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**BIOREMEDIATION OF INDUSTRIAL TOXINS THROUGH MICROBIAL  
IMMOBILIZED ENZYMES: A COMPREHENSIVE REVIEW**

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**Abstract**

Industrialization is a major cause of ecological disturbance. Industrial pollutant contains a wide range of recalcitrant pollutants, which destructive effects to human and other living beings. Therefore, their proper removal and management is necessary for the sustainable environment. Bioremediation is a dominant and sustainable technique of waste removal, which not only remediates the pollutants, but also produces safe and eco-friendly co-products. This chapter

introduces the industrial pollutants, their effects, and remediation techniques. Also discuss about the degradation by immobilized enzyme, their carrier material and application in the various pollutants degradation.

**Keywords:** Industrial pollution; Waste water; Dye; Plastics; Microbial enzymes; Immobilization technique.

## Introduction

Industries are the main source which improves the wealth of the developing country but it is also the most important cause of emitting the recalcitrant pollutants into the atmosphere. Rapid industrialization, released a huge amount of recalcitrant environmental pollutants into the ecosystem that cause a detrimental effect to the animals, plants and humans (Saravanan et al., 2021; Weralupitiya et al., 2021). Amongst the several ecological pollutants plastics, pesticides, dyes, heavy metals and polycyclic aromatic hydrocarbons (PAHs) are the chief environmental pollutants because of their mutagenicity, persistence and non-biodegradability (Carolin et al., 2021; Vardhan et al., 2019). The metal ion like iron (Fe), manganese (Mn), zinc (Zn), cobalt (Co) and copper (Cu) are the vital elements which are essential for the development and metabolic activity of the organism, at higher concentration these elements can cause detrimental effect to the organisms (Rathi and Kumar, 2021). Some other metal elements like arsenic (As), chromium (Cr), nickel (Ni), lead (Pb), cadmium (Cd) and mercury (Hg) which are released from the various industries like paint, electroplating, metal processing and mining are lethal yet at very low concentration (Singh et al., 2018; Zhang et al., 2020). Dyes are the most significant colourants which are used in numerous manufacturing processes like leather, paper, food, paint, cosmetics and textile industries. When unprocessed dye containing industrial waste released into the atmosphere that causes various toxic effects to the living person (Sivaranjane and Kumar 2021; Varjani et al., 2020). Plastics are organic industrial and food pollutants which are derived from the organic and inorganic substances like natural gases, cellulose, coal and crude oil. Plastics are non-biodegradable, long term persisting pollutant which produces polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) that cause toxic effect to the ecosystem animals (Schmaltz et al., 2020). Polyvinyl chloride (PVC), Lowdensity polyethylene (LDPE), polypropylene (PP), High-density polyethylene (HDPE) and Polyethylene terephthalate (PET) are the most common type of plastic. Other than these pesticides are the agricultural pollutants which decrease the soil fertility and after rainwater overflow cause water pollution affects the aquatic flora and fauna (Hassaan and El Nembr, 2020). Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants which are derived from the industrial activities like that coal, petroleum and gas industries after the combustion process. PAHs are highly recalcitrant, persistent organic pollutants cause carcinogenic and teratogenic effects (Agrawal et al., 2021a).

Different physical, chemical and biological processes are employed for the reduction of these xenobiotic pollutants from the environment. Among all of them bioremediation means removal of pollutants through microorganism are the most reliable, cost effective, ecofriendly, safe and suitable method of degradation (Rathi et al., 2021), microorganisms secrets various enzymes like

laccases, peroxidases, oxygenases, phosphodiesterases, oxidoreductases, hydrolases and lipases (Sharma et al., 2018). Enzymes lower the activation energy of the toxic pollutants (Karthik et al., 2021). Enzyme regulated oxidizing responses are promising method for the degradation of contaminants. But one of the main challenges associated to the enzymatic remediation is the weak catalytic sustainability. Different form of physiological environmental conditions like pH, temperature, presence of solvents, protein denaturants and inhibitors affects the enzymes activity. For the protection of enzyme activity we can encapsulate enzyme into nanoparticles (Zheng et al., 2018) and the main reason of these process is the reusability of encapsulated enzymes, improving enzyme thermostability, increased selectivity and activity, high resistant beside inhibition, quick reaction termination by lowering the activation energy and easily detachment of enzyme from the product (Es et al., 2015; Katuri et al., 2009; Liu et al., 2020). The encapsulation of enzymes within the nanoparticles is a promising technology (Yan et al., 2006) in the bioremediation approach. Various materials and immobilization procedures have been applied in the lab scale and there have a wide variety of applications in the not only bioremediation but also in the food processing, biodiesel production and textile industries (Es et al., 2015). The effect of immobilized nanoenzymes on the pollutants like dyes, polycyclic aromatic hydrocarbons (PAHs), pesticides, heavy metals and plastics have been described in the detailed.

### **Industrial pollutants**

Industrial pollutants or waste are those unwanted substances which are produced during the industrial activity or product formation process. These are two types: 1. Liquid, 2. Solid waste.

1. Liquid waste: waste water or effluents discharged from various industries. It is a very hazardous for living beings and environment.
2. Solid waste: waste generated from different industrial processing in the solid form.

