A COMPREHENSIVE EXPLORATION OF CHEMICAL, BIOLOGICAL, AND PHYSICAL APPROACHES FOR IMPROVING WASTEWATER BIOREMEDIATION

Abstract

There are significant risks to the environment and public health associated with the discharge of organic and inorganic pollutants into the environment as a result of residential, agricultural, and industrial activities. More effective and inventive treatment methods are being extensively researched because the current traditional wastewater treatment plants are unable to completely eliminate contaminants. Utilizing naturally occurring bacteria, fungus, or plants, often known as bioremediation, to remediate polluted wastewater has been shown to be successful and efficient. The versatility of microorganisms to remove a harmful contaminant makes bioremediation an invention that may be used in many water and soil situations. In this chapter, physical, chemical and microbial bioremediation for pollutant mitigation from various ecological lattices has received significant attention. This chapter provides a succinct overview of bioremediation along with a description of the many techniques used to treat soil and industrial waste. Lastly, several bioremediation-related experiences.

Keywords: Methanogenesis, Phytoremediation,
Microbial enzymes, Nanomaterial, Electrocoagulation.

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I. INTRODUCTION

Bioremediation (Fig .1) is the process of disinfecting up and extracting pollutants or contaminants from the environment using living organisms such as bacteria, fungi, or plants(D. Mani & Kumar, 2014). It is an environmentally beneficial and long-term technique to cleaning up polluted sites and restoring environmental quality(Akcil et al., 2015). Organic materials (e.g., hydrocarbons, pesticides, solvents) and inorganic substances (e.g., heavy metals, nitrates) can both be treated using bioremediation(Department of Chemistry1 , Jiwaji University, Gwalior (M.P.), India et al., 2014).Water is one of the most important and valuable assets on the planet, sustaining life and maintaining ecosystems. Its significance can be understood from numerous angles(Galli et al., 2012).The ever-increasing population places enormous strain on natural resources and it has adverse effects in water resources (Wassie, 2020). It is predicted that the world's population will more than quadruple in the next 30 years, as will the demand for potable water, culminating in global shortages. Furthermore, growing urbanisation and industrialization have led to improper wastewater discharge and disposal from medical, municipal, agricultural, and industrial sources(Yohannes & Elias, 2017). The developing countries are still lingered with more contaminated water in lakes, ponds and rivers. These are the major causes for human health resulting in, cholera, diarrhoea, typhoid and other water borne diseases (Dhara et al., 2013). The industry's such as textiles, leather and chemicals are continuously mixing their waste dyes, expired chemicals, and other waste products, these results in the continuous contamination of the water resources in their surroundings(Hynes et al., 2020). Wastewater management and treatment has been adopted throughout world to save the water resources and given awareness to people regarding the biodiversity importance of water, ill health problems caused by these contaminated water. Water treatment involves a combination of biological and physicochemical processes, and the treatment approach chosen is mostly decided by operational costs, the source and quality of influent wastewater, and the planned reuse of the effluent(Misra & Pandey, 2005). Recently, new machines have been developed to improve the efficiency of target pollutant removal from wastewater. In industrial wastewater treatment, for example, the new oxidation process provides a compelling option for reducing non-biodegradable contaminants(Crini & Lichtfouse, 2019; Saeed et al., 2015).

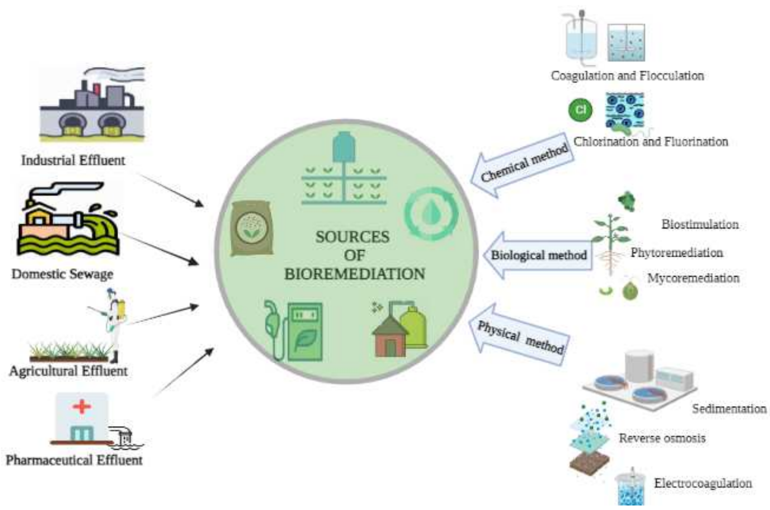

Figure 1: Approaches and methods of Bioremediation and their sources.

II. METHODS OF BIOREMEDIATION FOR WASTE WATER TREATMENT

1. **Chemical Methods of Bioremediation in WWT:** "Chem-bio" treatment refers to the combination of chemical precipitation and wastewater bioremediation in a treatment procedure (Fig .2). This combined strategy takes advantage of the benefits of both approaches to successfully remove a broader variety of contaminants from polluted wastewater(Ahmad et al., 2015; Herrero & Stuckey, 2015). The first stage involves the addition of chemical agents to the wastewater, such as coagulants and flocculants(Teh et al., 2016). These compounds, as previously stated, aid in the production of insoluble precipitates by neutralising charges on suspended particles and forcing them to agglomerate(Kurniawan et al., 2022; Sahu & Chaudhari, 2013). Heavy metals and some inorganic pollutants are very efficient at being removed by chemical precipitation. The effluent is allowed to settle in a sedimentation tank after chemical precipitation(Gutierrez et al., 2010). The produced flocs and precipitates, together with some organic debris and other contaminants, sink to the bottom as sludge during this process(Rodriguez et al., 2020).

