

BIOFILM AS A SIGNIFICANT TOOL IN BIOREMEDIATION

Abstract

Environmental pollutants and waste materials such as industrial waste, biomedical waste have always been a serious problem and becoming life threatening day by day. Bioremediation is a very helpful approach towards the waste management and removal of environmental pollutants that are hazardous, specifically microbial bioremediation. Microorganisms are utilized in bioremediation as biofilms which are important tools in bioremediation processes. Biofilms are used in bioremediation of various environmental pollutants, waste materials, contaminant water, and also used in the waste water treatment. Bioremediation is the process of conversion of all hazardous materials into less hazardous products and conversion of waste materials into reusable products by using biological agents such as microorganisms, plants and their products i.e., enzymes called as bioremediators. Bioremediation is a safest, cheapest and effective eco- friendly technique, used for the removal of environmental contaminants and toxic wastes from environment using the bio-mechanisms of microbes and plants.

Keywords: Bioremediation, biofilm, bioremediators,

Author

Komal Bhandari
Ph.D. Scholar
Department of Biochemistry and
Biotechnology
Sardar Bhagwan Singh University
Balawala
Dehradun, Uttarakhand, India.
komallbhandari619@gmail.com

I. INTRODUCTION

Natural disasters and human activities such as urbanization, rapid industrialization, intensification of agriculture and manufacturing industries has resulted in the accumulation of hazardous contaminants in environments with many harmful pollutants such as xenobiotic compounds, waste materials, dyes and heavy metals [1, 2]. Increased industrial and agricultural growth result in the release of various chemicals including toxic compounds, heavy metals into the water, air and soil and make them contaminated which are needed to be remediated, contaminant free and reusable [3]. Wastewater from the industries is the major source of heavy metals which are major environmental pollutants, increased concentration of heavy metals became serious threat to living organisms [4]. Various physical and chemical methods have been used for the management of contaminants and waste materials but these methods are insufficient and are not very much effective but bioremediation is very effective and eco- friendly technique for the removal of contaminants [1]. Bioremediation is economically more cost- effective for degradation and detoxification of complex pollutants at large scale as compared to conventional methods of treatment of pollutants [2, 5]. Bioremediation was first used to clean wastewater by the Romans (around 600BC), and was officially invented in 1960s by George Robinson. [5]. **Bioremediation** can be defined as” the process of converting waste materials into useful products, toxic chemicals into non or less toxic materials, and cleaning up the environmental pollutants from air, water, and soil by using microorganisms such as bacteria, fungi, algae, and green plants or their biological mechanisms & enzymes.” **Bio**= living things, **remediation** = improving /correcting or removing something that is bad or undesirable, hence we can say that bioremediation is a process of using living organisms to improve or remove undesirable substances or materials that are harmful for the environment. Bacteria, can be easily grown and change the texture and nature of the complex pollutants which makes them suitable for bioremediation, fungi are also used for removal of various pollutants. Genetically engineered microorganisms are also used in bioremediation which have very significant effect on the pollutants, rather than the normal microbes. Because certain biodegradable pathways or enzymes from different microorganisms are transferred into a single host to perform desirable biodegradation method for specific pollutants [6].

1. **Bioremediation** is a technology that relies on promoting the growth of specific microorganism that are indigenous to the contaminated sites that are able to perform significant activities and bioremediation methods involves the **biodegradation** or **decomposition** and **biotransformation** or recycling of the contaminants or waste materials [7,8]. Depending on the types, nature and composition of pollutants or contaminants the bioremediation either completely remove them or transform them into less contaminant or non-contaminant form or lowers their concentration [9]. Bioremediation is the most feasible approach to deal with the prevalent contaminants such as organohalides, heavy metals such as zinc, copper, cobalt etc., [10]. Most of the bioremediation processes uses microorganisms specifically to reduce, eliminate, or transform the contaminants present in soils, water, and air [11]. **Microorganisms** specifically bacteria are the primary choice for the bioremediation processes because of their biological mechanism of utilizing the substances from contaminants as nutrients, require for their growth, in doing so microorganism degrade or decomposed the contaminants [12]. In bioremediation, the damaging effect of complex and hazardous chemicals on the environment is reduced.

Bacterial biofilms are also used in the bioremediation as an advanced method to remove, degrade or decompose the environmental pollutants or contaminants. Although plants, algae, and fungi are also used in the bioremediation process. Based on the different biological agents used, bioremediation is also known as:

- **Phytoremediation:** Process that uses plants and associated soil microbes for bioremediation.
 - **Microbial Bioremediation:** Process that uses bacteria for bioremediation.
 - **Mycoremediation:** Process in which fungi based bioremediation methods are used.
 - **Phycoremediation:** It is the use of algae for bioremediation of pollutants.
2. **Biofilms:** Use of biofilms is the advancement of the bioremediation techniques. Biofilm is a community of bacteria and other microorganisms (algae, fungi etc.) [5]. Primarily used biofilm is bacterial biofilm, constructed by various species of bacteria with complexity and plasticity that allow the adaptability to diverse growth conditions [13]. It is an active, well-organized, multicellular and sessile community of bacteria constructed by both gram –positive and gram negative bacteria or bacteria that colonize plants and attach to a solid surface. The most common species of bacteria that form biofilms are *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus viridans*, *Staphylococcus epidermidis*, and *Proteus mirabilis* [13, 14]. The biofilm is composed of bacterial cells and embedded in an extra cellular matrix known as extracellular polymeric substance (EPS) that is primarily composed of polysaccharides which function in the attachment or adhesion to the surface, protection of cells from outer environment, due to which bacterial cell develop tolerance to many environmental stressors such as hazardous chemicals, toxins, fluctuating temperature and pH, etc. The surfactants of EPS are used in solubilizing non-degradable compounds such as organic pollutants [15].

