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Combatment of Aquatic Pollution through Biofilm Technique: A Review

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A B S T R A C T

Aquatic pollution occurs due to the discharge of harmful substances, chemicals or microorganisms into water bodies i.e. a stream, river, lake, ocean, aquifer, or other body of water resulting in contamination & degradation of water quality and rendering it unsuitable for human use or the environment. Many advanced water treatment techniques are followed before discharging this polluted water into the surrounding environment. However some limitations is there. In this context, Biofilms have become the important part of biological treatment of municipal waste water. Biofilm reactors are units where biofilm rich sludge are used in treatment of wastewater through many biological processes. Among all treatment methods Biofilm based bioreactors are considered as best available technologies for waste water treatment. Biofilms are complex structured porous and most tolerant communities of the aquatic ecosystem that includes environmentally efficient strains of algae, fungi, bacteria, actinomycetes and viruses. These microbial isolates have been extensively studied for assimilation, bio-sorption and biodegradation of almost all sorts of organic and inorganic pollutants in aquatic ecosystems. Further research is needed to bio-stimulate the promising strains of biofilms for pollution treatment. Considering the increased pollution stress, its negative impact on aquatic life and potential of aquatic biofilms to be used as indicators of pollution for controlling this pollution is reviewed in this study.

Introduction

Aquatic pollution causes due to the discharge of harmful substances, chemicals or microorganisms into water bodies i.e. a stream, river, lake, ocean, aquifer, or other body of water resulting in contamination and degradation of water quality and rendering it unsuitable for human use or the environment. It can be classified as surface water or groundwater pollution.

Marine pollution and nutrient pollution are subsets of Aquatic pollution. Sources of this are either point sources or non-point sources. Point sources have one identifiable cause of the pollution, such as a storm drain or a wastewater treatment plant. Non-point sources are more diffuse, such as agricultural runoff (Moss *et al.,* 2008). Worldwide causes of death and disease is due to this aquatic pollution, e.g. due to water-borne diseases (west *et al., and* Pink *et al.,* 2006). It is leading to the deaths of 1.8 million people in 2015 (Kelland *et al.,* 2017). India and China are two countries with high levels of water pollution.

An estimated 580 people in India die of water pollution related illness (including waterborne diseases) every day (CHNRI, 2010). About 90 percent of the water in the cities of China is polluted (Chinadaily. Com. 2005). As of 2007, half a billion Chinese had no access to safe drinking water (Kahan *et al.*, 2007). This pollution is measured by analysing water samples through Physical, chemical and biological methods. In this context Biofilms in aquatic ecosystems colonize various surfaces (sand, rocks, leaves) and play a major role in the biological control of pollution. Aquatic biofilms supply energy and organic matter to the food chain, they are important in recycling organic matter and contribute to water quality.

A biofilm is defined as "an assemblage of microbial cells that is irreversibly associated with a surface and enclosed in a matrix of primarily polysaccharide material. (Kelley and Firestein's Textbook of Rheumatology., Tenth Edition, 2017). For assessment of pollution stress and investigation in a faster rate at community level with numerous endpoints living communities are used in monitoring studies (Gold *et al.,* 2002; Mages *et al.,* 2004; Kropfl *et al.,* 2006; Xuemei *et al.,* 2010; DeForest *et al.,* 2016). Stress monitoring and restoration of aquatic ecosystems required the skill to label ecological change precisely using measurable indicators (Ryder and Miller 2005; Lear *et al.*, 2009). In streams which are passing through agricultural areas, mixtures of chemicals derived from agricultural activity may directly or indirectly affect community structure and function of Biofilm (Boivin *et al.,* 2006). Biofilms inhabit the base of the trophic level of the food chain of streams and help in fueling energy to higher trophic levels driving carbon and nutrient cycles (Battin *et al.,* 2008). Microbial biofilms actively take part in the degradation of plant and animal remains, cycling of nutrients and elimination of suspended sediments in the aquatic environment.