The type of waste generated showed in figure 1, it's totally dependent on the nature and characters of the industries. Industries frequently released waste water which are containing high chemical oxygen demand (COD), biological oxygen demand (BOD), Total dissolved solids (TDSs) and organic, inorganic pollutants (Bharagava et al., 2020). On the basis of their nature industrial waste are categorized into two types 1. Inorganic waste 2. Organic waste. Inorganic waste or pollutants includes heavy metals likes Nickel (Ni), Lead (Pb), Arsenic (As), Chromium (Cr), Cadmium (Cd) and Mercury (Hg) which are toxic and non biodegradable. While organic waste or pollutants consist of persistent organic pollutants (PAHs, PCBs), phenols (chlorinated, nonylphenols), phthalic esters, pesticides, azo dyes etc. both types of inorganic and organic pollutants cause serious toxic, carcinogenic, teratogenic and mutagenic effects into the living beings (Chandra et al., 2008; Saxena et al., 2017). Table 1 showed the different types of industrial pollutants and their effect in the environment. Due to the lethal nature of wastewater pollutants many environmental protection agency like World Health Organization (WHO), United States Environmental Protection Agency (USEPA) and Agency for Toxic Substances and Disease Registry (ATSDR) recognized as priority pollutants. Industrial waste water cause soil and water pollution along with show their bad affects into the fauna and flora of the terrestrial and aquatic ecosystem (Sharma et

al., 2021). Once industrial waste water generated, it not only toxic affects the ecosystem but also reduce the fresh land and water resources, therefore harmful effects shows on the future generations. As a result the toxic pollutants must be removed from the industrial wastewater to keep away their harmful effect and to restore their important products.

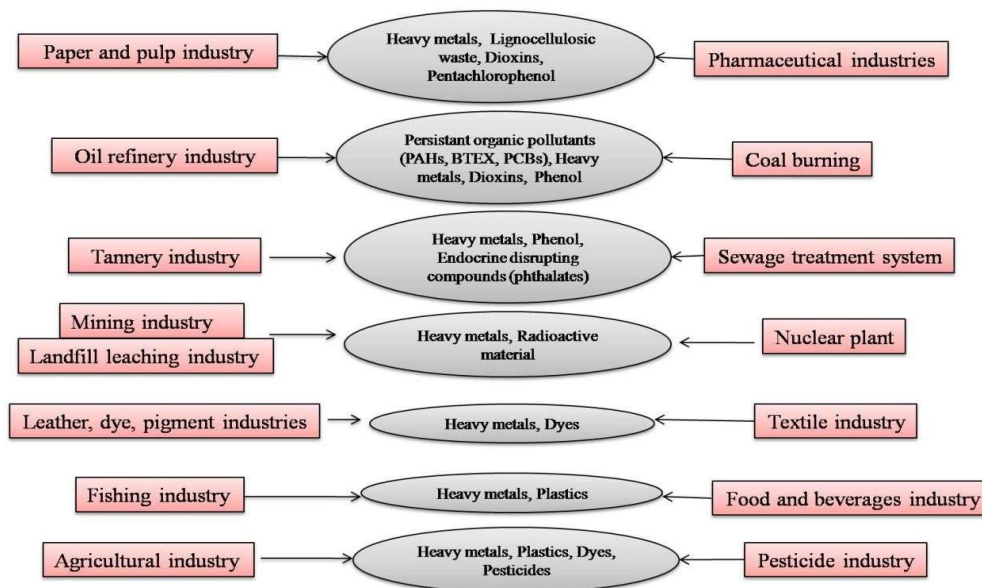


Figure 1: Types of Industries and their pollutants.

Table 1. Different types of Industrial pollutants and their effect in the environment.

S. No.	Types of Pollutants	Source	Environmental effect	References
A.	<b>Inorganic pollutants</b>			
1.	Heavy Metals			
a.	Arsenic (As)	Mining industries, landfill leaching, Agriculture	Cancer, skin problems, reduces soil fertility, damage pregnancy, stimulates the ROS (reactive oxygen	Muzaffar et al., (2022); Saravanan et al., (2021)
		wastewater, Combustion process	species) production, disturb the central and cardiovascular system.	
b.	Cadmium (Cd)	Agriculture wastewater, landfill leaching	Damage the kidney and bones (osteotoxic effect), itai-itai disease, hepatic injury, DNA damage, teratogenic effect.	Genchi et al. (2020); Kumar and Sharma, (2019)
c.	Mercury (Hg)	Mining, coal burning, cement	Behavioral and neurological disorders, blindness,	Engwa et al. (2019); Han et al.