In addition to polysaccharides biofilms also consist of lipids, phospholipids and other cell components, all major classes of macromolecules (i.e. proteins, polysaccharides, DNA and RNA) can also be observed in biofilm. The formation of biofilm is regulated by cell–cell communication, known as quorum sensing (QS) that connects the microbial cells to coordinate with diverse changes [13]. The QS system is common in soil bacteria that create associations with plants and are helpful during phytoremediation of different pollutants, mostly for metals. Due to the adoptability and surviving ability or tolerance capability of cells within biofilms, the biofilm mediated bioremediation is the best method in order to remediate the contaminants or hazardous substances as they can help immobilized and degrade the pollutants [16]. The biofilms play an important role in the removal of pollutants from the air, water, and soil hence it is beneficial for the environmental application. Nowadays biofilms can be produced in laboratory with the help of bioengineering techniques by using some specific bacterial species such as *Bacillus subtilis* it is a commonly used species for the production of biofilms commercially [17]. Hence biofilms are important tools in bioremediation due to their vast applications such as in removal of environmental pollutants, waste water treatment, removal of contaminants from water i.e., water purification, bioremediation of heavy metals from soil, degradation and decomposition of contaminants etc.

II. PRINCIPLE OF BIOREMEDIATION

Bioremediation is based on the principle of biodegradation of contaminants or pollutants by using biological mechanisms of microorganisms under controlled and appropriate environmental condition suitable for growth and activity of microorganisms [18, 12]. For effective bioremediation process the biological mechanism i.e., metabolism of microorganisms must be active that use various enzymatic reactions to degrade or convert the hazardous pollutants into less hazardous compounds [12]. Microorganisms may be indigenous to the contaminated site or can be transport or isolated from other places, action of multiple microorganisms require to degrade a compound and the rate of degradation of compounds depends on the environmental conditions i.e., if favourable to microbial growth and activity then the degradation will be faster [18].

Bioremediation process can occurs either under aerobic conditions (in presence of oxygen) or under anaerobic conditions (in absence of oxygen) depending on the type of microbial species used i.e., aerobic bacteria or anaerobic bacteria [12]. Aerobic bacteria degrade the pollutants in presence of oxygen and the rate of degradation is depends on the availability of oxygen and anaerobic bacteria does not require oxygen for growth and activity. Depending on the type of microorganism used, bioremediation can be aerobic bioremediation and anaerobic bioremediation. Hydrocarbons are readily degraded under aerobic conditions, whereas chlorate compounds are degraded only under anaerobic conditions [2].

III. TYPES OF BIOREMEDIATION

Depending on the site or location where remediation is processing i.e., either at the original or contaminated site or away from the contaminated site bioremediation process is of two types:

- In- Situ Bioremediation
- Ex- Situ Bioremediation

1. In- Situ Bioremediation: Most commonly used method involves the treatment of wastes or pollutants at the original contaminated site. This method involves the use of indigenous microorganisms that are already present at the contaminated site also enhance microbial activity by supplying nutrients to the microbes. Also use plants for degradation, removal, and immobilization of pollutants in soil (Phytoremediation). In situ bioremediation is generally used to degrade or eliminated the pollutants from soil and ground water [7].

2. Ex- Situ Bioremediation: Treatment of contaminants away from the contaminant site is known as ex-situ bioremediation. In this method contaminants are excavated from the contaminated site to somewhere else for the treatment.

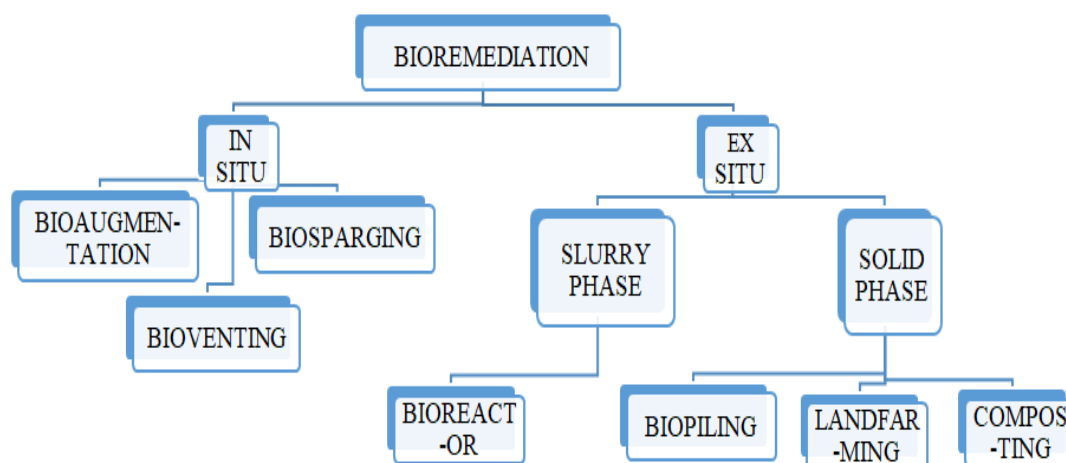


Figure1: Types and Strategies of Bioremediation

3. Strategies for In-Situ Bioremediation: Various strategies for in situ bioremediation of pollutants or contaminants from contaminated soil and groundwater are as follows:

- **Bioaugmentation:** The increased supply or addition of microorganisms (indigenous or exogenous) to the contaminated soil to enhance biodegradation of contaminants [19]. Exogenous microorganisms are those which are isolated from other places and transported to the original contaminated site. This method is commonly used in municipal wastewater treatment [18].
- **Biosparging:** Air injection in the saturated zone in the contaminated soil to promote microbial activity. Through this process oxygen and nutrients are supplied to microbes (indigenous) to promote aerobic biodegradation of contaminants [19].
- **Bioventing:** Use of indigenous microbes for biodegradation of organic pollutants found in unsaturated zone. The process involve injection of air into the soil for providing oxygen to microorganisms which enhance activity of microorganisms leading to accelerate the rate of degradation (aerobic degradation) of pollutants. Bioventing target some specific contaminants e.g. benzene, acetone, toluene, phenol or petroleum products [2, 20] .

4. Strategies for Ex-Situ Bioremediation: As the ex-situ bioremediation involve the removal of contaminant or waste material from its origin site to another site, it can be treated using different techniques based on the two phases which are:

- **Slurry Phase:** This treatment involve the formation of slurry by mixing excavated contaminated soil with water and nutrients and placed in a bioreactor, that continuously mix the slurry to promote interaction between microorganisms and contaminants for degradation of contaminants [7].
- **Solid Phase:** Solid phase treatment involves three processes which takes place on solid land or soil, are:

- **Composting:** Process of recycling or converting organic matters into useful fertilizers by using microorganisms. Contaminated soil excavated from the contaminated site and transfer to a composting pad where the contaminated soil is mixed with organic waste such as agricultural waste to promote the development of microbes, which degrade the soil contaminants [20].
- **Landfarming:** Waste materials are excavated from original site and spread on uncontaminated soil amended with nutrients to enhance biodegradation by naturally occurring bacteria. It is aerobic degradation of soil contaminants in which degradation of contaminants takes place by microbes using oxygen [2].
- **Biopiling:** Basically used to reduce petroleum concentration from soil, it is the combination of land farming and composting. It utilizes soil microorganisms to decompose the contaminants by depositing contaminant soil in pile with air, nutrients moist supply required for microorganisms [19].

Table 1: Bacteria involved in bioremediation of different contaminants present in soil

Bacteria Species Involves in Bioremediation of Soil		
	Pollutant Remediated	Bacteria
1	Heavy metals	<i>Bacillus spp.</i>
		<i>Pseudomonas spp.</i>
		<i>Endophyte bacteria</i>
		<i>Flavobacterium</i>
		<i>Enterobacter</i>
		<i>Rhodobium marinum</i>
		<i>Rhodobacter sphaeroides</i>
2	Petroleum hydrocarbons	<i>Rhodococcus</i>
		<i>Mycobacterium</i>
		<i>Acinetobacter</i>
		<i>Staphylococcus spp.</i>
		<i>Bacillus spp.</i>
		<i>Pseudomonas spp.</i>
3	Poly aromatic hydrocarbon(naphthalene)	<i>Pseudomonas stutzeri</i>
4	Pyrene and phenanthrene	<i>Stenotrophomonas acidaminiphila</i>

Table 2: Different bacteria species involved in the bioremediation of various contaminants present in water

Bacteria Species involves in Bioremediation of Water		
	Pollutant Remediated	Bacteria
1	Heavy metals	<i>Bacillus subtilis</i>
		<i>Pseudomonas aeruginosa</i>
		<i>Micrococcus yunnanensis</i>
		<i>Flavobacterium</i>
		<i>Enterobacter</i>
		<i>Agrobacterium</i>

		<i>Azobacter</i>
		<i>Lactobacillus plantarum</i>
	Perchlorate	<i>Vibrio</i>
		<i>Salinivibrio</i>
		<i>Nesiotobacter</i>
		<i>Staphylococcus spp.</i>
		<i>Bacillus spp.</i>
		<i>Pseudomonas spp.</i>
	Chlorophenols & Oil spills	<i>Pseudomonas</i>
		<i>Rhodococcus</i>
4	Hydrocarbons	<i>Halomonas</i>
		<i>eurihalina,</i>
		<i>Enterobacter</i>
		<i>cloacear,</i>

IV. PHYTOREMEDIATION

Using plants to clean up contaminated soil, water and air is known as phytoremediation, it is an in situ bioremediation method. Soil contaminated by the presence of pollutants such as, hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals etc., are harmful for soil [20]. Planting certain plant species in contaminated soil that have natural mechanism of biodegradation, utilizes enzymes instead of microorganisms, these enzymes are able to breakdown the pollutants as plants absorb them and enzymes convert them into less hazardous or non-hazardous compounds such enzymes are dehalogenase, phosphatase, peroxidase, nitro reductase [21].