Need of biofilm technology in aquatic pollution study

Biofilms having many of the important characteristics needed for community level monitoring studies: (1) they are extensively disseminated; (2) they are sessile, thus can imitate the actual circumstances of habitat; (3) they react more quickly to environmental fluctuations because of their short life cycle than higher level organisms; (4) these communities are composed of diverse taxonomic populations with varying environmental tolerance; (5) biofilm samples can be easily collected (Kropfl *et al.,* 2006; Nocker *et al.,* 2007; Porsbring *et al.,* 2007; Xuemei *et al.,* 2010). Biofilms are good bio-indicators and biomarkers, offering a suitable tool to screen metal pollution in water bodies. Benthic biofilms possess multiple functions in the development of stream and riverine ecosystems and production are frequently restricted by availability of dissolved inorganic nutrients i.e. N or P (Reisinger *et al.,* 2016). Autotrophic and heterotrophic organisms' living inside a given biofilm are often restricted by various nutrients despite experiencing analogous physical and chemical circumstances provided by superimposing river water (Johnson *et al.,* 2009; Hoellein *et al.,* 2010; Reisinger *et al.,* 2016). Biofilm helps in the buildup of metal pollutants that are sensitive to the defensive effect of major cations and protons (as for many aquatic living organisms) (Leguay *et al.,* 2016). The dominance of green algae in the biofilms is indicated by the the presence of quantity of pigments i.e. chlorophyll. Pigment composition changes after brief exposure to pollution load and can be used as a biochemical marker of toxic effects (Sabater *et al.,* 2007; Xuemei *et al.,* 2010). Stream ecosystems primarily derive nutrients and organic carbon from terrestrial ecosystems and this process is dependent on the land use of the adjacent landscape. (Qu *et al.,* 2017; Teittinen *et al.,* 2015; Smucker *et al.,* 2013; Ren *et al.,* 2013) and an influence of land cover conditions on biofilm stoichiometry (O'Brien and Wehr 2010; Qu *et al.,* 2017). Considering the increased pollution stress, its negative impact on aquatic life and potential of aquatic biofilms to be used as indicators of pollution for controlling this pollution is reviewed in this study.

Concept of biofilm

Biofilms are complex communities composed primarily of autotrophic (algae) and heterotrophic microbes (bacteria, fungi, protozoa) which accumulate at surfaces of man-made or natural substrata and are characteristically enclosed by their secretory products such as the milieu of extracellular polymeric substances (EPS) (Sekar *et al.,* 2002; Kropfl *et al.,* 2006; Denkhaus *et al.,* 2007). EPS regulate the structural and functional integrity of microbial biofilms and contribute significantly to the organization of the biofilm community (Branda *et al.,* 2005). EPS components presented in Table 1 are typically aggregates of extracellular polysaccharides, proteins and lipids and DNA (Daniel *et al.,* 2010; Hall-Stoodley *et al.,* 2004; Aggarwal *et al.,* 2016).

Process of biofilm technology

Biofilm is defined as communities or clusters of microorganisms that attached to a surface (O Toole *et al.,* 2000 and Singh *et al.,* 2006). Formation of biofilm could be possible by a single or multispecies of microorganisms that have the potential to form at biotic and abiotic surfaces (O. Toole *et al.,* 2000). As a general, there are few steps that important for development of biofilm, which starting with the initial attachment and establishment to the surface, followed by maturation, and finally, the detachment of cells from surface (O Toole *et al.,* 2000; Singh *et al.,* 2006; Watnick *et al.,* 2000).

Biofilm diversity and distribution patterns

Biofilms are the assemblage of group of microorganisms, such as bacteria, algae, fungi, protozoa and viruses and all of them form important part of the biofilm community and add to the diversity of aquatic ecosystems (Stoodley *et al.,* 2002; Battin *et al.,* 2007; Jackson and Jackson 2008). The diversity of biofilms often depends on the form of substrate and aquatic medium in which they are formed. Varity of microorganisms used as Biofilm are given below.

Waste water treatment through biofilm technique

Biofilms have become the important part of biological treatment of municipal waste water and there are various technologies used for treatment of waste water. A few of them are presented below. Biofilm reactors are units where biofilm rich sludge are used in treatment of wastewater through many biological processes. Biofilm reactors are differentiated according to - (1) The number of phases involved (2) As per biofilm attached to a fixed or moving carrier within the reactor and (3) How electron donors or accepters are used.