		industries	chlorosis, developmental defects.	(2022)
d.	Chromium (Cr)	landfill leaching, Tannery wastewater, leather, dye, pigment industries	Skin and nasal irritation, cancer, bone marrow depression, lung carcinoma	Saxena and Bharagava (2017); Hossini et al. (2022)
e.	Lead (Pb)	Agriculture wastewater, landfill leaching, mining industries	Damage in CNS (central nervous system), birth defects fatigue, paralysis, renal dysfunction, mental retardation	Martin and Griswold, (2009); Tualeka et al. (2022).
f.	Copper (Cu)	Acid mine drainage	Liver damage, nausea, stomach cramps	Lim et al., 2017
B.	<b>Organic pollutants</b>			
a.	Polycyclic aromatic hydrocarbons (PAHs)	Petroleum refineries industry, aluminium production	Cause mutation, cerebellar dysfunction, destroy soil fertility, affects biodiversity,	Mallah et al. (2022); Jesus et al. (2022)
b.	Dyes	Textile, paper, paint, pulp, leather and pharmaceutical industries	Long term exposure cause cancer, skin irritation, respiratory disease, reduce the transparency of water bodies and algal growth,	Al-Tohamy et al. (2022)
c.	Pesticides	Pesticide industries, sewage treatment system	Immune suppression, cause food poisoning, reproductive abnormalities, loss of biodiversity	Tudi et al. (2022); Kalyabina et al. (2021)
d.	Phenol	Paper, oil refineries, wood preservation, distillery industrial wastewater	Hemolytic anemia, pulmonary edema, gastrointestinal damage	Di Lorenzo et al. (2021)
e.	Radioactive materials	Mining industries, nuclear plant	Cancer, mutation, damage central nervous system, destroy soil fertility and biodiversity	Adeola et al. (2022)

f.	Plastics	Fishing industries, Agricultural industries, food and beverages industries	Endocrine disruption, Genotoxicity, carcinogenic effect	Rubio et al. (2020)
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### Bioremediation

The environmental pollutants are processed by various physical and chemical techniques. For instance the waste water first filtered, flocculated, and further treated with chlorine. Another example is the wastewater treatment with electro deionization (Mistry et al., 2022). However, these techniques incur so much cost to process the pollutants and accumulate the pollutant in nature. Bio-based techniques, such bioremediation, have demonstrated a strong potential to be environmentally friendly and long-lasting approaches to treat contaminated locations (Cecchin et al., 2017). Bioremediation is a type of biological degradation of recalcitrant pollutants in which through the action of microbial enzymes toxic recalcitrant compounds are converted into non-toxic compounds and carbon dioxide and water are obtained as the end product (Agrawal et al., 2021b; Rathi et al., 2021). The process of bioremediation involves enticing microorganisms to quickly break down toxic organic chemicals in groundwater, soil, pollutants, and sediments to levels that are safe for the environment (Sharma et al., 2021). Microorganisms remediate polluted sites, through their potential to utilize components of pollutants as terminal electron acceptors to produce energy for their development (Jugder et al., 2016). Using natural attenuation, bioaugmentation, biostimulation or a combination of these mechanisms, bioremediation of contaminants can be accomplished (Deshmukh et al., 2016). Microorganisms secrete various enzymes like hydrolase, phosphodiesterase, oxygenase, laccase, lignin peroxidase, manganese peroxidase, oxidoreductase, lipase etc. which are responsible for the degradation (Narayanan et al., 2023). Enzymatic degradation is a specific technique which depends on the enzyme substrate reaction. Enzyme decrease the activation energy of the mechanism therefore the reaction needs less time to complete (Karthik et al., 2021). Enzymatic degradation processes are highly specific, flexible, easily operated, low mass transfer and provide valuable outcome even in the adverse atmospheric conditions (Kensa, 2011). Table 2 represented the degradation of pollutants by different microbial enzymes. But the selection of appropriate microbial cell which produced enzyme is difficult. After selection enzyme production and purification is also complicated and high cost process for the industrial level. To overcome all over the difficulty, scientists have been invented enzyme immobilization technique for the cost effective, easy and sustainable remediation (Koyani et al., 2016; Liu et al., 2020). According to the requirement, several material like porous bead (Bilal et al., 2017a), nanofiber (Xu et al., 2014), microsphere (Jiang et al., 2014), nanotube (Zhai et al., 2013), nanoparticles (Kalkan et al., 2012; Tavares et al., 2018) are used for the immobilization purpose. Immobilization process not only provides sustainability but also reusability of enzyme also possible. In-depth research has been done in the last years on the genetic makeup and enzyme systems of the microorganisms that are used in bioremediation (Dash and



Osborne, 2023). Latest progress in metabolic engineering and synthetic biology have allowed for the practical application of genetically modified microbial scavengers for the bioremediation of harmful pollutants (Tran et al., 2021). The use of enzyme-based biosensor systems, catabolic enzyme expression and regulation, and gene editing and manipulation can improve bioremediation (Dash and Osborne, 2023). Emerging techniques and technologies created in the fields of genetically edited microbes and microbe-assisted nanotechnology have also demonstrated to be effective bioremediation tools with significant potential to remove environmental pollutants (Saravanan et al., 2022). Genetic engineering techniques are being used to modify or engineer microbial populations in order to expand the range of contaminants from which they can be used (Yaashikaa and Kumar, 2022). Enzyme modification is possible using conventional protein engineering techniques, which entail mutagenesis of the gene of enzyme and collection of the most effective enzyme (Tran et al., 2021).

The main categories of microbes secreted enzymes that be able to break down the majority of dangerous ecological pollutants are laccases, hydrolases, lipases, oxidoreductases, and oxygenases (Narayanan et al., 2023). Moreover, few organisms have ability to degrade several compounds. For example *Rhodococcus* spp. exhibit bioremediation of phenolics, polycyclic aromatic hydrocarbons, dye and heavy metals (Nazari et al., 2022). *Pseudomonas putida* can detoxify xylene, benzene, toluene by utilizing them as carbon source (Tran et al., 2021). The bioremediation procedure is influenced by the several factors such as types of microorganisms, their optimum pH, temperature and their interaction with the pollutants (Saravanan et al., 2022).