Phytoremediation exploit the use of plants to degrade, accumulate, extract, or remove various organic and inorganic contaminants from soil, water and air [11]. Phytoremediation is best choice of method for bioremediation of soil as various plant species are competent in immobilization and detoxification of soil contaminants, also microorganisms that associated with plants help in the degradation of contaminants [10]. Some plants provide nutrients for microorganisms associated with them to promote their growth and development [20]. Rhizosphere a region around plant roots, provide microenvironment for the plant-microbe interaction that result in the formation of biofilm on the surface of plant root [11]. Root associated biofilms involved in the degradation, immobilization of soil contaminants such as heavy metals, thus help in bioremediation of soil. Air pollution has a very dangerous impact on human health and wellbeing, and has become a global problem, various plants regulate the impact of air pollution by eliminating the hazardous components present in the air such as, carbon monoxide (CO), nitrogen oxides (NOX), volatile organic compounds (VOC) with most often listed in this group the polycyclic aromatic hydrocarbons (PAHs), heavy metals (HM) and sulphur dioxide (SO₂) [22].

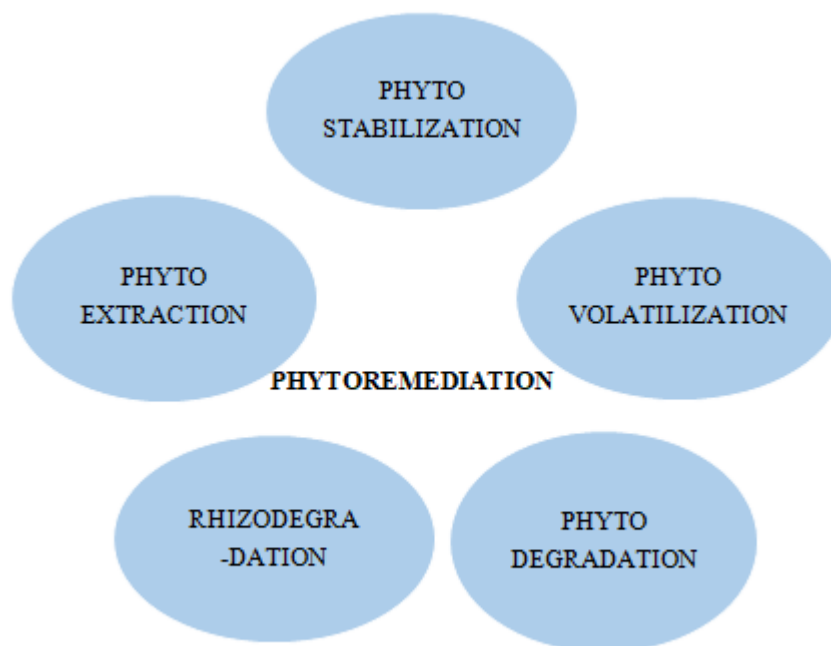


Figure 2: Various Methods of Phytoremediation

Plants remove the contaminants from the soil by extraction, degradation, immobilization, and volatilization processes hence phytoremediation is divided into different types such as:

- 1. Phytoextraction:** Specific plants are grown in the contaminated soil to extract the particular contaminant, in order to do that certain plants absorb the contaminants from the soil and store them in their root, shoot and stem [18]. By phytoextraction a mass of plants and contaminants (usually metals) is produced, that can be transported for disposal or recycling [2].
- 2. Phytodegradation:** Some plants breakdown or degrade the toxic contaminants and convert them into nontoxic or less toxic products, reaction takes place in rhizosphere with the help of enzymes, this method is known as phytodegradation [10].
- 3. Phytovolatilization:** Plants uptake contaminants and then transport them into plant tissues for breakdown by metabolism to convert them into volatile form then release the contaminant via transpiration process [23].
- 4. Phytostabilization:** This process occurs in roots, where plants first entrap the contaminants and then adsorb or absorb them to reduce their migration or to prevent the contaminants from spreading. It prevents water and soil erosion [20].
- 5. Rhizodegradation:** This process depends on the action of plant roots and associated microbes and their excretion products. The degradation of contaminants occurs in roots, plants involved in rhizodegradation utilize sugar, alcohol, acids, and other nutrients to promote a better living environment for microorganisms that breakdown the toxins [20].

Table 3: Different Plants Involved in Phytoremediation of Soil, Water, And Air.