Advantages of biofilm technique

Environmental cleanup

Biofilms play an important role in industrial and ecological significance and decontamination of polluted water. Biofilm have the ability to degrade the industrial chemical pollutants by using them as a carbon source (Sgountzos *et al.,* 2006). Biofilms helps in bioremediation of heavy metals and degradation of some harmful chemicals in the environment and can also be used as bio-indicators of pollution. There are different groups of microbes in aquatic bodies that have been tested for decontamination of waste water (Srivastava *et al.,* 2017).

Water decontamination

This can be possible by the process of removal of contaminants from polluted water by the use of living organisms such as microorganisms from biofilms. It includes the basic mechanism of assimilation, adsorption and biodegradation.

Assimilation of nutrients

Every organism requires some sort of nutrients such as nitrogen and phosphorous for better growth and survival. These nutrients are compulsory for microbial growth. Microbes use inorganic forms of nitrogen as a sole source of nitrogen for growth. Nitrate and phosphate assimilation occurs during the agitation period of the anaerobic-aerobic activated sludge process in the first reactor and during aeration and denitrification in the second reactor (Yariv 2001; Villaverde 2004; Wu *et al.,* 2012).

Adsorption of contaminants

Adsorption of microorganisms and their aggregates is generally called as bio-sorption. This process is needed for removal of metal from the water. Bio-sorption in biofilms is shown by bacteria (e.g. *Pseudomonas aeruginosa*), fungi (*Aspergillusniger*) and algae (*Chaetomorphalinum*) (Joo *et al.,* 2010; Fu and Wang 2011). The mechanism of bio-sorption of metals and other dies involves the use of special features of these microorganisms such as adhesion and flocculation properties. Bio-sorption does not produce toxic materials and biofilms maintain their heterogeneity by extracellular polymeric substance (Wu *et al.,* 2012). The structure of biofilms is important in metal adsorption and porous structure of microbial aggregates in biofilms enables active bio-sorption (Wu *et al.,* 2010) (Fig. 1 and 2, Table 2 and 3).

Table.1 Composition of a Biofilm

S. No.	Component	Percentage of Matrix
1	Water	97 %
2	Microbial cells	2–5%
3	Polysaccharides	1–2%
4	Proteins	<1–2% (includes enzymes)
5	DNA and RNA	<1–2%
6	Ions Bound and free	

Source: http://microbewiki.kenyon.edu/index.php/ Stream_biofilm

Table.2 The list of microorganisms as Biofilm for the removal of different kinds of pollutants

