Roane et al. 2001).
- Horizontal Transfer of Recombinant genes (HGT) to Native species- This is the transfer of foreign genes introduced in the ecosystem from the newly introduced GMM/GMP to the naturally occurring native species. The main concern for the chance of occurrence of HGT is that it becomes impossible to eliminate these interbred species if the phenotypic traits are harming the ecosystem (Wasilkowski et al. 2012; Roane et al. 2001; Congeevaram et al. 2007). Such HGT can occur via various methods such as transformation, conjugation or transduction. An example of the harmful effects of the HGT on the ecosystem can be the transfer of antibiotic resistance genes to a pathogen, potentially causing health hazards to humans (Roane et al. 2001; Congeevaram, et al. 2007). This transfer results in novel trait resistance to the antibiotic through pathogen, which could have been otherwise destroyed by an appropriate antibiotic treatment (Roane et al. 2001; Congeevaram et al. 2007; Chojnacka 2010).

- Impact on the non-targeted organism- Selective pressure can increase between the targeted species needing modification and non-targeted species not to be disturbed. The environment around the non-targeted species changes drastically due to the foreign genes entering the ecosystem via the GMM/GMP, causing a shift in the ecology, thereby increasing the survival pressure drastically on the non-targeted species. They need to adapt to the new ecological changes, or else they might not evolve and can become a distinct population (Blackmore and Reddish 1996; Bharagava and Mishra 2018; Singh et al. 2014; Wasilkowski et al. 2012).

9 Conclusion and Future Perspective

According to various studies and surveys, bioremediation has been considered the best alternative to other conventional techniques. Biotechnology application towards improving living standards and controlling environmental pollution has been recognised as one of our generation's growing and future technology, opening new opportunities and providing the future groundwork for innovation and application in various other fields. Furthermore, with bioinformatics and metagenomic studies, it has become much easier to generate valuable data from soil and sea, to identify and isolate the source of new genes, which show different unique capabilities, to be applied in developing transgenic organisms and microorganisms. Various new approaches are being applied to make bioremediation more efficient and more straightforward, such as genome shuffling for generating cross-species organisms, enzyme modification to alter its activity and modification in the metabolic pathway to provide a minor energy spending route for bioremediation. Advancement for developing new ways for handling the genome of any organism is still going on in combination with the different fields of sciences aiding in this venture to find common ground.

Presently bioremediation using bacteria and other microorganisms is still in development and is not widely used. Such limited application is the sole reason for the difficulty for these engineered microorganisms to survive in an environment outside the ideal lab conditions since no matter how many lab simulations these GMM can survive, the natural environment always holds a different condition altogether. Lesser survival rate could be due to the higher level of toxicity in the ground soil or the constant change of season, which also changes the pH, temperature and soil fertility. Hence, for these reasons, phytoremediation using transgenic plants has been pushed as the current choice for bioremediation, owing to their ability to require less nutrient input and protect water and soil from erosion. Genetic risk validation methods have also made it possible to assess, analyse and proceed with caution to ensure the transgenes inserted in plants or microorganisms do not escape or alter the environment, further contributing to pollution. The potential of phytoremediation by transgenic plants exceeds the limitation of typical plants involved in remediation. Experiments are also being conducted, giving suitable groundwork for the future development of a technique capable of using transgenic plants and transgenic organisms to carry out bioremediation at an efficiency level that has never been observed before. The combination being tested involved the plants for degrading the toxic contaminants from the environment and the rhizospheric microorganisms working towards enhancing the availability of hydrophobic compounds that can degrade a wide variety of toxic chemicals mixed with pesticides, herbicides and dyes.