Plants Involves in Phytoremediation of Water, Soil, & Air			
	Plants for Soil Remediation	Plants for Water Rmediation	Plants for Air Remediation
	Z. mays (Corn plant)	Eicharnia crassipes (Hyacinth)	Trees: Pinus sylvestris
	Brassica juncea (Mustard green)	Myriophyllum verticillatum (Whorl leaf)	Betula pendula
			Fraxinus pennsylvanica
	Helianthus (Sunflower)	Pistia stratiotes (Water lettuce)	Fraxinus excelsior
			Pyrus calleryana
	Vigna radiate (Mung bean)	Potamogeton (Pondweed)	Sorbus intermedia
	Lycopersicon esculentum (Tomato)	Typha latifolia	Shrubs: Pinus mugo
			Syringa meyeri
	Pteris vittata (Fern)		Spiraea
			Taxus x media
			Taxus baccata

V. BIOFILM MEDIATED BIOREMEDIATION

Biofilm mediated bioremediation is an advanced bioremediation technique in which biofilms produced by microorganisms are used to degrade, decompose, immobilize or to remove the contaminants from the environment. As we know that biofilm is a complex community of microorganisms encapsulated in an extracellular matrix composed of chemically and functionally diverse biomolecules primarily polysaccharides or glycan. Biofilms can be developed synthetically by genetic engineering, by using various potential bacteria such as *bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa* etc., and are used in bioremediation of various pollutants [17, 24]. The extracellular matrix is the most significant and functionally diverse, play major role in the bioremediation because of its composition that includes polysaccharides, nucleic acid, proteins, lipids and extracellular enzymes which help them in adherence, growth, cell to cell communication, provide protection from outer environment, and the catabolic activities of enzymes are used to degrade or hydrolysed the chemicals or pollutants e.g. in the wastewater treatment [15, 5].

Waste water is consist of various contaminants such as organic contaminants and ammonium, which is decomposed by bacterial biofilms into less toxic or non-toxic compounds via the action of enzymes. The enzymes convert ammonium (NH_4^+) into nitrite NO_2^- , acids, H_2O and ATP by utilizing O_2 . NO_2^- further oxidised into nitrate NO_3^- , as more surface area is present for biofilms the more NH_4^+ is removed.

Microbial glycoconjugate a complex compound produced by microbial strains also help in the formation of biofilms, is a combination of glycoproteins and glycolipids and involve in the bioremediation of organic pollutants, pesticides, hydrocarbons etc. Microbial

biofilm absorbs and immobilizes environmental pollutant due to the production of glycoconjugate [25]. Social interactions & heterogeneity in biofilms are very helpful for degradation of complex pollutants due to diverse catabolic pathways that exist in diverse environments with microbes [5].

Within the biofilm the various microbial communities are also responsible for differential gene expression of the substrate, showing a broad range of metabolic pathways for biodegradation [25]. The most important characteristic of bacterial biofilm is their flagellar based movement and their chemotaxis that play an important role in biodegradation of pollutants, because biofilms can sense the presence of xenobiotics and move towards them by quorum sensing hence increasing biodegradation [16]. Biofilms are also used as monitors of bioremediation and also used as an indicator for monitoring heavy metal contamination in water as their morphology and physiology changes due to presence of heavy metals and changes in surrounding environment [26]. Biofilm mediated bioremediation is a safer and competent technique in comparison to bioremediation with planktonic microorganism because cells within the biofilms have great adaptability, survival abilities and tolerance against various environmental conditions i.e., they can survive in diverse environmental conditions [16]. Planktonic microorganisms are free living bacteria that do not attached to surface. Specific gene expression by plants due to biofilm-forming bacteria performs a very significant role in the bioremediation of organic and inorganic waste [5]. Biofilm-mediated bioremediation can also be facilitated by enhanced intercellular gene transfer, using mixed population biofilms, bioavailability of the pollutants to the biofilm-residing bacterial cells via chemotaxis, and optimizing the physicochemical parametric conditions [27]. The presence of aerobic and anaerobic organisms, and different metabolic processes are survival strategies of a cell within biofilm due to genetic diversity, this diversity and metabolic range makes biofilm-forming bacteria hold more potential in the bioremediation of pollutants [5]. Indigenous biofilm forming bacteria in the soils perform bioremediation, which is part of the nutrient cycle and global self-purification system. Some bacteria produced bio surfactants, nontoxic, biodegrading agent that help to absorb and degrade the hydrophobic pollutants [13]. Bio surfactants contain many active compounds such as glycolipids, lipoproteins, neutral lipids, and ionic lipids which involved in the removal or degradation of many organic and inorganic pollutants [15].

- 1. Biofilms Engineering:** Biofilms engineering is an advanced biotechnology techniques for the production of important and controllable biofilms that can be used for various purposes including bioremediation. The term biofilm engineering was first introduced by the Centre for Biofilm Engineering, Montana state university [23]. Synthetic biology tools are widely used to design and control microbial communities by manipulating regulatory networks, regulating gene expression and engineering cell to cell interactions [17]. Biofilm engineering is concerned with exploiting the benefit of microbial communities by understanding the fundamentals of development of biofilms. Biofilms are mostly studied for the environmental biotechnology specifically for water remediation or purification or recycling of sources [23].
- 2. Periphytic Biofilms:** Periphytic biofilms are a type of biofilm which consist of different microorganisms such as fungi, algae, cyanobacteria and protozoa. Periphytic biofilms are found either freely floating in the freshwater or attached to a submerged surface. Periphytic biofilms are composed of phototrophic benthic biofilms, which are the assemblage of dead and living microbes, algae. Phototrophic benthic biofilms are serve as

primary producers and play an important role as source of food for aquatic lives. Periphytic biofilms are capable of using, converting, and modifying various pollutants such as heavy metals, organic pollutants, pharmaceutical wastes, micro plastic wastes. Periphytic biofilm has resistance against outer physicochemical variations in the surrounding environment that tends to maintain internal processes. Periphytic biofilms remove pollutants by three mechanisms which are, bio absorption, bioaccumulation, and biodegradation [28].