Bioremediation procedure can be performed *in-situ* and *ex-situ*. *Ex-situ* bioremediation methods are employed to manage garbage that has been removed from a polluted area and processed in different site utilizing physicochemical and biological procedures (Sharma et al., 2021). For instance petroleum hydrocarbons can be removed from groundwater at low cost via *in-situ* bioremediation (Pathania et al., 2023). Bioremediation is aided by the technology such as quantum chemistry, machine learning and biomolecular modelling etc (Xu et al., 2023).

### **Bacterial Bioremediation**

Microbes, particularly bacteria, are excellent attractive candidates for diverse *in-situ* and *ex-situ* remediation procedures due to their small genome size, quicker replication time, basic cellular organization, rapid evolution and adaptation in a contaminated atmosphere. Rhizobacteria that promote plant growth (PGPR) are one of the possible choices for organophosphate pesticide remediation and are frequently utilized to increase the phytoremediation capacity of plants (Dash and Osborne, 2023). Further, species of *Bacillus* and *Achromobacter* associated with *Cannabis sativa* plant helps to degrade heavy metals and organic pollutants from distillery sludge (Singh et al., 2023).

**Table 2: Degradation of pollutants by microbial enzymes.**

Type of pollutant	Microorganisms	Enzymes involved	References
Organohalide	<i>Sulfurospirillum multivorans</i>	Reductive Dehalogenases	(Jugder et al., 2016)
Organophosphate pesticide	<i>Agrobacterium radiobacter</i>	Organophosphate hydrolase	(Horne et al., 2002)
	<i>Brevundimonas diminuta</i>	phosphotriesterase	(Thakur et al., 2019)
polychlorinated biphenyl	<i>Bacillus subtilis</i>	Methyltransferase	(Sun et al., 2018)
Hydrocarbon	<i>Alcanivorax borkumensis</i>	Lipase, esterase, alkane hydroxylase	(Kadri et al., 2018)
Textile dyes	<i>Anoxybacillus</i> spp.	Azoreductases,	(Mishra et al., 2023)
	<i>Bacillus amyloliquefaciens</i>	Peroxidases	(Ren et al., 2022)

### Mycobioremediation

Mycoremediation, which uses fungi or its derivatives to remediate environmental pollutants, is a relatively economical, environmentally benign, and efficient process (Beltran-Flores et al., 2022). The fungus can be used to remove a wide range of contaminants since they have enzymatic machinery for doing so (Kulshreshtha et al., 2014). The degradation of pollutants by fungi involves several enzyme systems such as catalases, cytochrome P450 monooxygenases, laccases and peroxidases (Deshmukh et al., 2016). The *Aspergillus* species is well demonstrated in the bioremediation of triphenyl phosphate (Feng et al., 2022), heavy metals (Talukdar et al., 2020) and lead (Sharma et al., 2020). Additionally, Abd El Rehim et al. (2016) utilized six species of *Aspergillus* for the bioremediation of textile Azo dyes. The white rot fungi such as *Bjerkandera ajusta*, *Trametes versicolor* and *Phanerochaete chrysosporium* produces laccases and peroxidases enzymes and have been reported for their ability to detoxify pesticides (Deshmukh et al., 2016). Fungal community associated with the root of the plants are also helpful in the remediation of contaminants. For instance, for the bioremediation of petroleum-contaminated soils *Polygonum aviculare* and its penetrated (root-associated) fungal strains can be used (Mohsenzadeh et al., 2010). Further, *Fusarium* associated with *Ricinus communis* helps in removal of heavy metal pollution (Yao et al., 2023).

### Nano bioremediation

Nanobioremediation is the incorporation of nanotechnology and bioremediation, it is a promising and beneficial method for removing environmental contaminants (Bhatt et al., 2022; Chaudhary et al., 2023). In this technology enzymes and other biomolecules from microorganisms are immobilised in the form of nanoparticles. These nanoparticles have a higher capability for cleanup of contaminants than complete cells because they penetrate efficiently into a contamination zone.



Further, they exhibit higher reactivity redox redox-amenable pollutants (Rizwan et al., 2014). Recently, more focus has been placed on using nanomaterials, in particular iron nanoparticles, as an inventive technique for the removal of pollutants in the contaminated sites (Cecchin et al., 2017). Moreover, pesticides, heavy metals, organic dyes antibiotics and other pollutants are remedied *in-situ* using manganese oxide, ferric oxide and gold nanoparticles (Bhatt et al., 2022). Many studies have demonstrated that unique traits of nanoparticles, like enhanced catalysis and adsorption character with high reactivity, have been the focus of considerable research. For instance, chitosan coated nanotubes prepared by incorporation of bacterial peroxidases helped in the degradation of textile dye (Ren et al., 2022). On the other hand, nanoparticles pose several limitations such as their toxicity towards the natural microorganisms present in the pollutant site, their clustering properties, their involvement in food chain etc. (Cecchin et al., 2017). Another limitation is the cost of nanoparticle preparation and application in the polluted site. Moreover, for the synthesis of nanoparticles for nanobioremediation, knowledge of microbial enzymes and the bioremediation mechanism is required. But the almost useful microbes secreted enzymes and their capacity to efficiently break down several pollutants or their potential for transformation are unknown at this time (Narayanan et al., 2023).