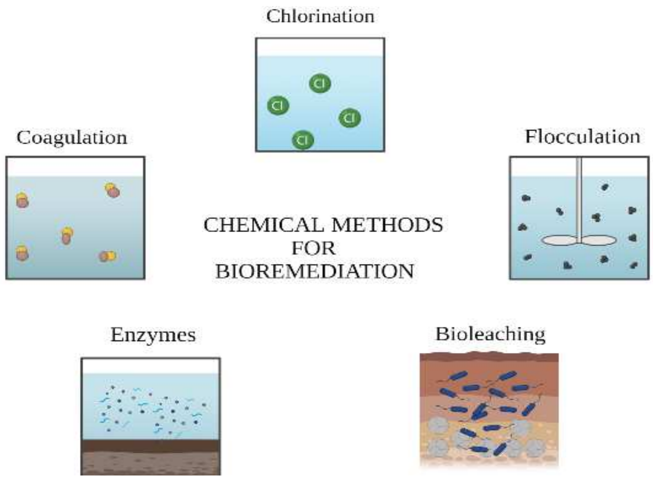

Figure 2: Chemical methods for waste water bioremediation.

2. **Coagulation and Flocculation:** Coagulation and flocculation are important pretreatment stages in wastewater bioremediation that improve process efficiency(Verma et al., 2012). These physical-chemical methods attempt to remove suspended particles and colloidal chemicals from wastewater, allowing microorganisms to biodegrade organic contaminants more easily(Saini, n.d.). Coagulation is the process of adding chemical coagulants to wastewater. Metal salts such as aluminiumsulphate (alum) or ferric chloride are commonly used as coagulants(Bakar & Halim, 2013). Coagulants balance the negative charges on suspended particles and colloids in wastewater. As a result, the particles begin to agglomerate and lose their electrostatic repulsion. The coagulant is added to the wastewater and interacts with the negatively charged particles, resulting in the formation of microscopicand destabilised particles known as microflocs(C. S. Lee et al., 2014). In addition, the coagulant neutralises the charge on organic particles, making them more prone to aggregation(Ghernaout & Ghernaout, 2012). Following coagulation, flocculation involves mild mixing of the water to stimulate microfloc collision and adhesion, allowing them to expand in size and create bigger, visible flocs(Mohd

Asharuddin et al., 2021; Wu et al., 2009). Mechanical mixing devices, such as paddles or propellers, are used in flocculation tanks or basins to accomplish this process(Iwuozor, 2019). A polymer flocculant may be applied at this stage in some circumstances to improve floc formation and development. Slow and moderate mixing of the coagulant-added wastewater is used to assist the collision and adherence of the destabilised particles, resulting in the production of bigger flocs(Owodunni & Ismail, 2021). As flocs develop in size, they entrap more suspended particles and organic materials, making it simpler to remove pollutants during following treatment procedures(Saravanan et al., 2021). Following coagulation and flocculation, the wastewater is subjected to bioremediation, in which microorganisms degrade organic contaminants into simpler, non-hazardous compounds(Singh et al., 2014). The flocs created during flocculation also provide surfaces for microorganisms to adhere and flourish, increasing the bioremediation process's effectiveness(Jagaba et al., 2021).

3. **Ion Exchange:** Ion exchange is another successful wastewater bioremediation technology, particularly for the removal of dissolved inorganic contaminants(Barakat, 2011). The exchange of ions between the solid phase of a particularly formulated resin and the liquid phase (wastewater) is a physical-chemical process(Pyrzynska, 2008). This exchange method aids in the selective removal of certain ions or toxins from wastewater, allowing it to be used in a variety of applications such as industrial operations or release into the environment(Katheresan et al., 2018). Ion exchange is based on the use of a resin material with certain functional groups capable of attracting and exchanging ions(Silva et al., 2018). These resins are primarily constructed of synthetic organic polymers and come in a variety of shapes, including beads and granules. The ion exchange resin used is determined by the contaminants to be removed and the chemistry of the water(Awual et al., 2013; Zaggia et al., 2016). The functional groups on the resin surface attract and bind particular ions present in the wastewater as it runs through a column or vessel containing the ion exchange resin(Kammerer et al., 2011; Rafati et al., 2010). Heavy metals (e.g., lead, cadmium, mercury), radioactive elements (e.g., uranium, radium), and other dangerous inorganic chemicals may be present in these ions(Brusseau & Artiola, 2019). To maintain charge balance, counter ions are released into the water when pollutants are adsorbed onto the ion exchange resin(Ochando-Pulido et al., 2018). In cation exchange, for example, hydrogen (H^+) or sodium (Na^+) ions may be released to replace adsorbed metal ions(Nouar et al., 2009). To replace adsorbed anions, hydroxyl (OH^-) or chloride (Cl^-) ions may be released during anion exchange. The ion exchange resin gets saturated with absorbed pollutants over time, and its capability for ion exchange decreases(Ortega et al., 2017; L. Zhu et al., 2017). The resin must be renewed to regain its functionality. Typically, this is accomplished by washing the resin with a regenerant solution that displaces the adsorbed ions, restoring the resin's capacity for future ion exchange. Because the regenerant solution containing the removed contaminants might be highly concentrated with pollutants, it must be properly treated before disposal(Pérez-González et al., 2012). Depending on the kind and quantity of pollutants, this solution may need to be treated further, such as by precipitation, filtering, or bioremediation, before it can be properly discharged or disposed of(Vardhan et al., 2019).

4. **Adsorption and Neutralization:** Adsorption and neutralisation are two significant processes in wastewater bioremediation that are used to remove contaminants and modify