Microbial isolates from biofilms	Role in environmental cleanup	References
Pseudomonas, *Chryseomonas*, *Sphingomonas* and *Burkholderiae* species	Biodegradation of phenol and pyridine and the highest biodegradation capacity (1700 mg/L and 3000 mg/L of phenol and pyridine respectively) is shown by *Pseudomonas MT1* isolate.	Rakaiby *et al.*, (2012)
Pseudomonas stutzeri, *Aeromonascaviae*, *Sphingobacterium thalpophilum*, *Fusarium udum* and *Hodotorula mucilaginosa*	Reduction of BOD and COD values of wastewater.	Bestawy *et al.*, (2014) and Rozitis and Strade (2014)
Bacillus amyloliquefaciens (S1), *E. coli* (Rz6) and their mixed culture. *Pseudomonas otitidis*	Removal of Total Suspended Solids, Fat, Oil, Grease and Total Coliform from waste water. *Pseudomonas otitidis* has been evaluated for crude oil degradation	Bestawy *et al.*, (2014) and Dasgupta *et al.*, (2013)
Pseudomonas stutzeri B. denitrificans B79 and *A. hydrophila L6*, *Rheinheimera pacifica*, *Thauera* sp.	Biological denitrification of wastewater and the river water	SriuNaik and PydiSetty (2011), Andersson (2009) and Jiang *et al.*, (2008)
Comamonas, *Thauera*, *Paracoccus*, *Paracoccus* sp. and *Azoarcus*	Act as heterotrophic nitrifiers in aquatic systems and at a laboratory scale for nitrification	Wang *et al.*, (2014), Cydzik-Kwiatkowska (2015) and Ma *et al.*, (2015)
Nitrosomonas sp. and *Candidatus kuenenia*	Removal of Ammonium from highly concentrated streams	Park *et al.*, (2014)
Phosphorous Accumulating Organisms (PAO) such as Accumulibacter sp., *Tetrasphaera* sp. and *Dechloromonas* sp.	Phosphorous removal from contaminated water	Oehmen *et al.*, 2007, Nielsen *et al.*,(2010), Nguyen *et al.*, (2011) and Kong *et al.*, (2007)
Marine strain P. mendocina NR802, *Pseudomonas paucimobilis*, *Sphingomonasbisphenolicum* and *Sphingomonas* sp. AO1	Biodegradation of PAHs from polluted water and other micro-pollutants such as PAH and Bisphenol A.	Mangwani *et al.*, (2013) and Kwiatkowska and Zielinska 2016
Pseudomonas putida, *Geobacter metallireducens*	Bioremediation of heavy metals from metal polluted water.	Singh and Cameotra (2004)
Acinetobacter sp., *Graphium* sp., *Fusarium* sp., *Candida tropicalis*	Decontamination of phenol and m-cresol containing wastewater	Wang *et al.*, (2007) and Santos and Linardi (2004)
P. aeruginosa ASU 6a (Gram-negative) and *Bacillus cereus* AUMC B52 (Gram-positive)	Low cost and effective bio-sorbants for removal of Zn (II) from wastewater	Joo *et al.*, (2010)
Acinetobacter calcoaceticus, *Erwiniaherbicola*, *P. aeruginosa* and *Pseudomonas maltophilia*	Affinity for bio-sorption of Gold (Au)	Tsuruta (2004)
Escherichia coli	Elimination of numerous heavy metals, such as lead (Pb), copper (Cu), cadmium (Cd), and zinc (Zn)	Kao *et al.*, 2006)
Microcystins degrading bacteria such as *Sphingpoyxis* sp. and *Sphingomonas* sp.	Removal of some specific compounds such as microsytin-RR, aliphatic homopolyesters and aliphatic-aromatic copolyesters.	Wu *et al.*, (2010) and Abou-Zeid *et al.*, 2004)
Basidiomycetes, *A. niger* and *Trichoderma* sp.	Biosorption of dyes from contaminated water such as Congo red, Orange G. etc.	Tatarko and Bumpus (1998) and Sivasamy and Sundarabal (2011)

Table.3 Types of biofilm reactors

Types of Reactor	Function
Trickling filter (Three-phase system – fixed biofilm-laden carrier, bulk water, and air).	Here water drips over the surface of the biofilm and air is subjected to pass upward or downward in the third phase Fig. 3a.
Submerged fixed bed biofilm reactor operated as up flow or down flow (Three-phase system – fixed (or semi fixed) biofilm-laden carrier, bulk water and air)	Water and gas bubbles (Aerobic and bioactive filter) flow through the biofilm reactor. Here gravel is immovable media and polystyrene beads act as semi-fixed media Fig. 3b, c.
Aerobic moving bed biofilm reactor or MBBR (Three-phase system – moving biofilm-laden carrier, bulk water, and air. Water flows through the Biofilm reactor)	Here water flows through the biofilm reactor and air is introduced with gas bubbles Fig. 3g. MBBRs can be used as single stage or multistage reactors. They are efficient enough to meet water quality standards for carbon oxidation, nitrification, and denitrification.
Denitrification fluidized bed biofilm reactor or FBBR (Two-phase system – moving biofilm-laden carrier and bulk water)	Water is allowed to flow through the biofilm reactor having electron donor and accepter Fig. 3g.
Denitrification filter (Two-phase system – fixed biofilm-laden carrier material and bulk water)	Water is allowed to flow through the electron donor and accepter Fig. 3b, c.
Membrane biofilm reactor or MBfR (Three-phase membrane system)	It is made of a microporous hollow membrane having water and biofilm on one side of the membrane and gas on the other side and gas is allowed to diffuse through the membrane to the biofilm Fig. 3h.
The biofilm-based microbial fuel cell or MFC (Two-phase membrane system)	It contains a proton exchange membrane which splits a classified biofilm-laden anode from a classified cathode with water on both sides. The electron donor is also separated from electron acceptor by this membrane. Other methods of waste water treatment include constructed wetlands and lagoons where the application of biofilm is vital in nutrient removal along with certain macrophytes.