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Nanotechnology for Bioremediation of Heavy Metals

Anu Kumar, Bhanu Krishan, Shivani, Sunny Dhiman, and Akshita Sharma

Abstract Heavy metals do exist as an important source for industrial processes such as textiles, power plants, and pesticide production plants but their discharge in the environment is a serious concern because of their high toxicity and non-biodegradable properties which can cause serious illness and dreadful effects to humans and animals including aquatic habitats. Due to their adverse effect, removal of heavy metals from any contaminated source through bioremediation with the inclusion of Nanotechnology has been an eloquent method in recent times. Nanomaterials exhibit unique properties such as selectivity; relative small size due to which they have been reported to act as an effective absorbent of heavy metals. Nanomaterials synthesized from microbes and fungi have been revealed to reduce the level of heavy metals contamination through enzyme-based routes or through absorption. Thus, this chapter will focus on different strategies employed using nanomaterials for heavy metal degradation.

Keywords Biogenic nanoparticles · Adsorption · Heavy metals · Nanocomposites · Nanofiltration · Nano-phytoremediation

1 Introduction

Starting from the era of industrialisation, numerous problems have been associated with the release of toxic contaminants into the environment. The main field where these contaminants are associated involves the natural water and soil sources which are further directly associated with the risks involved to human health as well as producing economic damages to the environment (Marcovecchio and Freije 2007). One of the most concerning contaminants in the environment is the release of heavy

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metals in high concentrations. The elements possessing metallic properties with a density greater than 5 g per cubic centimetre are defined as heavy metals; due to their toxicity these elements are also termed carcinogenic and well-known water-soluble toxins (Gunatilake 2015). Heavy metals include Arsenic (As), Lead (Pb), Selenium (Se), Tin (Sn), Copper (Cu), Silver (Ag), Zinc (Zn), Cadmium (Cd), Gold (Au), Mercury (Hg), Molybdenum (Mb), Nickel (Ni), Iron (Fe), Chromium (Cr), Manganese (Mn), Aluminium (Al), Thallium (Tl), Titanium (T), Thorium (Th) and Cobalt (Co). According to US-EPA, As, Cd, Cr, Cu, Pb, Ni and Zn are characterized as hazardous as well as toxic metals.

The release of such toxic metals into the environment is based upon two different sources of origin (a) Natural origin which may include mineralisation, leaching of ore and volcanism, (b) which includes the anthropogenic sources such as disposal of waste, industrial waste effluents, municipal waste and agricultural waste (Marcovecchio and Freije 2007; Gunatilake 2015). The largest recognised release of heavy metals resides near the urban and industrial areas. The industries associated with the release of heavy metals include tannery, semiconductor, mining, and refinement industry. The utilisation of these toxins in the industrial process is associated with shaping, forming, coating, and dyeing of products, metal surface treatment, installation of atomic energy and weaving of finished material into a finalised manufactured product (Ayres 1992). The toxicity and effects of metals are dependent upon the concentration and type of contaminant. Chromium is considered the dominant heavy metal in effluents,the use of Cr in industries is mainly associated with dyeing, paints, fabrication of steel and textiles.

The ineffective treatment and release of untreated effluent into water sources containing a higher concentration of chromium can significant changes in the aquatic ecosystem and life form, resulting in accumulation in aquatic plants, algae, invertebrates and fishes. Cr(VI) also has an adverse on humans which when present in the bloodstream can vandalise the blood cells, ingestion of Cr(VI) in abundance causes organ failure due to ruptured blood cells. Also, the accumulation of Cr in tannery workers are reported to cause altered iron metabolism. Other adverse effects include severe burns, systemic poisoning, eye damage, and respiratory problems (Singh et al. 2016). The presence of Zinc in water bodies is toxic to aquatic animals and upon ingestion, by humans,it is responsible to cause fatigue and dizziness (Awofolu 2005). Copper has the potential of forming a compound with sulphide ions which is directly harmful to humans. Copper sulphide can lead to kidney dysfunction, disruption of the capillaries, gastrointestinal, renal and respiratory anaemia (Gupta and Lutsenko 2009; SenthilKumar et al. 2011). A study was conducted on F344/N rats and $B6C3F_1$ mice by the US National Toxicology program in order to determine the effect of extreme concentration of $CuSO_4$ present in drinking water. The highest concentration 45 mg Cu/kg BW/day for male rats and 31 mg Cu/kg BW/day for females was found to be highly toxic with death and organ weight loss of the subjected animals (Lum et al. 2021). The main sources for exposure to Nickel are drinking water and food. Nickel, on the other hand, when ingested can cause an allergic reaction such as dermatitis, lung fibrosis, cardiovascular disease and cancer in the body

