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A REVIEW ON BIOFILM MEDIATED BIOREMEDIATION

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ABSTRACT

In this review, Bioremediation ability of Biofilms helps in the degradation of different types of industrial pollutants. The ability to form biofilms is a universal attribute of bacteria. Biofilms are microbial cells that have aggregated together on a surface. Biofilms can be made up of one type of cell or multitude of cell genera. Sites of biofilm formation include natural materials, metals, plastics and more. The mechanisms of different bacteria to form biofilms varies, depending on environmental conditions and specific strain attributes. Bioremediation is an eco-friendly, cost effective, sustainable technology that utilizes microbes to degrade a wide variety of pollutants into less harmful products. Relative to free-floating planktonic cells, microbes existing in biofilm mode are advantageous for bioremediation because of greater tolerance to pollutants, environmental stress and ability to degrade varied harsh pollutants via diverse catabolic pathways.

KEYWORDS: biofilm, bioremediation, extracellular polymeric substances, quorum sensing

INTRODUCTION

For decades, industrial processes have been creating waste all around the world, resulting in contaminated sediments and dispersal into environment.[1] Bacteria are able to grow adhered to almost every surface, forming architecturally complex communities termed biofilms. In biofilms, cells grow in multicellular aggregates that are encased in an extracellular matrix produced by the bacteria themselves.[2] Biofilm-mediated bioremediation presents a proficient and safer alternative to bioremediation with microorganisms because cells in a biofilm have a better chance of adaptation and survival, especially during periods of stress as they are protected within the matrix. Beneficial physical and physiological interactions among organisms in biofilms, the usage of xenobiotics is accelerated and, consequently, biofilms are used in industrial plants to help in immobilization and degradation of pollutants.[3]

Table 1: Difference between Planktonic & Biofilm producing bacteria.

Planktonic Bacteria	Biofilm Bacteria
Single cells	Aggregated Bacteria
Little capsular matrix	Surrounded by EPS
Physiologically Homogenous	Physiologically Heterogenous
Intracellular signaling not critical for cell division	Intracellular signaling critical for cell division & growth
Antibiotic Sensitive	Antibiotic Resistant
Affected by host immune responses	EPS protect this bacteria,not easily affected by host immune cells

STAGES OF BIOFILM FORMATION

It is a highly complex process, in which microbial cells transform from planktonic to sessile mode of growth.[4] It has also been suggested that biofilm formation is dependent on the expression of specific genes that guide the establishment of biofilm.[5] The process of biofilm formation occurs through a series of events leading to adaptation under diverse nutritional and environmental conditions.[6] This is a multi-step process in which the microorganisms undergo certain changes after adhering to surface. **(**Figure1**).**

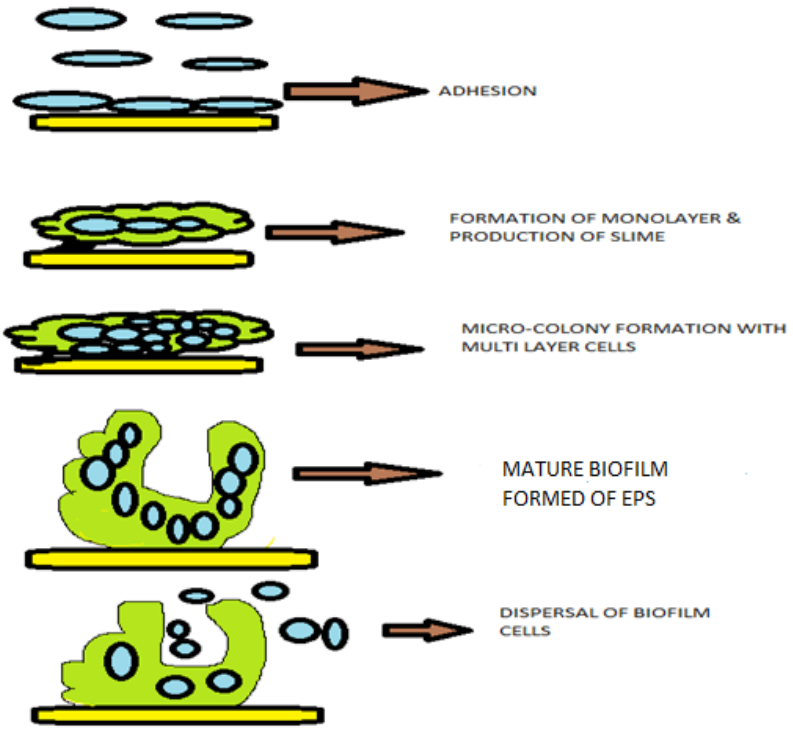

Fig 1: Different Stages of biofilm formation.

Biofilm formation has following important steps.

(a) Attachment initially to a surface -When a bacterium cell reaches to some surface very closely that its motion is very slow down, it make a reversible connection with the surface. For biofilm formation, a system of solid– liquid interface can provide an ideal environment for micro-organism to attach and grow (e.g. blood, water).[7]

(b) Formation of micro-colony- Multiplication of bacteria in the biofilm starts as a result of chemical signals. The genetic mechanism of exopolysaccharide production is activated when intensity of the signal cross certain threshold.[7]

(c)Three dimensional structure formation and maturation- After micro-colony formation, expression of genes related with biofilm take place. These gene products are needed for the EPS which is the main structure material of biofilm. Matrix formation is followed by water-filled channels formation for transport of nutrients within the biofilm.[8]

(d) Detachment- Detachment of planktonic bacterial cells from the biofilm is a programmed detachment. Dispersing of biofilm cells occur either by detachment of new formed cells from growing cells or dispersion of biofilm aggregates due to flowing effects or due to quorum-sensing.[9]

APPLICATIONS OF BIOFILM IN BIOREMEDIATION

Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria, fungi or plants to degrade or detoxify substances hazardous to human health and the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site.[10] Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is occurred by the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation, these process is known as Bioaugmentation.

