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BIOFILM : AN INSIGHT TO ITS DYNAMIC STRUCTURE

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ABSTRACT

Biological wastewater management schemes have a major impact on environmental safety and public health. This paper discusses numerous traditional and contemporary molecular approaches for investigating the structure, diversity, and dynamics of biofilms. The microbial species in the biofilm degrade various nutrients, such as phosphorous and nitrogen-containing chemicals, carbonaceous products, and pathogens embedded in the wastewater. Biofilm development is regarded as an important component of industrial effluent degradation. Once toxins are eliminated, biofilter filtered water is then discharged into the soil or used for irrigation and other recreational purposes. This paper discusses the factors that lead to biofilm heterogeneity. It also sheds light on the technical advances for evaluating Biofilm. Several strategies for developing biofilm in the lab are also explored. A kind of biofilm known as colony biofilm is also being studied, as well as its benefits and drawbacks. Cyanobacteria as a biofilm constituent is focused in this paper.

KEYWORDS: Biofilm, Technology, Colony biofilm, Cyanobacteria, Laboratory biofilm

INTRODUCTION

Biofilm is a solid and complex system that provides its members with numerous benefits such as adhesion or cohesion capabilities, mechanical properties, dietary sources, metabolite exchange platform, cellular connectivity, drug defence and resistance. A biofilm is characterized as a group of microorganisms that are irreversibly bound to a surface and encased in EPS and have high level of resistance to host cellular and chemical response. Attachment, maturation, and dispersion are the three phases of biofilm development. (Chew *et al.*, 2016)

There are certain bacteria in Biofilms which are restyled to develop on the surfaces. Many of them fabricate polymers (extracellular) and adhesins that permit cells to decisively cling to surfaces or to the cells at the next door (Flemming *et al.*, 2010)(Hung *et al.*, 2013) (Soto *et al.*, 1999). Biofilm's extracellular substance carry Proteins, DNA as well as polysaccharides (like Alginate, Levan, Cellulose, Colanic acid) which forms a cementive like stuff for adherence to the surface and for the 3D structure (Flemming *et al.*, 2010)(Limoli *et al.*, 2015). Individual cells create a matrix material that shapes various frameworks that benefit the whole population. These advantages include safeguarding of cells from numerous kinds of environmental tension (Fux *et al.*, 2005) (Singh *et al.*, 2009)(Van De Mortal *et al.*, 2004).

Biofilm cells are not considered to be static. Many of the microorganisms have been reshaped/ adjusted to surface associated motility which includes Twitching and swarming motility (Heydorn *et al.*, 2002)(Heurlier *et al.*, 2004)(O'Toole *et al.*, 1998)(Shrout *et al.*, 2006)(Wang *et al.*, 2004). Also, they are engaged in the communal activities. Communal activities includes construction of matrix and intercellular signaling (Costerton *et al.*, 1995) (Aguilar *et al.*, 2007). The reason behind this is that the bacteria in the biofilm compete with each other for the nutrients and also for space by inducing the toxic compounds to kill the proximal cells (Basler *et al.*, 2013) (Basler *et al.*, 2012)(Gibbs *et al.*, 2008) (Moscoso *et al.*, 2011).

Biofilms consists of bacteria (rigid), soft

viscoelastic ECM, small molecules that supply signaling or produce forces for unfurling and survival (Seminara *et al.,* 2012)(Wilking *et al.,* 2011).

A type of static biofilm is called as Colony Biofilm which are grown on the filters which are then kept on the surface of Petri plates of agar. At regular time interval of time, filters are moved to fresh media which in turn gives Biofilms somewhat the semi-continuous supply of the newly fresh nutrients. There are several advantages of this method but the major advantage is that these biofilms makes no use of expensive lab material and are very easy to grow. These biofilms can be used in various experiments like Cryoembedding and are generally considered as thick (Wentland *et al.,* 1996)(Huang *et al.,* 1996).

A number of studies can be performed by using static biofilm approach. It is demonstrated that *Archaeon Haloferax volcanii* when cultured under static condition, forms thick biofilms but fails to form when cultured in the continuous flow operation (Chimileski *et al.,* 2014).

Micro fluidics has now been extensively used in studying the growth and detachment of the biofilms. Biofilms can be of various thickness based on the flow velocity in the devices. Example; Under high flow velocity conditions, *Staphylococcus epidermidis* grow as monolayer and under low flow velocity conditions, it grows as multilayered (Lee *et al.,* 2008).

Micro fluidics can be used in the characterization of the physical properties of the biofilm. *S. epidermidis, Klebsiella pneumoniae, Vibrio cholerae* are some of the constituents of the biofilm (Hohne *et al.,* 2009).

Cyanobacteria have been recognized to be efficient in accumulation and degradation of varieties of toxic pollutants which includes xenobiotics, phenol, catechol, naphthalene, crude oil, pesticides, etc. Cyanobacteria are used expertly as a cost effective means for bioremediation of Industrial wastewater by metamorphosing the diffused supplements into the biomass. In contaminated networks, they are regarded as economically efficient and are considered as a minimal maintenance technology of remediation (Dubey, 2011). In remediation of Oil spills, species like *Oscillatoria salina, Plectonema terebrans, Aphanocapsa* sp., *Synechococcus* sp. proved to be efficient. In the aquatic environments, these species are known to develop as mats (Radwan *et al.,* 2001).

It was reported that cyanophyceae grows superabundantly with substantial diversification and bounty in calcium rich ponds (Kannan, 2006). It was also suggested that factors like dissolved oxygen, oxidizable organic matter and some bits of phosphate and nitrate favored growth of Cyanobacteria (Dubey, 2011).