Based on the substrate used for attachment periphytic biofilms are of five types: epiphyton (plants), epilithon (rocks), epipelon (sediments), epixylon (wood), and epipsammon (sand). Periphytic microorganisms are attach to each other and to the pollutants via extracellular polymeric substances (EPSs), they have been shown to effectively bio remediate heavy metals by absorbing positively charged heavy metals through negatively charged extracellular polymeric substance, and also degrade polyethylene [28].

3. Factors Affecting the Formation of Biofilms: Various environmental factors such as pH, temperature, nutrients, oxygen level and bacterial metabolites determines the formation of biofilms. The activity of biofilm is regulated by the level of adenosine triphosphate, dehydrogenase, deoxyribonucleic acid, and solid and volatile solid. The aging of biofilm reduced the capability of biofilm in wastewater treatment. Low salinity, low temperature, and hydrophobic surface are appropriate parameters for the formation of biofilms [15].

- **Effects of pH:** The pH of surrounding environment influence the formation of biofilm as well as the growth of microbe [29]. pH between 7-8 is favourable for biofilm formation also it alters the adhesion property of bacteria [15].
- **Effects of Temperature:** The formation of biofilm and bacterial growth is also influenced by temperature. As bacteria grow at an optimum temperature below or above of this temperature the growth of microbe is hampered so is the formation of biofilm [29].
- **Effect of Oxygen:** The availability of oxygen have an impact on the formation of biofilm and on the microbial growth. The availability of oxygen help in the growth of bacteria. Bacteria used oxygen and produced energy that utilized in the development of biofilm also the metabolic activities of biofilm can be reduce with inadequate oxygen supply [29].
- **Effects of Nutrients:** Nutrients play an important role in the formation and development of biofilms, as they are required for growth, development and various physical and biochemical activities [29].
- **Quorum Sensing:** The regulation of gene expressions by quorum sensing influence the formation and attachment of biofilms by upward regulation and downward regulation of genes [14]. Quorum sensing or cell to cell communication play an important role in the attachment or detachment of biofilm, also it triggered the proliferation of biofilms [5].

- **Effects of Extracellular Polymeric Substance:** The extracellular polymeric substance influence the growth and survival of microbe and biofilm. It also help biofilm in attachment to the surface and protects bacteria from hazardous chemicals and environmental stress.

4. Formation of Biofilm: biofilm formation involves following steps:

- **Reversible Attachment (adhesion):** In initial phase bacteria attached to a solid surface, due to the presence of adhesion molecules polysaccharides and adhesion forces such as Van Der Waals and hydrophobic interaction that help in the attachment, this is a reversible attachment [26].
- **Irreversible Attachment (adsorption):** Bacterial surface secrete various organic and inorganic molecules which help to reduce repulsion force between bacterial cell and attachment surface due to same charge between them this result into irreversible attachment [26].
- **Maturation:** This step involved in the growth and development of biofilm with the formation of extracellular polymeric substance as bacteria colonized and absorbed the nutrients and utilize them [26]. The mature biofilm perform various physiological and biochemical functions

5. Types of Biofilms: Biofilms are of various types depends on the microorganisms present within it such as bacteria, fungi, algae etc.

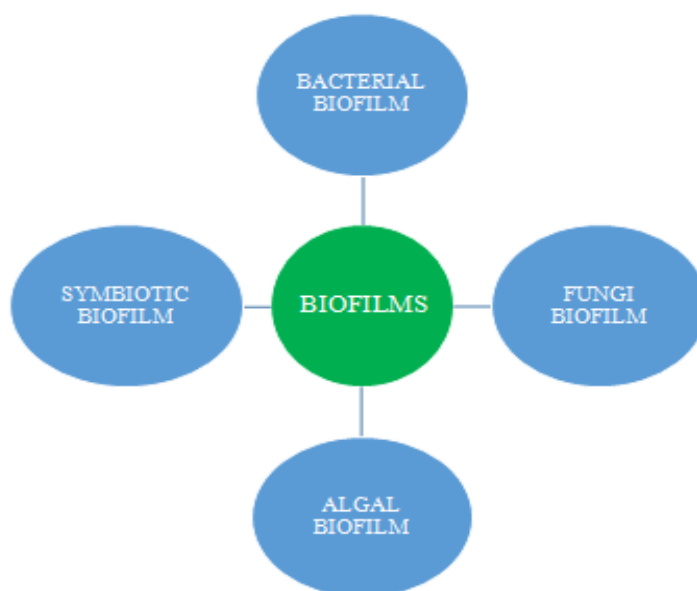


Figure 3: Classification of biofilms based on the type of microorganism present.