the pH of the wastewater(Nharingo & Moyo, 2016). Both methods are important in preparing wastewater for efficient bioremediation. Adsorption is a physical-chemical process that includes pollutants adhering to the surface of a solid substance known as an adsorbent(Afroze & Sen, 2018). Adsorbents in wastewater treatment are generally porous materials with a wide surface area that attract and trap contaminants in the water(Yahya et al., 2018). The specific contaminants contained in the wastewater are used to choose an appropriate adsorbent material. Adsorbents that are often used include activated carbon, zeolites, and different clays(Rafatullah et al., 2010). The adsorbent is introduced into the wastewater either by passing it through a packed bed of adsorbent particles or by mixing the adsorbent directly into the effluent(Mohammed et al., 2016). Pollutants in wastewater, such as organic molecules, heavy metals, or some inorganic substances, bind to the adsorbent's surface by physical forces such as Van der Waals interactions or chemical bonds(Gusain et al., 2020). Following adsorption, the effluent is removed from the adsorbent, which now retains the contaminants that were absorbed. Neutralisation is a chemical technique that is used to modify the pH of wastewater that is overly acidic or alkaline(Raschitor et al., 2014). In many circumstances, microorganisms utilised in bioremediation perform best within a narrow pH range. As a result, neutralising the wastewater to an optimum pH level is critical to the bioremediation process's effectiveness(Vitor et al., 2015). pHmetres or indicator sheets are used to determine the pH of the effluent. An appropriate neutralising agent is applied to the wastewater based on the observed pH value to bring the pH within the acceptable range(Suopajärvi et al., 2013). An acidic wastewater, for example, may require the addition of alkaline substances such as lime (calcium hydroxide) or soda ash (sodium carbonate), whereas an alkaline wastewater may require the addition of an acidic agent such as sulfuric acid or carbon dioxide(Q. Chen et al., 2018). To guarantee correct pH adjustment, the neutralising agent is fully combined with the effluent.

5. **Biological Approaches of Waste Water Bioremediation:** Bacteria, algae, plants and nanotecnology-mediated wastewater treatment, also known as biological wastewater treatment, is a popular and efficient form of wastewater treatment (Fig. 3). It is dependent on the action of microorganisms, specifically bacteria, to break down and eliminate organic and inorganic pollutants from wastewater before it is released back into the environment or reused for other uses.

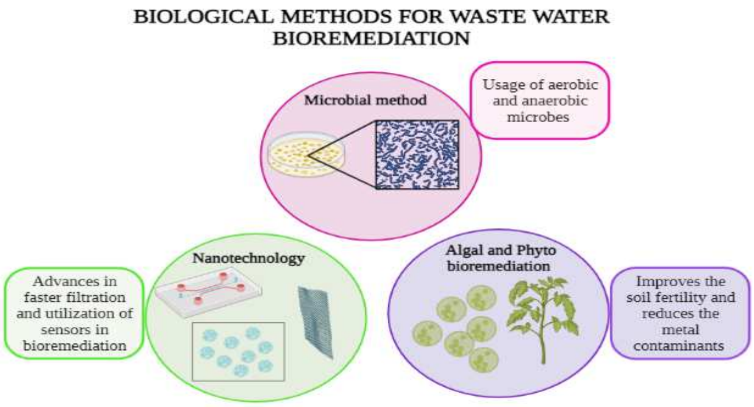

Figure 3: Different biological approaches of waste water bioremediation.

6. **Aerobic Wastewater Treatment:** Bacteria consume organic matter and other contaminants in the presence of oxygen in aerobic wastewater treatment. This process occurs in an aerated tank or pond, which provides an ideal habitat for aerobic bacteria to thrive(Stewart et al., 2008). These bacteria feed on organic molecules and transform them into carbon dioxide, water, and biomass. As a result, organic contaminants are reduced, and water quality generally improves(Xiao & He, 2014). Aerobic biodegradation of organic substrates is autocatalytic and beneficial, i.e., bacteria act as biocatalysts in this situation(Glueck et al., 2010). Depending on the pH, temperature, and biological process, different concentrations of aerobes are used, with the activated sludge process using the most bacteria(Besha et al., 2017). The activated sludge process is a simple and low-cost method for converting a large volume of substrate in aerobic wastewater treatment(Champagne & Li, 2009).

7. **Fixed bed reactors:** Fixed bed reactors are a form of bioreactor that is extensively used in wastewater bioremediation operations to clean contaminated water(Fernández et al., 2018). These reactors foster the growth of beneficial microorganisms such as bacteria and fungi that can breakdown and eliminate contaminants from wastewater(Naghdi et al., 2018). Fixed bed reactors are especially effective for treating wastewater with high levels of organic toxins and pollutants.They are made comprised of a container filled with support media, which offers a surface area for the microbial bio film to grow on(Harrison et al., 2010). Common support media include pebbles, gravel, plastic pieces, and other materials with a high surface area-to-volume ratio.When wastewater passes through the reactor, microorganisms in the water begin to cling to the top layer of the support media(Aslam et al., 2017). Over time, these germs develop a film known as biofilm, which is a gooey layer of microbial populations(Mohammadi et al., 2013).As the wastewater flows through the fixed bed reactor and comes into touch with the bio film, the microorganisms in the bio film start metabolising and breaking down the organic pollutants in the water(Joshiba et al., 2019). This biological degradation process breaks down complex organic chemicals into simpler, less toxic components like carbon dioxide, water, and biomass(Gumisiriza et al., 2017).The fixed bed reactor is meant to provide a continuous supply of oxygen to the biofilm during aerobic bioremediation, which uses oxygen-dependent microorganisms to breakdown contaminants(Khalil & Liu, 2021). This can be accomplished through aeration or by designing a flow pattern that encourages oxygen transport to the biofilm(Dias et al., 2018).Periodic backwashing or intermittent aeration is used to prevent blockage and maintain maximum reactor performance(Zhou et al., 2014). This aids in the removal of surplus biomass and the distribution of oxygen throughout the biofilm(Bassin et al., 2016).

   - **Reaction Rate Kinetics:** For bioremediation processes, the reaction rate kinetics of the microorganisms (μ) plays a crucial role in determining the biodegradation efficiency. The Monod equation is commonly used to describe the specific growth rate of microorganisms in response to the concentration of a limiting substrate(Ahmad et al., 2021; Kargi, 2009):

     $$\mu = \mu_{max} \times (S / (K_s + S))$$

     Where: μ = Specific growth rate of microorganisms (per time, e.g., per hour)
     μ_{max} = Maximum specific growth rate (per time)
     S = Substrate concentration (e.g., organic pollutants) (in mass per volume, e.g., mg/L)

K_s = Substrate half-saturation constant (in mass per volume, e.g., mg/L)