(Duda-Chodak and Błaszczyk 2008). The standard conventional methods chosen for the removal of these toxic heavy metals from wastewater includes bioremediation, solvent extraction, evaporation, ion-exchange, biosorption, advanced oxidation processes, photocatalysis and reverse osmosis (Gode and Atalay 2008). These processes are considered less efficient for the removal of such metals and create a challenging issue of inadequate removal with a cost-effective process. Thus, to resolve these challenges, principles of nanotechnology are applied in wastewater treatment, filtration to remove these metals from the environment even under minute concentration.

2 Nanotechnology in Heavy Metal Removal

Nanotechnology is an offshoot that involves the principles of nano-sciences, i.e., the study of structures and materials on a diminutive level. The study is based upon the manipulation and application of nanomaterials, a wide class of matter that encompasses particulate solidity, having atmost 100 nm as one of its dimensions (Balaji 2019). The demand for nanomaterials has become evident in many empirical applications. To define nanomaterials, it can be said a substance that possesses at least one dimension of lesser than 100 nm. The synthesis of such nanomaterials can be from bulk i.e. etching or mechanical milling; the process is referred to as the top-down approach. Another way is the bottom-up approach where nuclei are grown from atoms or molecules that can further be synthesized into nanomaterials by employing methods like green synthesis, laser pyrolysis, sol–gel process, spinning, etc. (Baig et al. 2021). There are various unique properties of NMs which makes them feasible for research studies. It is seen that nanomaterials may flatter magnetic properties even if their parent element is non-magnetic (Baig et al. 2021). The external magnetic field may help in exploiting these magnetic components. Magnetic nanoparticles have divergent characteristics, for instance, super magnetism, high-field irreversibility and shifting loop after field cooling (Sajid 2022). Nanomaterials acquire a high surface area ratio in contrast to their bulk counterparts. Due to the mentioned property, these NMs are highly beneficial in adsorption, catalysis as well as thermal and electrochemical sensing applications (Baig et al. 2021; Sajid 2022). Acting as a catalyst these NMs in 2D configuration provides an avenue for the catalyst to disperse atomically on it, inflating the catalyst activity (Baig et al. 2021). Based upon the size and shape these materials can be classified into carbon-based nanomaterials, metallic nanoparticles, ceramic-based nanoparticles, polymeric nanomaterials and biomolecules derived nanomaterials (Sajid 2022).

2.1 Metal and Metal Oxide Nanoparticles in Heavy Metals Removal

Metal and metal oxide nanoparticles (NPs) are of great study these days, this is because of the fact that the synthesis of such NPs is cost-effective, easy regeneration and exhibit high adsorption capacity, some of the metal NPs are illustrated in Fig. 1. Metal oxides such as Iron Oxide (Fe_2O_3) NP, Zinc oxide (ZnO) NPs, Nickel Oxide (NiO) NPs, Copper oxide (CuO) NPs and Titanium Oxide (TiO_2) are the extensively applied nano-adsorbents for the removal of toxic heavy metals. The surfactant functionalization of metal oxide NPs enhances the absorption capacity and is reported to increase with the decrease in the size of NPs. The studies related to the use of CuO, NiO and ZnO are found to efficiently remove heavy metals from the water (Parvin and Rikta 2019) but the use of bare metallic NPs is a less common process as the separation of NPs from the water is a deliberately difficult task. Therefore, to overcome this problem, capping and functionalization of nano-adsorbents are opted to enhance the structural stability and enables the removal of NMs from water easily. CuO NPs synthesized from natural sources as well as using precipitation method are reported for an excellent potential for absorption of heavy metals. A study regarding plant-mediated synthesis of CuO from *Catharanthus* leaf extract showed 2.11% and 2.91% removal efficiency for chromium and cadmium respectively when incubated with stock solutions of respected metals for up to 24 h (Verma and Bharadvaja 2021).

