Membrane-Based Hybrid Processes for Wastewater Treatment

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CHAPTER

3

Biofilms, filtration, microbial kinetics and mechanism of degradation: a revolutionary approach

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3.1 Introduction

Water is important for our quality of life. The increase in freshwater due to rapid growth in population and industries leads to decrease the freshwater storages and increases wastewater (Ramakrishnaiah et al., 2009). Water contamination is now a major problem in the world because of globalization, industrialization, urbanization, growing population and growing economy increases pressure on water resources, because of this amounts of wastewater increased and this demands for new wastewater treatment process. Water pollution and the destruction of ecosystems are increased day by day and to overcome these problems wastewater treatment is need of the environment (Acharya, 2020). Wastewater is a mixture of liquid and solid waste that is discharged from domestic residences, commercial areas, industries, and agriculture fields. Wastewater contains a wide range of contaminants of different concentrations. Most of the drinking water comes from rivers, but today these rivers are polluted, so protecting river water quality is extremely important before the water is used as drinking water. Traditionally, wastewater is treated to remove organic matter, insects, pathogens and nutrients to decrease the environmental impact of its discharge. If such untreated wastewater is discharge directly into environment it can contaminate the

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drinking water sources. To handle such situations, wastewater management is the best solution. Recent trends of waste water treatment are to remove the contaminants using nanoparticles (Bhairappanavar, 2020; Dave and Sharma, 2015). It may also contain a high concentration of toxic substances, from which some are natural while some are synthetic, which can create problems in biological treatment (Lin et al., 2012). There are two main types of water pollutants present: organic water pollutants such as insects, bacteria, organic volatile compounds, food processing wastes, and organic chemical forms; inorganic water pollutants containing heavy metals, nitrates and phosphate run-off from agriculture, and chemical waste release by industries (Singh and Gupta, 2018).

One of the main important techniques used for wastewater treatment include sedimentation techniques, when insoluble particles get settled down, then the upper layer of water is separated and the bottom layer is further used for sludge treatment. For such treatments, sand filters are mainly used. At many of these places, chemical substances are used for wastewater treatment such as chlorine used to kill bacteria.

There are so many different methods used for wastewater treatment, such as

1. Biofiltration (Cervantes et al., 2006)
2. Recycle and reuse (Asano and Levine, 1996)
3. Zero liquid discharge system (Bouwer, 1993)
4. Physicochemical treatments (Liu et al., 2009)

Filtration is the most important process used for water and wastewater treatment, and it is mainly used to purify the surface water. Such filtered water can be reused for various purposes. Biofiltration is a relatively new technology, applied to wastewater purification so as to remove organic, inorganic compounds, toxic compounds, and odorous compound (Soccol et al., 2003) and process has been reported as a cost-effective method. Any type of filter that is attached with biomass is considered to be a biofilter. It was first introduced in England in 1893 as a trickling filter in wastewater treatment (Metcalf and Eddy Inc., 1991); after this, it has been successfully used for treatment of domestic and industrial wastewater. Biofiltration can effectively remove organic matter that is not able to be removed from water and biologically treated sewage effluent in conventional sewage treatment (Carlson and Amy, 1998). Biofiltration process involves biodegradation of pollutants with the help of microorganisms present in biomass attached to the filter. Some studies noticed some pharmaceuticals as pollutants in urban and industrial wastewater (Mompelat et al., 2009). If such compounds enters into an animal or human body, sometimes compounds get metabolized while some other remains unmetabolized and can be toxic to that living being. To overcome with these problems, innovations in wastewater treatment is required. This requires special types of biofilters that contain unique biomass and also involves activated sludge treatment processes. For best results, combination techniques are used, which involves activated sludge process and biofilters; such bioreactors are mainly used for anaerobic as well as aerobic processes.

Any material contains a certain amount of moisture, a biofilm can form and can be visible as a slimy layer. Therefore biofilms can easily discovered in any natural environment but the problem is that natural biofilms are slow growing. The biological treatment cost is much lower than that of physicochemical treatment (Kumar et al., 2011). The composition of the biofilm matrix in both natural and artificial or engineered form are different. Nutrient availability, temperature, ionic strength, pH, light, carbon source, and water content can alter the structure of biofilms (Flemming and Wingender, 2010; Wilking et al., 2011). The minimum requirement of biofilm is proper support or surface, nutrients, and water. The main important steps for biofilm formation are (1) attachment, (2) colonization, and (3) growth of biomass. When microbial biomass is used for wastewater treatment, then it is necessary to prevent them from being wash off. For the biofilm, the very important substances is the support medium, and the materials need to meet the following criteria: insoluble, nonbiodegradable, nontoxic, nonpolluting, lightweight, flexibility in overall shape, high mechanical and chemical stability, high diffusivity, simple immobilization procedure, high biomass retention, minimal attachment of other microorganisms, and less expensive (Leenen et al., 1996; Zacheus et al., 2000). Attachment of biomass to surface is mainly depending on adherence property of biomass. Microbes first reversibly attach to the surface and then irreversibly with the help of adhesive parts or chemicals released by that microbes. At first, microbes grow in small colonies and then colonized with each other to form a biofilm. Biological wastewater treatment is mainly involves use of prokaryotes, even if fungi, protozoa, algae, and rotifers may also use. (Bitton, 2005). The mechanism is mainly based on the process that microbes remove carbon and nutrient from wastewater with help of various metabolic and respiratory processes. The most probably used prokaryotes in biofiltration techniques belongs to Actinobacteria, Bacteroidetes, and proteobacteria (Wagner and Loy, 2002). At some places algal biofilms were used which showed promising result for wastewater treatment. Now a days rotating algal biofilm reactors are used for raw sewage water treatment (Gera et al., 2016). The performance of biofilter is mainly depend upon the organic loading rate, filter design and temperature (Niquette et al., 1998).