8. **Moving Membrane Reactors:** Moving membrane reactors (MMRs) are a form of sophisticated wastewater treatment technology that combines bioremediation and membrane filtration principles(Azubuike et al., 2016). MMRs are intended to improve biodegradation efficiency while also providing solid-liquid separation using submerged or connected membranes(Friha et al., 2014). This novel technique has a number of advantages in wastewater bioremediation applications. Like other biological wastewater treatment processes, MMRs rely on the activity of microorganisms, primarily bacteria, to degrade organic pollutants present in the wastewater(Marimuthu et al., 2020). These microorganisms form a bio film on the surface of the membranes or on carriers within the reactor, creating a favourable environment for efficient pollutant removal(Zhong et al., 2019). When compared to standard biological treatment procedures, the combination of bioremediation with membrane filtration in MMRs improves process efficiency and dependability(Oller et al., 2011). MMRs transcend the limits of conventional clarifiers by integrating membrane filtration, resulting in improved solids-liquid separation and higher-quality effluent(Wang et al., 2021). MMRs are distinguished by their compact design, which requires less room than separate bioreactors and sedimentation tanks used in conventional treatment methods(Qyyum et al., 2020). Because of this space-saving characteristic, MMRs are appropriate for applications with restricted land availability(Visvanathan et al., 2000). MMRs frequently create less surplus sludge than conventional activated sludge systems due to the efficient solid-liquid separation achieved by the membranes(Bernardo et al., 2021). This may result in lower sludge treatment and disposal expenses. MMRs can be used with other advanced treatment techniques including as anaerobic treatment, denitrification, and phosphorus removal to remove additional pollutants and meet stringent effluent regulations(Bashar et al., 2018; Fulazzaky et al., 2015).

   - **Reactor Volume Calculation:** The volume of the MMR can be calculated based on the influent flow rate and the desired hydraulic retention time (HRT) for effective bioremediation. The HRT is the average time a wastewater particle spends inside the reactor(Chakraborty & Veeramani, 2006; Healy et al., 2012). The formula for reactor volume (V) is:
     V = Q × HRT

     Where: V = Volume of the reactor (in cubic meters, m^3)
     Q = Influent flow rate (in cubic meters per hour, m^3/hr)
     HRT = Hydraulic retention time (in hours, hr)

9. **Anaerobic Waste Water Treatment:** In the absence of oxygen, bacteria break down organic matter in anaerobic wastewater treatment. This method is very beneficial for high-strength industrial effluent containing complex organic components. As a by-product of anaerobic treatment, biogas, primarily methane, is produced, which can be used to generate electricity. However, anaerobic treatment is slower than aerobic treatment and may require additional polishing procedures for further purification.

- **Hydrolysis:** Hydrolysis plays a significant role in wastewater bioremediation, especially in the initial stages of organic matter degradation(X. Li et al., 2018). It is a biological process where complex organic compounds, such as proteins, carbohydrates, and lipids, are broken down into simpler, soluble compounds through the action of hydrolytic enzymes produced by microorganisms(Cammarota & Freire, 2006). Hydrolysis is a crucial step in the overall biodegradation process, as it converts large, difficult-to-degrade molecules into smaller, more accessible substrates that can be further metabolized by other microorganisms(Nikel et al., 2014). In wastewater treatment systems, a diverse group of microorganisms, including bacteria, fungi, and protozoa, play a crucial role in hydrolysis(Song et al., 2021). These microorganisms can be present naturally in the incoming wastewater or can be introduced deliberately in bioremediation processes(Wang & Yang, 2014). Microorganisms attach to surfaces, such as suspended particles or support media in bioreactors, and form bio films. Within the bio film, certain microorganisms secrete hydrolytic enzymes, which are specific enzymes that catalyze the breakdown of different types of complex organic compounds(Gaur et al., 2018). For example, proteases break down proteins, lipases break down lipids, and amylases break down starches and carbohydrates(Goodman, 2010). The hydrolytic enzymes act on the complex organic compounds present in the wastewater, breaking them down into simpler forms(Parawira, 2012). For instance, proteins are hydrolyzed into amino acids, carbohydrates into simple sugars, and lipids into fatty acids and glycerol("Lipid and Carbohydrate Metabolism in Caenorhabditis Elegans," 2017).

- **Acidogenesis:** Acidogenesis is an important stage in wastewater bioremediation, especially in anaerobic treatment systems. It is the second step of anaerobic digestion after hydrolysis and is critical in converting the simpler organic molecules generated during hydrolysis into volatile fatty acids (VFAs) and other intermediate products(Singhania et al., 2013). Acidogenesis prepares the way for the ultimate step of anaerobic biodegradation, methanogenesis, in which methane (biogas) is produced. The first phase in anaerobic bioremediation is hydrolysis, which happens before acidogenesis. The activity of hydrolytic enzymes generated by microorganisms breaks down complex chemical substances into simpler forms such as amino acids, carbohydrates, and fatty acids during hydrolysis(Dent et al., 2004; Liang et al., 2021). The acid-forming bacteria ferment the soluble substrates, turning them into VFAs and other intermediate products(W. S. Lee et al., 2014). VFAs are required for the next stage of anaerobic biodegradation, methanogenesis. Acidogenesis produces VFAs, which serve as precursors for the last stage of anaerobic biodegradation, methanogenesis(Sekoai et al., 2021). Methanogenic microbes use VFAs to create methane (CH_4) and carbon dioxide (CO_2) through a series of metabolic processes during the methanogenesis stage(D' Silva et al., 2021).