- **Bacterial Biofilms:** [29] These are self -regulating community of bacteria that have colonized a surface encapsulated by an extracellular polymeric matrix. The proficiency of bacterial biofilms is influenced by various factors such as pH,

temperature, ionic concentration, nutrition, surface proteins, extracellular proteins, capsular polysaccharides, adhesins, autolysin, anaerobicity, carbon dioxide level, glucose, osmotic levels, and the presence of surfactants. Within the bacterial biofilms the useful and beneficial species play a vital role in the wastewater treatment [29].

- **Fungi Biofilms:** Fungi biofilms consist of fungal communities and have same basic requirements for the formation of biofilms as bacteria, such as a surface for attachment and an extracellular matrix. Fungi have different biosynthetic enzymes [29].
- **Algal Biofilms:** These biofilms are consist of various algae species and colonize illuminated surface areas in the presence of moisture and nutrients [29].
- **Symbiotic Biofilms:** Multispecies synergy is particularly important in the case of solid substrate breakdown. In nature there is a lot of synergy between bacteria and fungi. Fungi are much more effective than bacteria in utilizing resources available through enzymatic hydrolysis. Bacteria and fungi are the only species that can degrade large plant polymers such as chitin and lignin, and break down cellulose into small molecules. Another example of symbiotic biofilm is bacteria and protozoa [29].

VI. APPLICATIONS OF BIOFILMS IN BIOREMEDIATION

Biofilm mediated bioremediation depends on the interaction of microorganisms with xenobiotic substances in the environment, cells within the biofilms synthesized various enzymes and cofactor which contributed to bioremediation [15]. Successful application of a bioremediation depends on the understanding the interactions among microorganisms, organic contaminants and soil or aquifer materials [16]. Bibliometric analysis based on search engines such as PubMed, revealed the applications of biofilm in bioremediation of environmental pollutants, water, and soil, [15]. Biofilm-based reactors are commonly used for treating large volumes of dilute aqueous solutions such as industrial and municipal wastewaters [16].

1. **Biofilms in Bioremediation of Organic Pollutants:** Several bacteria are involved in the degradation of various organic pollutants such as aromatic compounds, hydrocarbons, naphthalene, anthracene, benzo (b) fluoranthene, polychlorinated dibenzo-p-dioxins and indeno (1, 2, 3-c, d) pyrene, and phenanthrene. Biofilms produced by *Pseudomonas*, *Rhodococcus*, *Alcaligenes*, *Sphingomonas*, and *Methylosinus* can degrade variety of organic pollutants [15]. Hydrocarbons has become big problem with the rapid increase in petro- chemical industries. Biofilm forming *Pseudomonas sp.* is able to form biofilm in the presence of crude oil and can degrade crude oil efficiently under ex situ conditions [13]. In addition polycyclic aromatic hydrocarbons, a major natural pollutant present in water, air and soil, formed due to incomplete combustion of fossil fuels [30]. The degradation of polycyclic aromatic hydrocarbons takes place by the action of *P. mendocina*, which is induced by calcium dependent extra cellular polymeric substance [15]. Using *Pseudomonas* biofilms increased the degradation of naphthalene in a soil microcosm [13].

Among the major soil and ground water contaminants, chlorinated hydrocarbons are another class of persistent pollutants released from industrial wastes and are efficiently remediated by using biofilms under sequential anaerobic–aerobic biofilm reactors [13]. Pesticides are another important group of organic pollutants presents mainly in soil, such pollutants are organochlorine, organophosphorus, carbamates, and nitrogen based pesticides, among which mostly degraded by bacterial enzymes and organophosphorus compounds are degraded by *Pseudomonas* species [31]. Another class organic pollutants are nitro- aromatic compounds. These are an important group of chemicals which are widely used in various industries including agrochemicals, textile, and pharmaceutical industries, are highly resistant to degradation due to the presence of nitro groups. Lendenmann, Spain, and Smets (1998) used aerobic fluidized-bed biofilm reactors for the degradation 2, 4-dinitrotoluene and 2, 6-dinitro- toluene. More than 90% degradation was observed at all loading rates. Polychlorinated dibenzo-p-dioxins are highly toxic compounds present in soil, is dechlorinated by a group of microorganisms called dechlorinating *Chloroflexi*, under anaerobic conditions and leaving less chlorinated structure for aerobic degradation [13].