### **Enzyme immobilization technique**

Enzyme immobilization is the technique of fixing or immobilizing any substances (enzyme) into the matrix therefore it can be able to perform any reaction without any type of substance loss and reusability of them (Katchalski-katzir and Kraemer, 2000). Some of the enzymes have high charge value and sometimes the separation of enzyme and products happen to very difficulty. Immobilization process makes easy separation of enzyme from the product because it converts liquid form of enzyme into solid form hence immobilization technique is considered as a potent tool (Kulkarni, 2002). The enzyme immobilization is a rising technique which facilitates many advantages over free enzyme use.

When free enzyme present in the reaction mixture it's have complete mobility and no any type of other barrier which prevent the reaction between the substrate and enzyme but in the case of immobilization, the mobility of enzyme dependent on the matrix which have been used for the immobilization. Sometimes matrix has been fragile and diffusion boundaries therefore nanomaterials are used as the matrix for the immobilization process which are prepared by the different types of degradable polymeric substances (Amaral and Felipe, 2013). Other than this free enzyme have their own kinetic model, when the enzyme is immobilized, the parameters like pH, temperature, reaction mixture and  $K_m$  can be changed therefore its need to be defined new model and check properly. Sometimes immobilization process can hinder the active site of the enzyme and restrict the specific activity of enzymes (Ramakrishna and Matsuura, 2011). The matrix applied for the immobilization, also support the growth of microbes because polymeric substrates used by microbes as their carbon source (Krishna, 2011). In the various industrial process pellet

formation, flocculation and surface attachment are considered as the immobilized form (Akin, 1987).

### **Support matrix for enzyme immobilization**

For the immobilization process appropriate support matrix and technique for the enzyme is also necessary. Immobilization technique and support matrix should be applied carefully for successful result according to the enzyme. A support material should contain high loading capacity and stability for the chemically and mechanically damage during the immobilization process. A perfect support material permits the easy and viable separation of enzyme from their support matrix. Different variety of support material available for the immobilization process. Their chosen process depends on the type of enzyme, overall production cost, biocompatibility, product specificity and availability (Es et al., 2015).

### **Types of different support matrix**

#### **Collagen**

Collagen is more applicable material for the immobilization process because of their availability and biocompatibility. The sources of collagen includes bone, skin, tendon and cartilage, contain abundant amount of collagen tissue. This natural collagen polymer binds with the porous films and sponges. Collagen attached the cell strongly but the high cost of processing like purification makes the use of collagen as the immobilization matrix most challenging (Nedovic and Willaert, 2004).

#### **Alginate**

Alginate is a type of heteropolymer consists of L-guluronic and D-mannuronic acid. The alginate is obtained from marine algae and after processing it is extracted in the form of sodium salt. Alginate are highly available, easily gelation with calcium and biocompatible in nature therefore it is used as an immobilization matrix, but due to the loss of ion (divalent) in the solvent limits the use of alginate.

#### **Chitosan**

Chitosan is achieved by the deacetylation process of chitin. Animal shells like crustacean shells, insects, mollusk and fungi are the major source of chitin. Chitosan can be enzymatically hydrolyzed, and also formed gel with the help of crosslinking (ionic/chemical) with glutaraldehyde. Chitosan have biocompatible therefore abundantly used in the food, pharmaceutical industries and other biotechnology application like remediation etc. but its show low mechanical strength therefore other material like collagen, calcium phosphate can be used with the chitosan (Nedovic and Willaert, 2004).

#### **Agarose**

Agarose is a gel like appearance of agar and constructed after the purification process. Agar and agarose are a polysaccharide which is used in the immobilization process. Agarose gel formed pentagonal pore which allow diffusion of many molecules. They have generally soild in cold temperature and also formed thermal reversible gel beads.

### **Polyacrylamide**

Polyacrylamide gel is generally used for the partition of compounds according to their shape and size (Tampion and Tampion, 1987). Polyacrylamide is a synthetic polymer, can be used for the recovery and binding of metals like Zn, Cu, Au and Hg.

### **Enzyme immobilization technique**

For the each immobilization process specific technique required it's save time and cost of the reaction means process yield. Immobilization technique also increases enzyme stability, their 3D structure rigidity, hydrophilic conditions and also reduces enzyme inhibition (Cho et al., 1981).

### **Types of immobilization technique (table 3)**

#### **Physical adsorption**

Physical adsorption involves the physical attachment of enzyme with the substrate (organic or inorganic). Physical adsorption is an easy, low cost technique which is occurred by the Vander Waal force, hydrophobic interaction, hydrogen bond and dipole-dipole interaction. It depends on the nature and quality of the enzyme and substrate (Ligler and Taitt, 2011). Physical adsorption permits the reusability of substrate which helps in the reduction of operational cost.

#### **Encapsulation/ Entrapment**

Encapsulation or entrapment method involves the entrapment of enzyme into the polymeric substrate. The encapsulation method is depends on the biomolecule entrapment in a polymeric base substrate, which allow the movement of less molecular mass substrate into the porous matrix. The solid matrix or gel is used for the entrapment process which will be suitable with the enzyme and substrate (Cao, 2006).