- **Acetogenesis:** Acetogenesis, which occurs after acidogenesis but before methanogenesis, is an important intermediate stage in anaerobic wastewater bioremediation. Certain bacteria transform the volatile fatty acids (VFAs) generated during the acidogenesis stage into acetic acid (commonly known as vinegar) and other simple organic compounds during acetogenesis(Y. Li et al., 2015). This process prepares the way for the ultimate phase of anaerobic biodegradation, methanogenesis, in which methane (biogas) is produced(Krzysztof Ziemiński, 2012). Acidogenesis and Hydrolysis the first phases in anaerobic bioremediation are hydrolysis and acidogenesis, which occur before acetogenesis. Complex organic substances, such as amino acids, carbohydrates, and fatty acids, are broken down into simpler forms during hydrolysis(Dignac et al., 2000). In the acidogenesis stage, acid-forming bacteria ferment these simpler molecules, producing VFAs as a result. During the acetogenesis stage, the VFAs serve as substrates for acetogenic bacteria. Acetogenic bacteria are microorganisms that convert VFAs, specifically acetic acid precursors, into acetic acid and other simple organic compounds(Merlin Christy et al., 2014).Acetogenic bacteria use the Wood-Ljungdahl route to convert VFAs, particularly longer-chain VFAs such as propionic acid and butyric acid, into acetic acid (CH3COOH)(Im et al., 2018). The reduction of carbon dioxide (CO2) using hydrogen (H2) or other electron donors results in the production of acetic acid as a metabolic byproduct(Parshina et al., 2010). As an intermediate product of acetogenesis, hydrogen (H2) and carbon dioxide (CO2) are produced. These gases are important in the later methanogenesis stage, where methanogenic microbes use them(Bassani et al., 2015).

- **Methanogenesis:**Methanogenesis is the final and most important stage in anaerobic wastewater bioremediation. During methanogenesis, methanogens use intermediate products created in earlier stages, such as acetic acid (from acetogenesis), to make methane (CH4) and carbon dioxide (CO2)(G.-F. Zhu et al., 2008). This technique is critical in the production of biogas, a sustainable energy source, while also lowering the organic pollution load in wastewater(Shen et al., 2015). Prior to methanogenesis, the primary phases in anaerobic wastewater bioremediation include hydrolysis, acidogenesis, and acetogenesis. Complex organic substances, such as amino acids, carbohydrates, and fatty acids, are broken down into simpler forms during hydrolysis(S. Mani et al., 2016). Acid-forming and acetogenic bacteria ferment VFAs and create acetic acid in the ensuing acidogenesis and acetogenesis phases, respectively. Acetic acid (CH3COOH) and other intermediate metabolites from acetogenesis serve as substrates for methanogenicarchaea during the methanogenesis stage("Retracted," 2017). Methanogens are a kind of anaerobic bacteria that may produce methane (CH4) as a metabolic byproduct. For methane generation, methanogenicarchaea use two basic pathways: the acetoclastic pathway and the hydrogenotrophic pathway(Guo et al., 2015). Acetoclastic Route: Acetoclastic methanogens employ acetic acid (CH3COOH) as a direct substrate and convert it to methane (CH4) and carbon dioxide (CO2)(Laloui-Carpentier et al., 2006). Hydrogenotrophic methanogens use hydrogen (H2) and carbon dioxide (CO2) to create methane (CH4) in this process(Dong et al., 2019). Hydrogen is produced throughout the acetogenesis and acidogenesis phases and is a required substrate for this process. Biogas is created by collecting the methane (CH4) and carbon dioxide (CO2) generated during methanogenesis. This biogas is a useful renewable energy source that may be harvested and used to generate power, heat, or for other purposes(Atelge et al., 2020).

10. **Phytoremediation**: Phytoremediation is a subset of wastewater bioremediation in which plants and related microbes are used to extract, degrade, or immobilise contaminants from

polluted water. Specific plant species are chosen for their ability to absorb, translocate, and metabolise toxins in wastewater, thus lowering the concentration of contaminants(Susarla et al., 2002). This environmentally safe and sustainable approach may be used to treat a wide range of contaminants, including heavy metals, organic compounds, minerals, and even certain viruses(Brunner & Rechberger, 2015). The first stage in phytoremediation is to choose plant species that have a high affinity for the particular contaminants present in the wastewater. Because various plants have differing capacity to absorb and tolerate different toxins, the selection procedure is critical to the project's success(Seth, 2012). The chosen plants absorb toxins from the water through their roots after being planted in wastewater. Contaminants in water can be soluble or particulate, and plants can absorb both types depending on their qualities(Abdel-Shafy & Mansour, 2016). Following absorption, pollutants travel via the plant's circulatory system (xylem) and are distributed to various plant components such as leaves, stems, and roots(Wild et al., 2006). Plants may collect pollutants in their above-ground tissues in some situations, allowing for simple removal from the system by harvesting the plants. In addition to direct absorption and accumulation, certain plants may metabolise and destroy contaminants via phytodegradation. Pollutant breakdown and transformation can also be aided by microorganisms found in the rhizosphere (root zone) of plants. Through a process known as phytovolatilization, some plants can release pollutants in a volatile form(Limmer & Burken, 2016). This is especially useful for volatile organic compounds (VOCs). Pollutants are held and filtered by the plant roots during rhizofiltration, thereby lowering their concentration in the water(Dushenkov et al., 1995). Once the plants have fulfilled their function in some phytoremediation applications, they are collected and removed from the wastewater. Depending on the level of contamination in the plants, they may need to be appropriately disposed of as hazardous waste or utilised for phytomining, which extracts precious metals from plant biomass(Rascio&Navari-Izzo, 2011).

11. **Microbial enzymes bioremediation:** Enzymes may also be superior to both microbial remediation and conventional treatments. Indeed, enzymes act against a specific substrate (microorganisms may prefer more easily degradable compounds than the pollutant), are not inhibited by inhibitors of microbial metabolism, can be used under extreme conditions limiting microbial activity, are highly effective at low pollutant concentrations, and are active in the presence of microbial predators or antagonists. They are also more mobile than microorganisms due to their smaller size.Because of all these qualities, enzymes are helpful in environmental bioremediation (Saxana et al., 2020).

According to reports, microbial enzymes have a variety of functions in different industrial applications. Due to their high specificity to a wide range of substrates (pollutants), use under conditions so harsh that microbes cannot survive, high effectiveness at low pollutant concentration, high activity in the presence of inhibitors of microbial metabolism, and high mobility (small size) than microorganisms, microbial enzymes are also helpful in bioremediation of environmental pollutants from industrial wastes. Microorganisms produce a wide range of enzymes that can be employed in the detoxification and breakdown of a variety of organic and inorganic contaminants (Saxana et al., 2020).