Factors like exposure time, concentration of pollutants, native environment of microbes directly

Table 1. Factors contributing to the heterogeneity of biofilm:

Physiological Heterogeneity	Genetic Variability	Stochastic Gene Expression
Bacteria refashioned to their localized environmental surroundings. Physiology of discrete cells differs from proximal cells (Rani *et al.,* 2007).	Mutations do happen in initial cloned occupants of the cell. Communal cells may possess mutations (Boles *et al.,*2004) (Hansen *et al.,* 2007).	Although cells encounter same environmental conditions, even cell subsets manifest similar genes at distinct levels (Baty *et al.,* 2000)(Baty and Eastburn *et al.,* 2000).

Table 2. Technological Advances in Biofilm Determination: (Franklin *et al.,* 2015)

Next Generation Sequencing (NGS)	Provide an insight towards the genetic coding prospective of organisms in Biofilms.
RNA Sequencing, RT-qPCR	For apprehension of process of gene expression that are happened in the biofilms globally as well as locally.
Mass Spectrometry	For proteomics of biofilm and also for biofilm's metabolic profiling.
Fluorescent Staining	Gives 3D structural view of the extracellular substance of biofilm.
FISH (Fluorescent in situ hybridization)	Accounts for the biofilm's community structure.
Nuclear Magnetic Resonance	Provides facts on the water dynamics in the biofilms.
FT-IR (Fourier Transform Infrared Spectroscopy)	Permits identification of the cellular and extracellular compositions.
Micro fluidics	For understanding dynamics of formation and detachment of biofilm and also for sensing of biofilm.

affects the rate of growth and capability of biodegradation. Cyanobacteria are termed to be as efficient degraders and the exemplary removers of the pollutants. It was reported that even at high concentration of pollutant (up to 10ppm), cyanobacterial species showed magnificent deletion standards (Dubey, 2011).

Species like *Synechococcus*, *Oscillatoria*, *Nostoc*, *Nodularia* and *Cyanothece* are found to be efficient in degradation of Industrial effluent/ pollutant at steady rate. They also proved to be effective in illustrating the resistance against the toxicity of pollutant. These cyanobacterial species are tremendously endorsed for advantageous bioremediation for pollutants removal (in-situ as well as off-site) (Dubey, 2011).

All industrial effluents bequeath that they contains significant quantity of nitrates and phosphates with an enhanced level of Chemical Oxygen Demand and Biological Oxygen Demand and a stunted level of Dissolved oxygen (Ebtesam, 2008)(Larsson *et al.*, 2009).

The enterprise of the microbes in the Biofilms are not consistent over intact biofilm and cells adjust to their circumscribed environment.

Future Prospective

Biofilms are the aggregate of several kids of microbes that emerges from distinct surfaces. These are now efficiently cast off as a remedy of wastewater treatment. A process called Nitrification can also be employed along with the usage of biofilm. Microbes in the biofilm are able to degrade several kinds of wastewater/ effluent from various kinds of Industries. The composition of biofilm can be altered so as to have enhanced effect in Bioremediation. One step ahead understanding of microbial communities and the interaction between them will help to infer Biofilm's exact role and mechanism in the process of Bioremediation. Comprehending the elements and the genes convoluted in the formation of Biofilm will help out to blossom more potent master plan for eco-friendly as well as sustainable agriculture. This will also help to enhance the application of biofilm in Bioremediation.

CONCLUSION

Biofilm formation is a basic adaptation and survival mechanism used by bacteria. The EPS protects the bacteria in the biofilm from unfavorable environmental conditions and immunogenic responses. Chemical gradients created in biofilm allow bacteria to survive in a variety of physiological states, providing insurance in the changing environments. Biofilms may be constituted of many organisms that communicate with one another. It is known as a consortia of microbes that will aid in the degradation of effluent in various kinds of industries. The structure of biofilm may be altered to improve its performance in bioremediation. The transition is defined in terms of the microbe consortiums that were used. Numerous

Table 3. Methods for Biofilm growth under Laboratory conditions: (Franklin *et al.*, 2015)

Operating of Bioreactors	
Static Condition	Continuous Flow Condition
1. Biofilms bacteria undergo all the phases of growth i.e., lag, log, exponential as well as stationary and there is no or lowest shear force. 2. Neither replacement of the medium nor wash out of cell.	1. Operated under the condition called 'wash out'. The doubling time of the bacteria is longer than the residential time of the chamber of bioreactor. 2. There is a continuous supply of the medium.

Table 4. Advantages and Disadvantages of Colony Biofilm : (Huang *et al.*, 1996)

Advantages	1. Easy to grow 2. Thick 3. To obtain Biofilm's vertical cross sections. 4. For assessing gene expression varieties in the biofilms. 5. To evaluate diffusion rates of Antibiotics. 6. Inexpensive lab material usage.
Disadvantages	1. As there is no wash out, obstruction of planktonic cells with biofilm assay takes place. 2. Bacteria are not exacted to stick to the matrix material or to surface because there is no continual flow of the media.

Table 5. Cyanobacteria as a constituent of Biofilm : (Dubey, 2011)

Applications	1. In wastewater treatment 2. In industrial effluent treatment 3. Aquatic and Terrestrial habitat bioremediation. 4. Biofertilizer 5. Fuel, food, feed6. Source of fine chemicals 7. Pollution control agent.

consortiums may be effective at degrading effluents from a variety of industries. Understanding microbial communities and their interactions can help to infer Biofilm's exact function and mechanism in the Bioremediation phase.

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