#### **Covalent binding**

Covalent binding dependent on the development of covalent bond between the support material and biomolecules (Kok et al., 2001). It is a strong bond therefore does not permit the enzyme free movement; therefore decrease the activity of enzyme and also the reusability of enzyme and substrate are difficult due to the heat stability (Aehle, 2006).

#### **Cross linking method**

This method is the combination of encapsulation and covalent binding and works in the present of cross linking agents like bis isodiacetamide and glutaraldehyde (Panesar et al., 2010).

**Table 3. Techniques of enzyme immobilization.**

S. No.	Types of immobilization technique	Advantages	Disadvantages
1.	<b>Physical Adsorption</b>	1.Simple and not expensive technique 2.Enzyme does not change their conformation 3.High catalytic action 4.Reusability is possible	1. Less stable and low yield 2. Weak interaction therefore cause desorption of enzyme 3. Microbial attack is possible into the enzyme
2.	<b>Encapsulation/ Entrapment</b>	1.Easily protected technique and chemical modification not possible 2.Permit controlled discharge of products and simple downstream process 3.Easily handled with low molecular mass compound and maintained cell mass	1. Sometimes enzyme can be leaked out from the pores 2. Limit bulk transfer is possible
3.	<b>Covalent Binding</b>	1.Strong bond and highly heat stable 2.Avoid the discharge of enzyme from the substrate	1. Loss of enzyme activity 2. Reusability not possible
4.	<b>Cross-linking</b>	1.Strong binding 2.Higher stability and leak prove	1. Loss of enzyme activity due to alteration in the active site

### **Application of immobilized enzyme in the remediation of industrial toxin**

#### **Waste water treatment**

Industrial wastewater effluent is believed as the major cause of soil and water pollution as compared to various different sources of environment pollution (Goutam et al., 2020; Saxena and Bharagava, 2017). Biological treatment of wastewater has been relevant in various industries since the twentieth century. Biocatalysts immobilization such as microbial adhesion to matrix has been intensely applied for wastewater pollution management. Naha et al. (2023) reported that in recent years synthetic biology has been explored where incorporation of both engineering and biological thoughts to improve available wastewater management techniques. They also stated that there are novel techniques and certain hypothesis can be applied on multi bedded wastewater management techniques which is more cost effective, sustainable as well as effortless handling and installation.

Mulinari et al. (2023) studied that use of aqueous polydopamine (PDA) solution is a suitable alternative for immobilization of enzyme. Lipase Eversa Transform 2.0 (ET2) was formed from GM (genetically modified) strain of *Aspergillus oryzae* used with cellulose membrane the lyophilized ET2 was immobilized by coating of PDA with two and one step method and concluded that PDA was excellent bonding agent because the modified membrane exhibited 35% oil fouling activity and higher 90% customized by two step method showcased 40% and 74% respectively. Zdarta et al. (2023) reported that new fabricated biosystem prepared by  $\text{CaSiO}_3$  and laccase used for elimination of 17  $\alpha$ ethynylestradiol (EE2) from aqueous systems. They also reported that Treatment of EE2 (100 mg) showcased 100% removal efficiency with 12h at 25°C at pH 5 and remove EE2 more than 40% from real wastewater samples. Sharma et al. (2023) stated that water pollution is caused by micropollutants (MPs) which increase toxicity in the environment. He also reported that microbial enzymes specially immobilized biocatalysts is a potent approach for the reduction of environmental pollution.

Maghraby et al. (2023) reported that various enzymes play a major role in industrial applications such as lipase reported for oil fouling activity in industrial effluents due to ability to degrade lipids, phospholipids and many more. Similarly, protease, amylase and cellulase are major enzymes used for remediation of environmental pollution. Iqhrammullah et al. (2023) stated that due to emerging contaminants results in global threat to human health so to overcome these problems laccase a multicopper enzyme has been applied for its ability to treat wastewater effluent from industries through the reduction reaction in the presence of phenolic and non-phenolic compounds. Verdel et al. (2023) investigated that pulp and paper manufacturing industry is the third largest producer of industrial wastewater so here microbial enzymes can remove pollutants or can also convert polymers such as cellulose, hemi-cellulose and lignin into new and value added products.

Yu et al. (2023) reported that accumulation of metronidazole (MNZ) inhibits wastewater treatment biosystems. They also discovered that immobilized *Aspergillus tabacinus* LZ-M could eliminate 77.39% of 5mg/L MNZ. Preethi et al. (2022) investigated that enzyme based bioremediation technology plays a keen role now a days. Oxidoreductases act as a biocatalyst and a promising candidate for wastewater treatment. Salehipour et al. (2022) reported metal-organic frameworks work as a good candidate for supporting material of immobilization of enzymes. These composites (MOF-enzyme) play a very vital role in treatment of wastewater and elimination of hazardous organic pollutants. Zdarta et al. (2021) reported the use of immobilized oxidoreductases in wastewater management for the elimination of phenolic compounds which is a promising choice for future of wastewater decontamination. Sharavanan et al. (2021) reported that enzymes such as hydrolases, dehalogenases, transferases, oxidoreductases and oxygenases are the important microbial enzymes which are responsible for the elimination of recalcitrant pollutants present in the atmosphere for example heavy metals, pesticides, dyes etc.