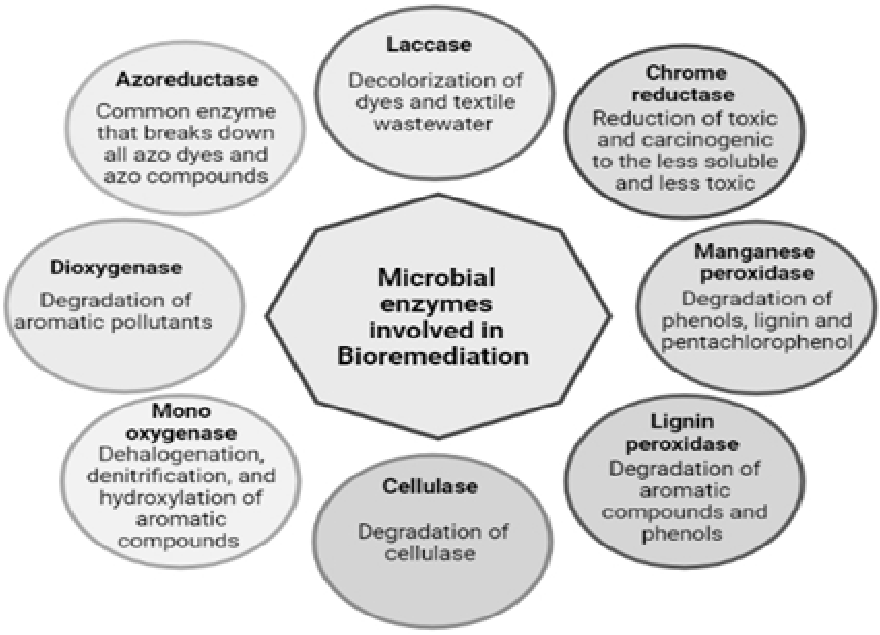

Figure 4: Microbial Enzymes and its applications in Bioremediation

12. **Algal Bioremediation:** Algal bioremediation is a potential strategy in which algae or microalgae are used to extract, degrade, or sequester contaminants from wastewater. Algae are photosynthetic microorganisms with a rapid growth rate and the ability to efficiently absorb and digest nutrients such as nitrogen and phosphorus, as well as heavy metals and organic contaminants(Abdel-Raouf et al., 2012). Algal bioremediation has various advantages, including the capacity to remove a wide range of toxins, the ability to produce biomass, and its involvement in decreasing greenhouse gas emissions via photosynthesis(Maity et al., 2014). The initial stage in algal bioremediation is to identify suitable algae species depending on the properties of the wastewater and the impurities to be remedied(Fazal et al., 2018). Because different algae have different affinities for different contaminants, the selection procedure is critical to attaining efficient cleanup. Algae are extremely efficient in absorbing nutrients, notably nitrogen and phosphorus, which are major pollutants in wastewater due to their role in eutrophication(Lin et al., 2021). As algae develop, they absorb these nutrients, lowering their amounts in the water. Certain organic contaminants can be transformed and metabolised by algae via processes such as biodegradation and bioaccumulation. They have the ability to degrade complex chemical molecules into simpler, less hazardous forms. Some algae have metal-binding properties that allow them to absorb and sequester heavy metals from water(Priya et al., 2014). Biosorption is a method that can lower the concentration of hazardous metals in wastewater. Photosynthesis occurs in algae, which creates oxygen as a byproduct. This is very useful for aerating wastewater, promoting aerobic biodegradation, and improving water quality. Once enough contaminants have accumulated in the algal biomass, it may be collected from the wastewater. Depending on the level of contamination and the algae species utilised, the collected biomass can be used for a variety of applications, including bioenergy generation, biofertilizers, and feed for livestock or aquaculture(Siddiki et al., 2022). To produce algae in a controlled environment, algal bioreactors can be configured as open ponds or closed systems. Light intensity, temperature, and nutrient supply may all

be optimised in closed systems to boost algal growth and pollutant absorption(Muñoz & Guieysse, 2006).

13. **Biofillers:** Biofillers are biologically generated natural materials that may be utilised as fillers or additives in wastewater treatment processes. Examples include agricultural leftovers, plant fibres, and microbial biomass. Because of their large surface area, porosity, and capacity to absorb contaminants, these biofillers provide several advantages in wastewater bioremediation(Razzak et al., 2022). When biofillers are used in wastewater treatment systems, they can improve pollutant removal, increase treatment efficiency, and provide a more sustainable and environmentally friendly approach to wastewater treatment. Because biofillers have a porous structure with a wide surface area, they can absorb and exchange ions with contaminants in wastewater(Xiang et al., 2020). They have the ability to collect and hold impurities including heavy metals, organic molecules, and nutrients, thereby eliminating them from the water. The contact duration between the water and the adsorbent material is increased by adding biofillers to wastewater treatment units(Paredes et al., 2016). This increased contact time enables more effective pollutant adsorption and higher removal rates. Some biofillers, particularly those formed from microbial biomass or activated sludge; contain microorganisms that can help with nutrient removal via processes such as nitrification, denitrification, and phosphorus absorption(Cohen, 2001). Through physical adsorption and microbial decomposition, biofillers can help reduce organic matter, BOD (biochemical oxygen demand), and COD (chemical oxygen demand) in wastewater(Sinha et al., 2008). Biofillers are more ecologically friendly than synthetic fillers since they are sourced from natural and renewable sources(Fombuena et al., 2014). Furthermore, certain biofillers are biodegradable, which contributes to the treatment process's overall sustainability. Biofillers can be employed in a variety of treatment systems, including as activated sludge, biofilters, built wetlands, and bioreactors(Md Anawar & Chowdhury, 2020). Depending on the application, they can be combined with other filter media or used as standalone fillers. Biofillers are cost-effective alternatives to standard synthetic fillers because they may be supplied locally from agricultural wastes or other biomass(Budzianowski, 2017; Echeverria et al., 2017).