Another study related to the green synthesis of CuO NPs from mint leaves and orange peels showed the shreds of evidences for the removal of Pb(III), Ni(II) and Cd(II) from contaminated water. The optimum uptake capacity was calculated after the treatment of the metal oxide NPs with metal-contaminated water to determine the adsorption capacity of the NPs. The optimum uptake capacity for Pb(III), Ni(II) and Cd(II) was found to be 88.80, 54.90 and 15.60 mg/g with a sorbent dose of 0.33 g/L at pH 6 (Mahmoud et al. 2021). Iron oxide NPs with excellent adsorption

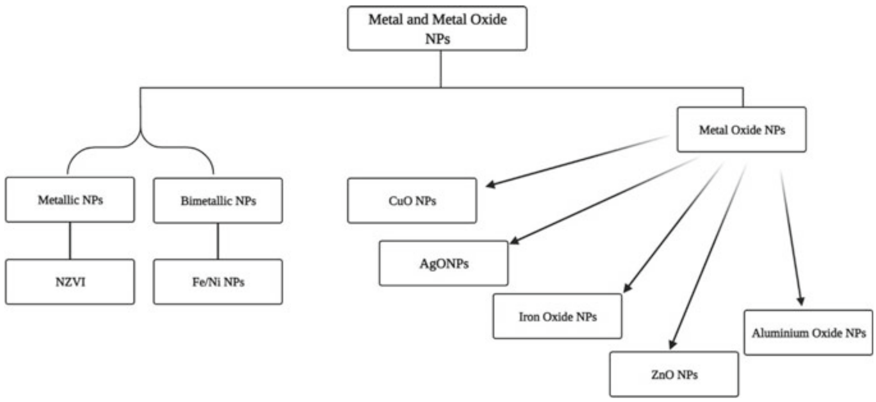

Fig. 1 Illustration of various metal and metal oxide NPs

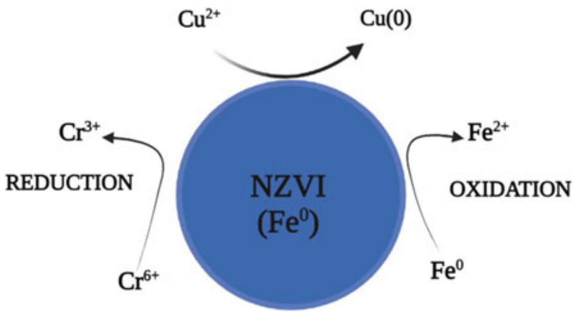

Fig. 2 Surface adsorption phenomenon of NZVI against toxic heavy metals

efficiency has been reported to adsorb Cr, Pb and Zn with an efficiency of 92.26%, 75.57% and 89.36% (Venkatraman and Priya 2021). Iron oxide NPs synthesized from *Taranjabin* have been analysed to remove lead from wastewater with an efficiency of 96.73% at 50 mg/L of the highest concentration (Miri et al. 2021). Bimetal oxide NPs tends to show higher adsorption capacity than single metal oxide NPs from wastewater. These NPs absorb the heavy metals by sites binding, electrostatic interaction, and selective adsorption. Bimetal NPs such as Fe-magnetic NPs has been found eloquent for the removal of Arsenic (As) from wastewater in a short contact period by the interaction of the hydroxyl group of the NPs with As (Parvin and Rikta 2019; Deliyanni et al. 2006).

Metal NPs such as nano-size zero-valent ions (NZVI) with improved stability are found effective for the elimination of heavy metals. In a study, NZVI were entrapped in a non-toxic biodegradable stabilizer composed of chitosan carboxymethyl β-cyclodextrin complex was found to remove Cr^{6+} and Cu^{2+}, following the principle of physisorption reduction of Cr^{6+} to Cr^{3+}, oxidising Fe^0 to Fe^{3+} (illustrated in Fig. 2) (Kanel et al. 2006). Other metal and metal oxide NPs which have been studied for their extensive removal properties against toxic metals includes dendrimer nano-absorbents, chitosan NPs, inorganic molecules functionalized NPs and polymer functionalized NPs (Wadhawan et al. 2020).