Source: (Lewandowski *et al.*, 2011)

Figure.1 Stages of biofilm development (Cogen and Keener, 2004)

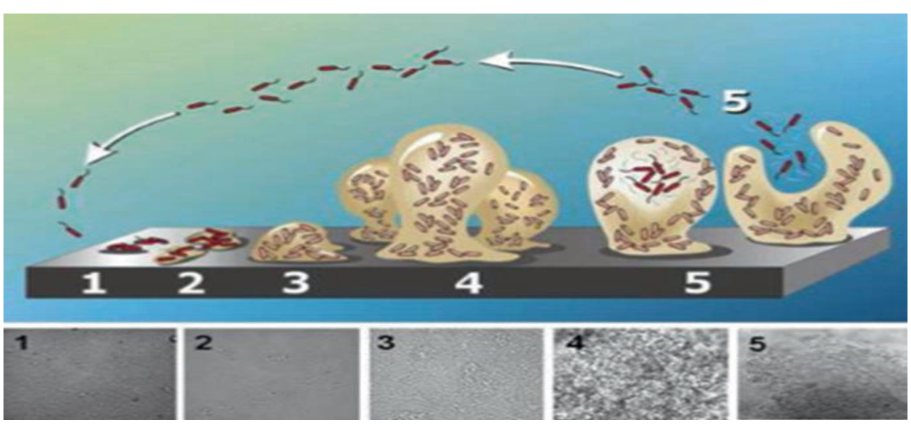

Table.4 Lists of biofilm reactors used for the wastewater treatment

Description		Type of wastewater	References
Aerobic membrane bioreactor (MBR)			
	functions as dual mechanism which membrane filtration occurs along with biodegradation processes water and small solution molecules pass through the membrane while solid materials, biomass, and macromolecules are retained in the reactor	Can treat high-strength synthetic wastewater	(Dhaouadi *et al.*, 2008)
Rotating	**biological contactor (RBC)** operates by attaching microorganisms to an inert support matrix to form a biofilm support matrix and a sequential disc configuration is placed partially or totally submerged in the reactor and it will rotates around a horizontal axis slowly where the wastewater flows through into it	Can treat high-strength synthetic wastewater with chemical oxygen demand (COD) concentration up to 12000 mg/L	(Von sperling *et al.*, 2005)
Anaerobic−aerobic granular biofilm bioreactor			
	granular biofilm bioreactor consists of an upflow anaerobic sludge bed (UASB), having an aeration column or sparger placed in the middle of the reactor anaerobic and aerobic populations of the biofilm co-exist closely in the same reactor offers a good strategy to complete mineralisation of highly substituted compounds	Treat various chlorinated pollutants	(Tartakovsky *et al.*, 2005)
Anaerobic-aerobic fixed film bioreactor (FFB)			
	combination of two fixed-film bioreactor with arranged media (anaerobic and aerobic) connected in series with recirculation system gives advantages as less sensitivity to environmental variations and higher growth rate due to the used of immobilised cells on the surface of the media	Treat wastewater that have high content of oil and grease	(Del Pozo *et al.*, 2003)
	use a cylindrical fluidised bed with pulverised pumice-stone as support material for microorganisms to attach aeration is performed by four cylindrical fine bubble membrane diffusers offers good stability despite variations in organic load and delivers short start-up time for operation	Eliminates organic carbon and nitrogen from municipal wastewater	(Fdez-Polanco *et al.*, 1994)