14. **Bioaugmentation:** Bioaugmentation is a type of bioremediation approach that includes introducing particular microorganisms or microbial consortia into wastewater treatment systems to improve pollutant breakdown(Omokhagbor Adams et al., 2020). The purpose of bioaugmentation is to increase the overall performance and efficiency of biological treatment procedures by adding microorganisms with specialised metabolic capabilities, such as those for digesting certain pollutants(Juwarkar et al., 2010). This method is especially beneficial when the natural microbial population in the wastewater is inefficient in removing specific pollutants(Ahmed et al., 2022). The initial step in bioaugmentation is to identify the best microorganisms or microbial consortiums for the specific contaminants in the wastewater. These bacteria are often chosen based on their demonstrated capacity to breakdown or metabolise certain pollutants(Myers et al., 2018). To verify viability and activity, the chosen microorganisms are cultured in the laboratory under controlled circumstances(X. Li et al., 2014). The microorganisms are acclimated and adapted to the specific wastewater conditions they will encounter in the treatment system throughout this phase. Once suitably developed, the microbes are inserted or inoculated into the wastewater treatment system(Vázquez-Padín et al., 2009). This can be

accomplished by directly adding microorganisms to the wastewater, including them into the activated sludge process, or employing specialised bioreactors. The injected microbes begin interacting with the wastewater's existing microbial population(Kan et al., 2011). They add additional metabolic capacities to the system, allowing for more efficient breakdown of certain contaminants. Because foreign microorganisms can outcompete native microbes for accessible contaminants, pollutant clearance rates rise(Hamme et al., 2003). The effectiveness of bioaugmentation is dependent on adequate treatment monitoring and optimisation. Monitoring microbial population dynamics, pollutant removal effectiveness, and other pertinent metrics helps guarantee that the bioaugmentation process is successful and long-lasting(Cecconet et al., 2020). In certain situations, the imported microorganisms may become established and incorporated into the native microbial population, resulting in long-term gains in pollutant degradation even after the initial bioaugmentation process has completed(El Fantroussi & Agathos, 2005).

15. **Nanomaterials for Adsorption and Catalysis:** In the realm of wastewater bioremediation, nanomaterials have showed considerable potential, notably in adsorption and catalytic processes. Their distinct features, such as large surface area, customizable surface chemistry, and increased reactivity, make them extremely effective in removing pollutants from polluted water and catalysing particular processes to breakdown or change pollutants(Tijani et al., 2014). Nanomaterials with a high surface area per unit mass, such as nanoparticles and nanotubes, provide more active sites for pollutant adsorption. Nanomaterials' surface chemistry may be modified by functionalizing them with different chemical groups, making them extremely selective for certain contaminants(Y. Chen et al., 2017). The unique features of nanomaterials allow them to adsorb a diverse spectrum of pollutants, including heavy metals, organic chemicals, colours, and new toxins(Naseem & Durrani, 2021). Because of their tiny size and wide surface area, nanocatalysts are more reactive, enabling quicker and more efficient catalytic reactions(Söğütlü et al., 2021). Nanomaterials' surface characteristics may be tailored to have particular catalytic activity, allowing them to selectively breakdown some contaminants while sparing others(Rasmussen et al., 2010). To create highly reactive species those breakdown pollutants, nanocatalysts can be utilised in AOPs such as photocatalysis (using light) or heterogeneous catalysis (using multiple phases)(Q. Chen et al., 2014).

16. **Physical Methods of Bioremediation:** Utilizing biological agents (like microbes) to degrade or change contaminants in polluted environments is the main goal of bioremediation. However, physical techniques can also contribute to the support and improvement of bioremediation procedures Fig. 4. These physical bioremediation techniques are frequently utilized to improve the biological agents' ability to function.

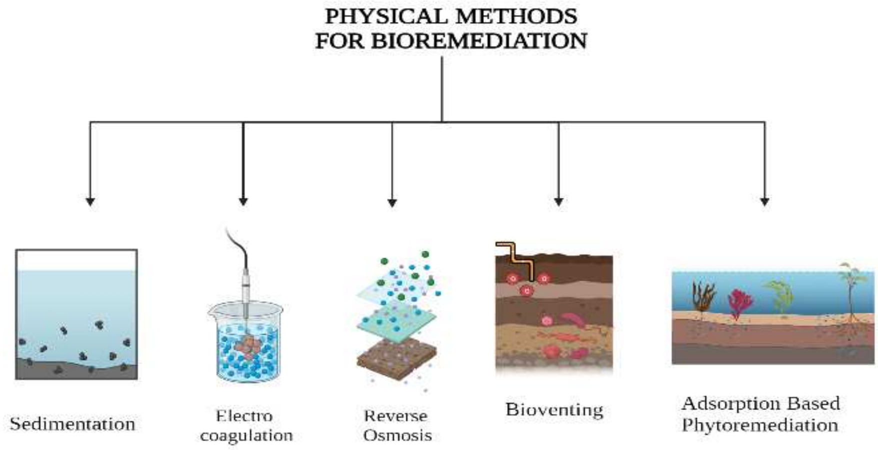

Figure 5: Different methods involved in bioremediation using physical approaches

17. **Sedimentation:** Sedimentation is a physical water treatment procedure that includes letting suspended particles to settle in water under the action of gravity(Goula et al., 2008). Sedimentation tanks, also known as clarifiers or settlers, are used to aid in wastewater treatment. The sedimentation tank receives wastewater, and the flow velocity is slowed to allow the particles to settle(Bürger et al., 2011). Sludge is formed when heavier particles, such as sand, grit, and organic debris, sink to the tank's bottom. Lighter particles, such as grease and oil, float to the top, where they create a scum layer(Samal et al., 2022). The cleared water, which is substantially free of suspended materials, is collected and treated further using techniques such as biological treatment, disinfection, and so on(Z. Li et al., 2010). Following sedimentation, the partially treated wastewater is sent to a bioreactor, which can be an activated sludge system, a sequencing batch reactor (SBR), or another sort of biological treatment unit(Ni et al., 2009). Microorganisms such as bacteria and protozoa eat organic materials as a food supply in the bioreactor, transforming it into biomass and energy(Udayan et al., 2022). This biological breakdown of organic contaminants greatly decreases the content of organic components in wastewater. Before release, the treated water is subjected to further treatment, such as sedimentation, filtration, and disinfection, to fulfil the needed effluent requirements(Naidoo & Olaniran, 2013). DAF is a water treatment technique that uses small air bubbles to remove suspended particles, oils, and greases from wastewater(Rubio et al., 2002). It is particularly useful in treating wastewater with a high concentration of small particles or compounds that are difficult to settle using ordinary sedimentation(Khoufi et al., 2007). Air is dissolved under pressure into the wastewater in a DAF system, forming small bubbles. The wastewater is then discharged into a flotation tank or basin, where the lower pressure allows the dissolved air to escape, generating micro bubbles (Niaghi et al., 2015). These micro bubbles cling to the suspended particles and float them to the surface, where they create a froth layer (the float) (Landels et al., 2019). The float is skimmed off the surface, eliminating the wastewater's suspended particles, oils, and greases. DAF improves the effectiveness of downstream processes such as bioremediation by removing suspended particles, oils, and greases from wastewater(Jafarinejad & Jiang, 2019). DAF protects the bioreactor from clogging and inhibitory compounds by lowering organic load and solid particles, enabling steady and optimal biological treatment performance(di Biase et al., 2019).Following DAF, the