3 Nanocomposites in Heavy Metal Removal

Nanocomposites are the inorganic or organic composite materials having one dimension less than a nanometre, these nano-scale composites can be classified into five major groups which are (i) sol–gel composites, (ii) intercalation-type nanocomposites, (iii) entrapment type, (iv) electroceramics and (v) structural ceramics. These types of nanocomposites differ in production strategies and overall structural integrity (Komarneni 1992). The approach that makes nanocomposites feasible for their application in the removal of heavy metals is surface functionalization through the introduction of functional groups such as hydroxyl, sulphates and animo groups. These functional groups, therefore, supports the adsorption and chelation of the heavy metals, thereby, increasing the adsorption capacity of the nanocomposites (Wang et al. 2018). These NCs are incorporated with different solid substrates which enhance the overall physical and chemical properties of the NCs. Solid substrates such as carbon nanotubes, zeolites, graphene oxide, bentonite, magnesium oxide and silica are studied for size control of magnetic iron oxide NPs (Hayati et al. 2018). Because of the easy solid–liquid separation under magnetic field and reusability, various studies have been reported for nanocomposites in heavy metal removal from wastewater. Magnetic carbon nanocomposites such as Zr-Magnetic organic frameworks (MOFs) composites, these composited prepared in a study consisted of a nano-sized Fe_3O_4@SiO_2 core coated with Zr-MOFs, to study the efficiency of these adsorbents for the heavy metals, Fe_3O_4@SiO_2@UiO-66 and its amino derivatives (Fe_3O_4@SiO_2@UiO-66-NH_2 and Fe_3O_4@SiO_2@UiO-66-Urea) were also synthesized. The obtained Zr-MOFs were reported to exhibit high adsorption capacity and fast adsorption kinetics for metals ions (Huang et al. 2018).

Novel carbon nanotubes coated with poly-aminodoamine dendrimer have also been reported to absorb As(III) and Co^{2+} and Zn^{2+} from aqueous solutions. The reaction was carried out in a fixed-bed column under controlled pH and flow rate. The maximum absorption observed was 432 mg/g for As(III) (Hayati et al. 2018). Hydrothermal fabrications of TiO_2- MoO_3 nanocomposites synthesized by precipitation method were studied to remove selected heavy metals from standard solution. The nanocomposites showed the highest adsorption of 59 mg/g for Cr(VI under elevated room temperature, therefore, making it a considerable good choice for the removal of toxic heavy metals (Zhao et al. 2018).

4 Nanofiltration and Nanofilter Membranes

A recent advancement involving well-developed pressure-driven membrane filtration has been utilised for the separation of molecules from the liquid phase. The property of NFs which makes it a special methodology for treatment and removal of heavy metals is fixed charge possessed due to the surface charge dissociation, the charge groups can be either sulphonated or carboxyl groups. This property enables this technique to be involved in the treatment of pharmaceutical waste, metal recovery and removal of organic and inorganic pollutants present in surface water (Bowen and Welfoot 2002; Abdel-Fatah 2018). Another property of NFs which makes them an efficient choice is of smaller pore size of membranes. With a high recovery rate and low cost these NFs are studied for removal of heavy metals, studies have reported 92% of copper removal from the high volume of wastewater, with 150–35 mg/L of COD removal and 80% salt rejection (Khedr 2008; Liu et al. 2008). The separation mechanism involved for NF rejection is defined by the following steps by Macoum (Macoun 1997).

(a) *Wetted Surface*: The transport of molecules occurs through the membrane due to the formation of hydrogen bonding between the water and the NF membrane.