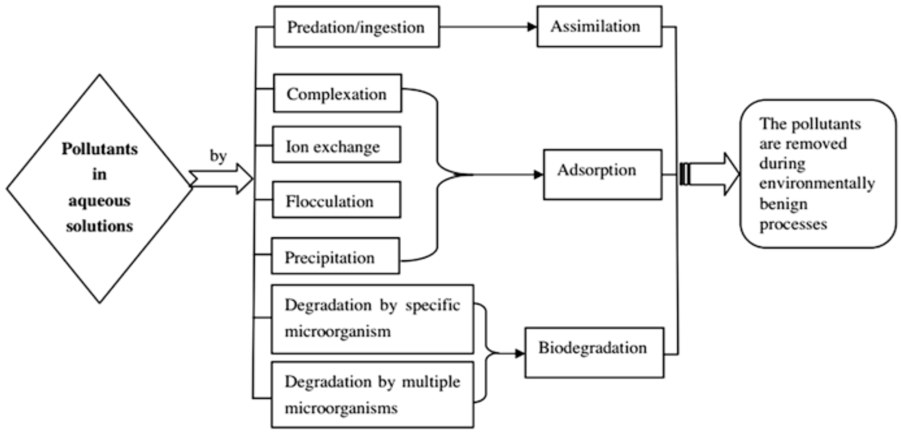

Figure.2 The method of contaminant removal from aqueous solutions by the conjunct mechanisms of assimilation, adsorption and biodegradation (Wu *et al.*, 2012)

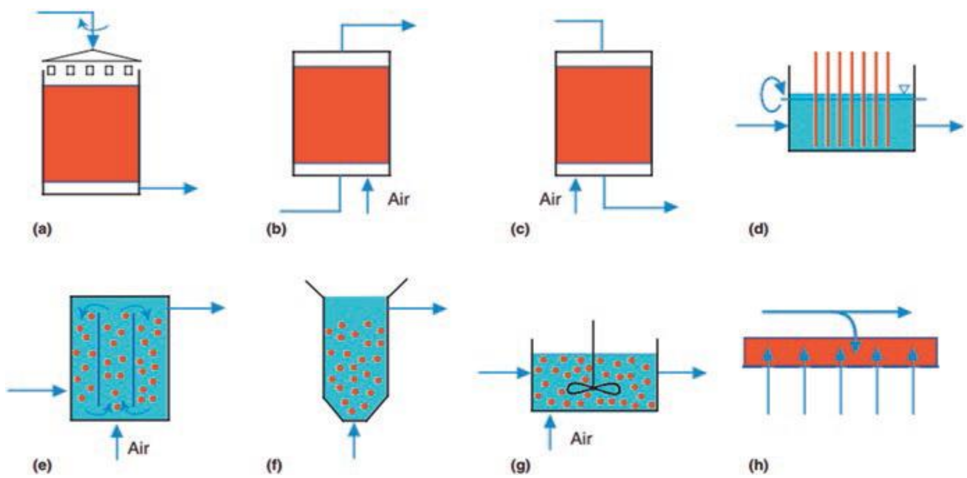

Figure.3 Types of biofilm reactors: (a) trickling filter; (b) submerged fixed bed biofilm reactor operated as up flow or (c) down flow mode; (d) rotating biological contactor; (e) suspended Biofilm reactor including airlift reactor; (f) fluidized bed reactor; (g) The moving bed biofilm reactor; and (h) The membrane attached biofilm reactors. (Morgenroth 2008; Lewandowski *et al.*, 2011)

Disadvantages of biofilm technique

Some Biofilm based bioreactors are costly.

Needs continuous watch and timely maintenance. However, this drawback can be subsidized by using several physical methods like back washing, mechanical scrubbing etc.

Another problem is biofouling of pipes. Despite of limitations, this technology is suitable and feasible way of combating aquatic pollution.

Control of aquatic pollution requires appropriate infrastructure and management plans. The infrastructure may include wastewater treatment plants, Sewage treatment plants and industrial wastewater treatment plants etc. Agricultural wastewater treatment for farms, and erosion control at construction sites can also help to prevent water pollution. Nature-based solutions are another approach to prevent water pollution. Among all these treatments Biofilm based bioreactors are in use from decades and are considered as best available technologies for waste water treatment. Biofilms are complex structured porous and most tolerant communities of the aquatic ecosystem that includes environmentally efficient strains of algae, fungi, bacteria, actinomycetes and viruses. These microbial isolates have been extensively studied for assimilation, bio-sorption and biodegradation of almost all sorts of organic and inorganic pollutants in aquatic ecosystems. Further research is needed to bio-stimulate the promising strains of biofilms for pollution treatment.

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