wastewater enters the bioreactor for additional treatment, where microorganisms break down the residual organic contaminants biologically. The combined procedures produce high-quality effluent that fulfils environmental regulations or may be utilised for a variety of uses(Luque et al., 2008).

18. **Electro coagulation:** Electro coagulation (EC) is a useful and successful pre-treatment technology for wastewater bioremediation. Electro coagulation improves the overall effectiveness of the treatment system by eliminating specific impurities and preparing the wastewater for greater biodegradation by microorganisms when paired with bioremediation techniques(Othmani et al., 2022). An electrochemical water treatment technique that employs an electric current to destabilize and agglomerate suspended solids, colloidal particles, and certain dissolved chemicals in wastewater is known as electro coagulation(Mollah et al., 2001). The procedure is carried out in an electro coagulation cell with metal electrodes (often aluminium or iron). When an electric current is given to the anode, metal cations are produced, which then neutralise the charged particles in the wastewater, resulting in the creation of coagulated flocs(Harif & Adin, 2007). Electro coagulation (EC) can be used as an efficient and helpful procedure. Electro coagulation efficiently eliminates colloidal and suspended particles that might otherwise obstruct or impede microorganisms' access to organic contaminants during the biological treatment process(Ammar et al., 2023). Heavy metals may be precipitated and removed from wastewater via electro coagulation. Because heavy metals can be hazardous to microorganisms during the bioremediation process, removing them beforehand enhances biodegradation effectiveness(Mao et al., 2015). The pH of wastewater may be adjusted via electro coagulation. Some bioremediation processes perform best in specific pH ranges, and EC can assist in bringing the pH to an acceptable level(Savage & Tyrrel, 2005). Organic molecules in wastewater can be partially broken down by electro coagulation, lowering the organic load that must be handled by the bioremediation process. This makes the bioremediation process more efficient(Valli Nachiyar et al., 2023).

III. EMERGING BIOREMEDIATION TECHNIQUES

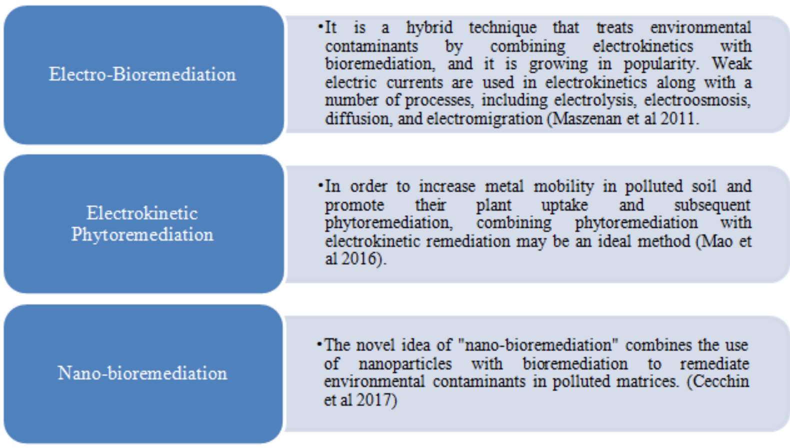

Figure 6: Emerging Bioremediation Techniques
Bhargava et al., 2020, Saxena and Bharagava (2017)

IV. CHALLENGES

Compared to traditional remediation methods, which are expensive, ecologically harmful, and produce secondary pollution that harms the ecosystem, bioremediation has arisen as a low-cost substitute. The main difficulties with enzymatic bioremediation technology are the toxicity of the result of the enzyme-mediated reaction, which should be less toxic than the substrate, which are problems related to the employment of enzymes in bioremediation. If an enzyme needs a cofactor, using it may be difficult unless a preparation that includes the cofactor and the enzyme is utilised.Their practical implementation is severely hampered by the high expense of enzyme extraction and purification, particularly when their continuous feeding is required (Roa et al 2010, Saxana et al., 2020).Enzymatic remediation technology can offer the greatest cleanup option for polluted settings by overcoming the restrictions mentioned above.

V. CONCLUSION

Wastewater is a significant contributor to environmental pollution and toxicity, and bioremediation is an environmentally beneficial method for managing such hazardous waste. Finding an environmentally acceptable waste management system is constantly a crucial component of sustainable growth. Globally, scientists are putting a lot of effort into developing environmentally safe remediation technologies. In order to counteract the risks to the environment, microbes are frequently seen as the environmentally benign instruments for the treatment and management of industrial wastes comprising extremely harmful organic and inorganic contaminants. Using genetic engineering methods, we may design organisms particularly for the bioremediation procedure. By using this technology, we may introduce two different types of genes into the organism: first, derivative genes, which may encode the protein necessary for the decomposition of contamination, and second, reporter genes, which may assist in detecting the degree of pollution. Therefore, site characterization is essential for the bioremediation approach to succeed as it aids in the development of a more appropriate and workable technology. To make bioremediation methods, such as phytoremediation, economically viable in the field, however, ongoing efforts are needed.

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