(b) *Capillary rejection*: This occurs due to the electrostatic repulsions between the solution and membrane.
(c) *Diffusion of solution*: The solute and solvent gets dissolved in the active layer of the membrane and diffusion of solution through the NF layer
(d) *Charged capillary*: Same charged ion gets attracted into the membrane and the counter ions are rejected due to streaming.
(e) *Finely porous*: The dense material of NF is punctured by pores. Therefore, partitioning between bulk and pore fluid determines the transport.

A novel type of NF membrane synthesized using chitosan and 1,3,5-triglycidyl isocyanurate gradient cross-link on polyethersulfone ultrafiltration membrane showed a high rejection performance to $MgCl_2$, Na_2SO_4, $MgSO_4$ and NaCl. This positively charged membrane manifested superior permeability and mechanical strength as compared to the earlier reported membranes (Yang et al. 2021). A cost-effective NF composed of thin-film composites were studied to separate selected heavy metals from the solution. These optimized NF membranes successfully rejected 93.9%, 97.9% and 87.7% of Cu^{2+}, Mn^{2+} and Cd^{2+} (Cheng et al. 2021). Fe_3O_4 NP dispersed uniformly on the surface of MXene sheets was studied to exhibit greater removal efficiency of heavy metals as compared to virgin MXene sheets. The prepared NM membrane were found to achieve removal of 63.2%, 64.1% and 70.2% removal of Cu^{2+}, Cd^{2+} and Cr^{6+} from wastewater. These membranes were reusable after washing with HCl solution (Yang et al. 2021).

Application of NF in heavy metal treatment is an effective strategy because of its operation at low pressure, high salt concentration tolerance and cost-effectiveness. The only limitation to this method is membrane fouling due to colloidal substances and high molecular weight components (Abdel-Fatah 2018).

5 Nanobioremediation and Nanophytoremediation

An emerging concept of Nanobioremediation (NBT) has been widely used for soil, water and air pollutants removal and treatment. This technique involves the integration of nanotechnology and bioremediation for an efficient treatment process under minimum time as bioremediation alone is a time-consuming process. Thus, this method has served potential benefits in the removal of pollutants, organic compounds and toxic metals (Carata et al. 2017). Metal-microbe interaction has excelled the fabrication of various NPs with the involvement of microbial and plant extracts. Such biogenic NPs are the hybrid strategy for the sustainable removal of toxic heavy metals. The synthesis of Biogenic NPs (BNP) is accomplished through two different techniques—biologically controlled mineralization, where the microbes control the nucleation of intracellular BNPs and biologically induced mineralization, where the synthesis of NPs takes place when metal ions are precipitated or reduced after interacting with cell wall or membrane filtrate (Misra and Ghosh Sachan 2021; Yue et al. 2016; Su et al. 2014). The synthesis of BNPs including their shape and size is greatly

Table 1 The biogenic NPs summarized for the removal of toxic heavy metals

NPs type	Microorganism/ Plant used	Heavy metal removed	Removal efficiency	Findings
CuS	*Shewanella oneidensis MR-10*	Cr^{6+} biosorption	94.1%	Xiao et al. (2017)
CdS	*Pseudomonas aeruginosa JP-11*	Cd removal	88.7%	Raj et al. (2016)
Pd	*Enterococcus faecalis*	Cr^{6+} reduction	100%	Ha et al. (2016)
CuO	Mint leaves and orange leaves extract	Pb^{2+} removal	88.80 mg/g uptake capacity	Mahmoud et al. (2021)
Ag	*Convolvulus arvensis*	Pb and Cd removal	82%, 77%	Arjaghi et al. (2021)

influenced by variables such as aeration, pH, temperature and metal ion concentration (Misra and Ghosh Sachan 2021). Several NPs synthesised biogenically from microbes and plants have been studied for an eloquent eviction of heavy metals by biosorption or reduction mechanism summarised in the table 1 (Misra and Ghosh Sachan 2021).