## Dye

Iqhrammullah et al. (2023) stated that laccase (multicopper) enzyme has been reported for its also ability to break harmful contaminants such as azo dyes. Alsukaibi et al. (2022) investigated the use of nanoparticles coated with enzyme based technology provide an excellent result for the removal of wide variety of dyes from wastewater, laccase, lignin peroxidase and many more in combination with metallic nanoparticles. Al-Tohamy et al. (2022) reported that enzyme immobilization appears a promising approach in textile dye degradation. They also reported the application of charcoal, calcium alginate and biochar pellets for immobilization of enzymes. The immobilized HRP used to degrade azo dye (acid red) upto 61% similarly, remazol brilliant blue, reactive black-5, congo red, and crystal violet degraded upto 82.17%, 97.82%, 94.35% and 87.43% respectively. The degradation of acid-red1 upto 88% via immobilized lacasse on epoxy-functionalized polyether sulfone beads.

Morshed et al. (2021) reported the used of various immobilized enzymes such as laccase, glucose oxidase, horse radish peroxidase showcased their stability for reusability and for the degradation of heavy metals, dye and various pollutants. Saravanan et al. (2021) investigated that enzymes such as dehalogenases, hydrolases, oxygenases oxidoreductases, and transferases are the important microbial enzymes which are applicable for elimination of dyes, heavy metals, pesticides toxic pollutants. Paisio et al. (2012) also reported isolation and characterization of a dioxygenase-producing bacterium *Rhodococcus* strain with phenol-degrading capacity used in the bioremediation of tannery effluent. Similarly, Jogdand et al. (2012) reported that use of coconut coir, loofah sponge, bagasse and jute can be used as low-priced matrices for immobilization of *Aspergillus terreus* showed great reduction in biological oxygen demand (BOD) and chemical oxygen demand (COD). Amongst all of these matrices, jute was the more suitable immobilizing matrix for *A. terreus* used to reduce the pollution level in terms of the removal of the dye and organic pollutants present in the effluent. In a current investigation, horseradish peroxidase (HRP) on calcium-alginate beads via covalent immobilization with a crosslinking agent – glutaraldehyde applied to remove synthetic reactive dye. Bilal et al. (2016) reported that, high decolorization efficiency of reactive orange (RO) and reactive blue (RB) and reactive red (RR) were observed with immobilized HRP, ranging from 70% to 80%. He also reported that, it conserved 40% of its primary enzyme activity after seven cycles.

The removal of crystal violet dye through the ligninolytic bacteria *Aeromonas hydrophila* investigated by Bharagava et al. (2018a). The crystal violet and methylene blue dye degradation by laccase immobilized peroxidase which was mimicking with magnetic metal-organic structures (MMOF), was reported by Ladole et al. (2020) in batch and continuous approach. They were observed upto 90– 95% degradation and 89% enzyme activity was sustained by laccase immobilize MMOF all over the 10th cycle for 25 days. In one study reported by Aslam et al. (2021), fungus *Pleurotus nebrodensis* used for isolation of purified laccase enzyme, which was immobilized with chitosan beads and applied for the management of industrial wastewater dye



with a degradation efficiency of about 84–90%. Jankowska et al. (2021) observed that the adsorptive and covalent type of immobilization of HRP on polyamide 6 electrospun fibers (HRP-PA6) has been exploiting for the handling of Reactive Black 5 (RB5) dye and Malachite Green (MG) dye degradation and upto 70% decolorization efficiency of dye was achieved. Table 4 showed list of enzymes and their maximum removal of industrial dye-based pollutants.

**Table 4: List of enzymes and their maximum removal of industrial dye-based pollutants.**

Peroxidase type	Source	Pollutant/dye	% Removal	References
Soybean peroxidase	Commercial Bio-Research Products	Sulforhodamine B dye	100	Alneyadi et al., 2017
Chloroperoxidase			100	
Manganese peroxidase	<i>Ganoderma lucidum</i> 00679	Drimaren Yellow X-8GN	90.2	Xu et al., 2017
		Drimaren Red K4Bl	70.1	
Phytase	<i>Aspergillus niger</i> NCIM 563	Chlorpyrifos	72	Shah et al., 2017
Horseradish peroxidase	Horseradish roots	indigo	84.35	Bilal et al., 2017b
		Methyl orange	>90	Bilal et al., 2018
Turnip peroxidase	Commercial	Crystal Ponceau 6R	>97	Almaguer et al., 2018
Ginger peroxidase	Ginger	Reactive Blue 4	99	Ali et al., 2018
Laccase	<i>Oudemansiella canarii</i>	Congo red dye	80	Iark et al., 2019

### Plastic polymer Bioremediation

Plastics, is a readily accessible and convenient substance, are usually utilize in industries and in our everyday lives around the earth. Together with its convenient application, it has been recognized as a global environmental hazard (Ali et al., 2021; Cheng et al., 2021). It is certain that as a result of the rising demand and utilization of plastic products, which has resulted in an addition of a huge number of old or spent plastics in the surroundings (Kumar et al., 2021). Plastic waste becomes an environmental contaminant that, even after 100 years, cannot be degraded in the natural environment (Ricardo et al., 2021). By 2025, 11 billion tones of plastics are imagined to collect in the atmosphere (Brahney et al., 2020). The current rate of plastic recovery, however, is less than 5%.