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.[11]

Biofilm producing micro-organisms are very effective to degrade various pollutants. For example-Recalcitrant chemicals like harmful dyes used in dyeing industries are degraded by biofilm producing bacteria They have developed enzyme systems for the decolourization and mineralization of azo dyes under certain environmental conditions.[12] In the case of enzymatic remediation of azo dyes, azo reductases and laccases seem to be the most promising enzymes. Laccases have been shown to decolourize a wide range of industrial dyes.[13]

Biofilm producing micro-organisms also degrade hydrophobic compounds. These micro-organisms secrete polymers & form biofilms on the surface of hydrocarbons, are used for treatment of slow degrading compounds because of biosorption, bioaccumulation & biomineralization.[14] Biofilm based reactors are commonly used for treating large volumes of waste waters also.[15]

BACTERIAL SOCIAL INTERACTIONS IN BIOFILMS (QUORUM SENSING): Bacteria present in biofilms are interact with each other in a competitive or in cooperative manner. When cell density of bacterial population is high, then certain chemical signals are secreted that helps the expression of specific genes of bacteria to communicate with each other. This process is called as quorum sensing and it is dependent on bacterial population density.

Quorum sensing was first reported in the marine bioluminescent bacterium *Vibrio fischeri.*[16] In case of Gram-negative bacteria, when population density is high, then signaling molecules are secreted called as autoinducers in the form of acyl homoserine lactones by Lux-I gene product, autoinducer synthase, then these signaling molecules diffuses through the cell membrane, Lux-R gene which act as transcriptional regulator that binds to the diffusing AHL and in turn activates the transcription of its target genes. In case of Gram-positive bacteria, small peptides act as signaling molecules.[17](Figure 2)

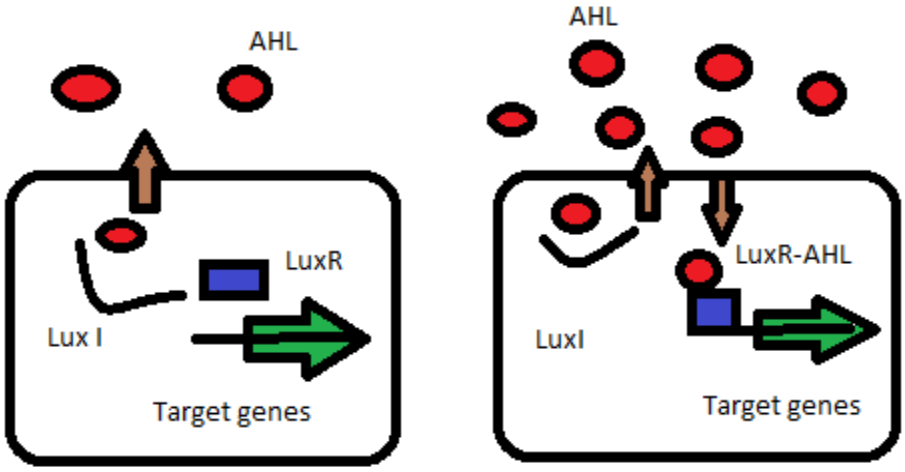

Fig.2: (a) Transcription is not activated at low cell density (b) Transcription is activated at high cell density.

MICROBIAL EXTRACELLULAR POLYSACCHARIDES AS AN INTEGRAL PART OF BACTERIAL BIOFILMS

A biofilm consists of a mixture of polymeric compounds, primarily polysaccharides, generally referred to as extracellular polymeric substance (EPS). Over 99% of microorganisms on Earth live within these biopolymers. The formation of biofilms is a prerequisite for the existence of all microbial aggregates[18] as an essential step in the survival of bacterial populations.[19] The proportion of EPS in biofilms can comprise between approximately 50-90% of the total organic matter.[20]

In Gram-negative bacteria, some of the polysaccharides are neutral or polyanionic. The presence of uronic acids or ketal-linked pyruvates enhances their anionic properties, thus allowing the association of divalent cations such as calcium and magnesium to increase the binding force in a developed biofilm. In some gram-positive bacteria, the chemical composition of their EPS could be slightly different due to their primarily cationic nature.[21] Aside from polysaccharides, biofilms also consist of proteins, nucleic acids, lipids and humic substances. Often the composition and quantity of the EPS will vary depending on the type of microorganisms, age of the biofilms and the different environmental conditions under which the biofilms exist.[22]

ROLE OF EPS IN BIOLEACHING

The recovery of metals from low-grade mineral ores using microorganisms being the economical and environmentally friendly bioprocesses termed bioleaching, relies on the

ability of microbes to oxidize solid compounds, resulting in soluble and extractable elements. Bioleaching is now an established biotechnological technique for the recovery of heavy metals.[23]

A. ferrooxidans is an acidophilic, obligately chemolithoautotrophic, gram-negative rod that oxidizes ferrous iron for energy generation.[24] It is one of the most commonly used microorganisms in bioleaching.[25] However, despite many studies of biofilms formed by *A. ferrooxidans* in bioleaching processes.[26]

CONCLUSIONS

Although microorganisms predominantly exist as multi-cellular communities within biofilms in most environments, scientists are still exploring these complex systems in order to understand the complexity of the interactions within the biofilms, and also their function in bacterial attachment and proliferation. Intense research of many different polysaccharides produced by a diverse range of bacteria has been commercially applied in food and biomedical fields. However, little is known about the role of EPS and biofilms in bioleaching applications. In order to improve the understanding of biofilm systems, the development of sensitive analytical techniques is required. Significant advances have been made to reveal new insights into biofilms and their constituents.

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