Nano-phytoremediation (NPT) is a greater potential study against pollutants that are poorly degraded by phytoremediation. This method is effectively been utilized in the fields of textiles, paints and cosmetics for the treatment of contaminants that includes heavy metals, organic and inorganic pollutants such as atrazine, PCBs, PAHs and organic solvents. This novel strategy is based upon the percipience of NPs by the plant roots, which upon entering the plant root transports in two ways-Apoplastic transport (outside the plasma membrane and xylem vessels), Symplastic transport (the water movement between cytoplasm and sieve tubes) (Srivastav et al. 2018; Roberts and Oparka 2003). The interaction between NPs and plants is studied to stimulate propitious effects to the plant,AgNPs can increase the ABA and GA phytohormones, whereas magnetic NPs can enhance the nitrogen assimilation and improvement in plant metabolism. Also, these NPs lead to an increase in the chlorophyll amount and the seed germination ability of the plant (Srivastav et al. 2018). Certain NPs assisted plant-based remediation studies are mentioned in the Table 2.

Although these biogenic NPs are eloquent in the control and degradation of heavy metals, certain challenges that limit their usage. BNPs form reactive oxygen species inside the microorganism that can lead to defective cellular structure and inhibition of growth. In the case of nano-phytoremediation, an efficacious degradation requires long experimentation studies; also, these studies are suitable only for a moderate level of toxic metal contamination.

Table 2 NMs utilisation with the plant in order to facilitate removal of heavy metal by combined Nano-phytoremediation

Nanomaterial	Plant species involved	Heavy metal	Removal efficiency	Finding
Nano-scaled zero-valent ions	Oak	Cu immobilization	76%, 73%	Slijepčević et al. (2021)
Salicylic acid NPs	*Isatiscappadocica*	Arsenic	705 ppm and 1188 ppm accumulation of metal in roots and shoots of plant	Souri et al. (2017)
Nano-scaled zero-valent ions	*Tradescantia spathacea*	Pb, Cd	73.7%	Jesitha and Harikumar (2018)
Nano-scaled zero-valent ions	*Alternanthera dentata*	Pd, Cd	71.3%	Jesitha and Harikumar (2018)

6 Conclusion

The most concerning contaminant in the environment is the release of heavy metals in high concentrations. These heavy metals are toxic to the environment as well as human health causing severe disease and long term health problems. The conventional strategies exploited for the treatment and removal of these toxic metals are less eloquent and found to release secondary toxic compounds. The employment of nanotechnology in the fields of treatment and degradation is an effective process that includes the exploitation of nanomaterials because of their unique properties. Due to their small size and high adsorption capacity, these NMs targets specifically these toxic metals and therefore, serve as an alternative for the removal of these heavy metals. Metal and metal oxide nanoparticles such as CuO, Fe_2O_3, ZnO and TiO_2 synthesized from plants and chemically are studied for their high adsorption capacity. Metal Oxide NPs are found to be highly eloquent for the removal of Cu, Cr and Pb from wastewater samples. Also, NZVI which are effective metal nanoparticles with unique magnetic properties is found to stabilize toxic metals from water sources. Novel carbon nanotubes coated with poly-aminodoamine dendrimer and nanocomposites composed of metal oxides are studied for removal of metals such as, Co and Zn through adsorption under controlled pH and temperature with high kinetic properties. The other effective and cost-effective process is the exploitation of NF membranes which are highly porous with efficient rejection capability. These membranes are coated with NPs or equivalent particles which increase their efficiency in order to separate heavy metals from the solution. Nanobioremediation and nanophytoremediation on the other hand is an emerging field under the principles of nanotechnology which aims to use the biogenic NPs synthesized through microbial routes or exploited with their aid. Such NPs are non-toxic to the environment and facilitate the complete removal of toxic heavy metals under controlled experimentation. The exploitation of nanotechnology is not only focused on the removal but recently these principles are used for sensing and detection of the toxic levels of contaminants which includes inorganic pollutants, organic pollutants, heavy metals and xenobiotics. Thus, the field of nanotechnology is a promising concept for the pollutant-free environment.

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