Furthermore, plastics known as "white pollution" will disintegrate into smaller fragments identified as MNPs. Plastic fragments with a diameter of below 5 mm are referred to as microplastics (MPs) (Kumar et al., 2021). Because nano-plastics (NPs) are smaller in size (100 nm), they have a greater effect on living tissue than MPs. NPs can simply pass through the cell membrane and have an adverse effect on the cell and tissues. MNPs are often complicated to handle and

eliminate, but they also have a greater impact on living organisms (Kumar et al., 2021). MNPs not only discharge organic toxins, but they also collect in living organisms and work as a carrier for both organic and inorganic pollutants (Bradney et al., 2019; Wang et al., 2020).

Only a few studies and analysis have looked into the biological removal of MNPs (Priya et al., 2021; Sun et al., 2021; Yuan et al., 2020), but the method and potential routes of MNP removal in aquatic and terrestrial environments need to more study. Some studies on the biotic and abiotic biodegradation of a large range of synthetic polymers have reviewed the microbial degradation of plastics. The extracellular enzymes capable of degrading PE have been found in the *Rhodococcus ruber* and *Penicillium simplicissimum*, as well as the thermophilic bacterium *Brevibacillus borstelensis* and *Streptomyces* sp. Several microbes degrade polyhydroxy alkanates (PHA), including polyhydroxy butyrate (PHB). Several microbes metabolise PHA; PHA depolymerases have been investigated in *Pseudomonas stutzeri*, *Alcaligenes faecalis*, and *Streptomyces* sp. Basidiomycetes, Deuteromycetes (*Penicillium* and *Aspergillus*), and Ascomycetes are the most common PHA-degrading fungi collected from soil and marine environments (Table 5). Polycaprolactone (PCL) is artificial polyester that is simply degraded by microbes such as *Alcaligenes faecalis*, *Clostridium botulinum*, and *Fusarium*.

Polylactic acid (PLA) is a polymer that is normally used in biodegradable plastics; it's degraded by *Bacillus brevis* (thermophilic bacterium) and *Fusarium moniliforme* and *Penicillium roqueforti* (fungal strain). Fungal species, *Fusarium solani* and *Aureobasidium pullulans* sp., degrade polyurethane, polyester PUR degrading enzyme produced by the bacterium *Pseudomonas chlororaphis* was isolated. The bacterium *Pseudomonas putida* degrades PVC, and the actinomycete *Rhodococcus ruber* degrades polystyrene (Caruso, 2015; Chow et al., 2023).

**Table 5: Different types of enzyme and their applicability in plastic degradation.**

Enzyme	Type of Plastics	Source microorganism	Molecular weight (kDa)	References
Cutinase	Poly- (butylene succinate) (PBS), poly-(butylene succinate-coadipate) (PBSA) and poly-(lactic acid) (PLA) PET Film	<i>Aspergillus oryzae</i> <i>Thermobifida alba</i> AHK119	21.6	Maeda et al. (2005) (Thumarat et al. 2015)
Cutinase like enzyme	Poly-(butylene succinateco-adipate) (PBSA)	<i>Paraphoma</i>	19.7	(Suzuki et al. 2014)
Cutinase	Poly- (butylene succinate) (PBS) , poly-(butylene succinate-co-adipate) (PBSA)	<i>Cryptococcus s magnu</i>	21	(Suzuki et al. 2013)

MHETase	Polyethylene terephthalate (PET)	<i>Ideonella sakaiensis</i>	65	(Palm et al. 2019)
Lipase	Plastics poly( $\epsilon$ -caprolactone) and poly(1,4-butylene succinate)	<i>Amycolatopsis mediterannei</i>	48	(Tan et al. 2021)
Laccase	Polyethylene (PE) , Polyethylene microplastics	<i>Escherichia coli</i>	66	(Zhang et al. 2023)
Esterase	Polyester and poly-(lactic acid) (PLA)	<i>Escherichia coli</i>	29	(Tchigvintsev et al. 2014)
PETase	Polyethylene terephthalate (PET)	<i>Ideonella sakaiensis</i>	32	(Son et al. 2019)
PHB depolymerase	Poly [(R)-3hydroxybutyrate] (PHB)	<i>Ralstonia pickettii</i> T1	42.7	(Hiraishi, Komiya, and Maeda 2010)
Polyamide	Polyurethane polyester copolymers	<i>Nocardia farcinica</i>	10-50	(Gamerith et al. 2016)

## Conclusion

Industrialization is a most important source of contamination in the environment and disturbs the ecological balance. Bioremediation is an eco-friendly process that applies the biological agents for the transformation and degradation of organic and inorganic pollutants from industrial effluents. However, the efficiency of remediation effects by the ability of enzymes secreted by the microbes which perform the detoxification of environmental pollutants. A huge amount of enzymes secreted by the bacteria and fungi, involved in the elimination of pollutants. Enzymes such as ligninolytic enzymes [such as laccase, manganese peroxidase (MnP) and lignin peroxidase, (LiP)], monooxygenase, dioxygenase, and reductase have been investigated in the degradation of various pollutants from industries. Further with the use of genetic engineering of pollutant degrading enzymes, we can enhance the proficiency of degradation of industrial pollutants.

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