- 2. Bioremediation of Heavy Metals:** Industrial and urbanization activities such as mining, metallurgy, electroplating, distilleries, pesticides, fertilizers, tanneries, photography, wood products, energy, and atomic energy installations are an active source of heavy metal contamination to aquatic and terrestrial environments [27]. Metals at molecular density higher than 5gm/cm^3 are known as heavy metals, are potential pollutants of air, water and soil and are not easily degradable, hence biofilms are used to degrade or remove the heavy metals from the environment [30]. When present in trace amount heavy metals are essentials for physiological and biochemical functions, but in higher concentration they are toxic and cause serious health and environmental hazards. The non biodegradability properties of the various heavy metals result in a prolonged presence in the environment and became recalcitrant [27]. Various mechanisms are involved in the biofilm-mediated bioremediation of heavy metals such as bio sorption, bio precipitation, bioaccumulation, bio reduction, complexation, and irreversible adsorption [32]. The extracellular matrix has metal binding capacity it entrap the metal ions as metal ions are cations and extracellular matrix is polyionic. The negatively charged site of extracellular matrix involve in the adsorption of metals [26]. Bio sorption is a complex method which include uptake of contaminants, used in the bioremediation of heavy metals, it involves various mechanisms such as ion exchange, bio precipitation, and complexation [1]. As the biofilm introduced to the contaminated water or soil the extracellular matrix binds to the heavy metal ions through ion exchange process, in which cationic heavy metals bind to anionic groups of the extracellular matrix as more heavy metals binds to extracellular matrix so the concentration of heavy metals decrease from the contaminant site and increased in the biofilm. In complexation the heavy metals are complexed with cell surface groups and then further removed, bio precipitation takes place in the conversion of metals into precipitation resulting in decrease the toxicity of metals [5].
- 3. Wastewater Treatment:** Waste water contain various hazardous chemicals, especially waste water from industry contain many organic, inorganic and heavy metals which contaminate the water and cause water pollution. These contaminants are hazardous for environment and terrestrial & aquatic lives, hence the degradation or removal of these hazardous contaminant is necessary from the water [29]. Biofilm is a significant tool for wastewater treatment, as biofilms effectively degrade or remove contaminants from waste

water due to the presence of their extracellular matrix which composed of important biomolecules and enzymes with diverse functions [13]. Hence the extracellular matrix of biofilms is able to degrade or decompose various contaminants effectively. The enzymes present in biofilms are involved in the degradation of various organic pollutants present in the wastewater such as petroleum aromatic hydrocarbons, acetonitrile, ammonium etc., and the inorganic pollutants or heavy metals are removed or degraded by extracellular matrix that binds to heavy metals in order to convert them into less toxic form [29].

- 4. Bioremediation of Micro Pollutants:** [29] Micro pollutants are hazardous compounds released from pharmaceutical, cosmetic, and electronic industries pose a serious threat to environment resulting in water pollution. Biofilms mediated bioremediation is best solution for removal of micro pollutants because microorganisms present within biofilm are able to absorb the pollutants and degrade them via catabolic processes. Titanium dioxide a nanoparticle found in all cosmetics and household products is most hazardous nanoparticle, biofilm mediated absorption or removal of titanium dioxide convert it into less toxic form [29].

Removal of various toxic dye from dye contaminated waste is also achieved by biofilm mediated bioremediation. Major dyes such as crystal violet, methyl orange, amaranth dye, malachite green are removed or degraded by various bacteria such as *E. coli*, *B. subtilis*, *Staphylococcus aureus*, and *Pseudomonas* sp. *Enterococcus faecalis* [27].

- 5. Bioremediation of Marine Hydrocarbon:** [33] Hydrocarbons are major composition of most of the compounds such as petroleum, crude oil, natural gas, coal etc., they are ubiquitous. Marine hydrocarbons are found in the ocean, via their release from natural sources such as volcanos and from oil spills during the transportation of oils via ships, this result into the release of hydrocarbons into the marine water and cause the pollution and a threat to the marine lives. Due to the action of many hydrocarbon degrading bacteria present in marine environment, hydrocarbon are removed from the marine system. Crude oil and its refined products (diesel and gasoline) are a highly complex mixture of hydrocarbons, which required a complex community of microorganisms (biofilms) to degrade them properly. Marine hydrocarbons can be degraded under aerobic and anaerobic conditions. The rate of degradation of hydrocarbons in aerobic environment is faster than in anaerobic condition. The degradation of marine hydrocarbon under aerobic conditions is result via the action of bacteria belong to the class Gammaproteobacteria in the phylum Proteobacteria, as these bacteria solely feed on hydrocarbons, and anaerobic degradation of hydrocarbon result via the action of bacteria belong to class Deltaproteobacteria [33].

VII. CONCLUSION

Bacteria that live in polluted environment have the ability to form a biofilm for better survival. Within biofilm bacteria are protected from the outer environment and hazardous organic and inorganic chemicals because of the presence of extracellular polymeric substance, quorum sensing, and chemotaxis properties, that makes biofilm potential agent for bioremediation. Biofilms of different microorganisms are involved in the removal of different pollutants effectively. Due to the presence of different types of bacteria and their mechanism, pollutants can be bio remediate under aerobic as well as anaerobic conditions. The biofilm-mediated bioremediation process has the advantage of reusability of bacterial biomass and a

low cost and the bioremediation can be monitor by using biofilms. The development of industries and increasing human activities result in the aggregation of xenobiotic substances and hazardous pollutants in the environment which are needed to be removed from the environment. Biofilm Mediated Bioremediation one of the potential and eco-friendly method for the degradation and decomposition of pollutants from the environment. Various metabolites secreted from bacterial cells are help in the degradation of different pollutants, makes microbes the better agents for bioremediation. Utilizing microorganisms to reduce the concentration and toxicity of various chemical pollutants, bioremediation has become one of the most effective and rapidly developing field of environmental biotechnology.

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