The main role of this study is to analyze the biofiltration method used for wastewater treatment. Innovations in the biofilms and biofiltration can increase the potentiality of wastewater treatment. The biofiltration-based methods for wastewater treatment are easy and safe for handling. Due to its wide-range of use and application, many studies have been done on biofiltration system. Genetically engineered organisms are used for biofilm production, which shows promising results in wastewater treatment.

The biological treatment is used in the secondary treatment processes where the major objective is the removal of organic matter. Ultimately the biological treatment systems are governed by the ability and actions of microorganisms to decompose and degrade the target organic contaminants. The properties of the microbial species which is involved in degradation or decomposition process decides how the organic contaminants are going to degrade or decompose a specific type of contaminant or a type of particular type of organic matter. Additionally, it will include how many cells are present; at what rate they can degrade or decompose the organic matter; what are the kinetics of substrate utilization or the pollutant degradation; and what is the kinetics of biomass growth? The microbial growth and basic requirements for the microbes to grow as well as the kinetics of the microbial growth are an important part of study during a wastewater treatment plant. This study reveals about the various models and the parameters of microbial growth kinetics in wastewater treatment.

3.1.1 Characterization of wastewater

Water is a very important part of living organisms. Water pollution means the addition of unwanted substances or nutrients as an energy forms that directly or indirectly enter into waterbodies which negatively affects the environment. Water pollution in the world has reached a critical point. Almost all the rivers, lakes, ponds, and other water bodies are now polluted by domestic or industrial wastewater. Main important sources of water pollution are sewage discharge, industrial effluents, and surface run-off of agricultural land (chemical fertilizers, pesticides, and insecticides).

Many of the industries are situated near the rivers because of such increasing industries effluents are release directly into river (Ray and David, 1966). Chemical effluents release in water bodies studied by (Ganpati and Alikunhi, 1950). Pharmaceutical wastes release in water is studied by (Ajmal, 1980). Industrial wastewater is mainly depends upon the type of industry and is mainly characterized by parameters such as chemical oxygen demand (COD), biological oxygen demand (BOD), suspended solids (SS), and dissolved oxygen (DO). Components of wastewater are nutrients, organic matter, heavy metals, and microbial pollution. Nitrate and phosphate are most commonly found in rural as well as urban areas, which act as a nutrient source for most microbes present in water bodies (Tripathi et al., 1998). Such nitrogen and phosphate are beneficial for growth of most microbes, and because of this number of microbes increase in waterbodies, more study on nitrogen level in the ecosystem was done by Mitchell et al. (2001). Blue baby is a type of symptom caused by increased level of nitrogen in the environment, which kills the new generations. Some industries discharge hot water directly into waterbodies from nuclear power plants or thermal power plants; this affects the biodiversity of the surrounding area, and this was studied by Zeikus and Brock (1972). Most industries release different types of chemicals like toxic metals in water bodies that interfere with DO, BOD, and COD (Mittak and Ratra, 2000). Turbidity of water also affected growth of microbes and it also affected natural processes such as photosynthesis of chloroplasts. The pH of water is an also important characteristic of water pollution. Most industries release large amounts of chemicals in water bodies and because of this the nature of water bodies turns acidic because of acid rain. The pH of water turns acidic, which is harmful for plants and other living organisms. Because of some human activities, sometimes radioactive materials release in water bodies and even small doses of such radioactive material can cause mutations (Singh and Gupta, 2016).

Most industries release some organic as well as inorganic substances, which cause eutrophication (Goldman, 1972). Industrial wastewater may contains small or large, freely suspended, and dissolved organic solids. Dead parts of living organisms can also increase the organic content of waterbodies this was detected by (Villar et al., 2001). Heavy metals are mostly present in effluents. Most of heavy metals are toxic in nature and can increase the toxicity of water. These heavy metals are uptake by some living beings such as hydra that uptakes chromium and manganese (Sinha et al., 1993). Accumulation of lead, copper, and iron was detected in algal species by (Rai and Chandra, 1992). The more advanced techniques helps to study that such heavy metals pollution can increase the risk of cancers (Fig. 3.1).

3.1.2 Biofilm mechanism

There are several different types of methods involved in wastewater treatment, but most of the time some unwanted pollutants remain in water after the treatment. In such

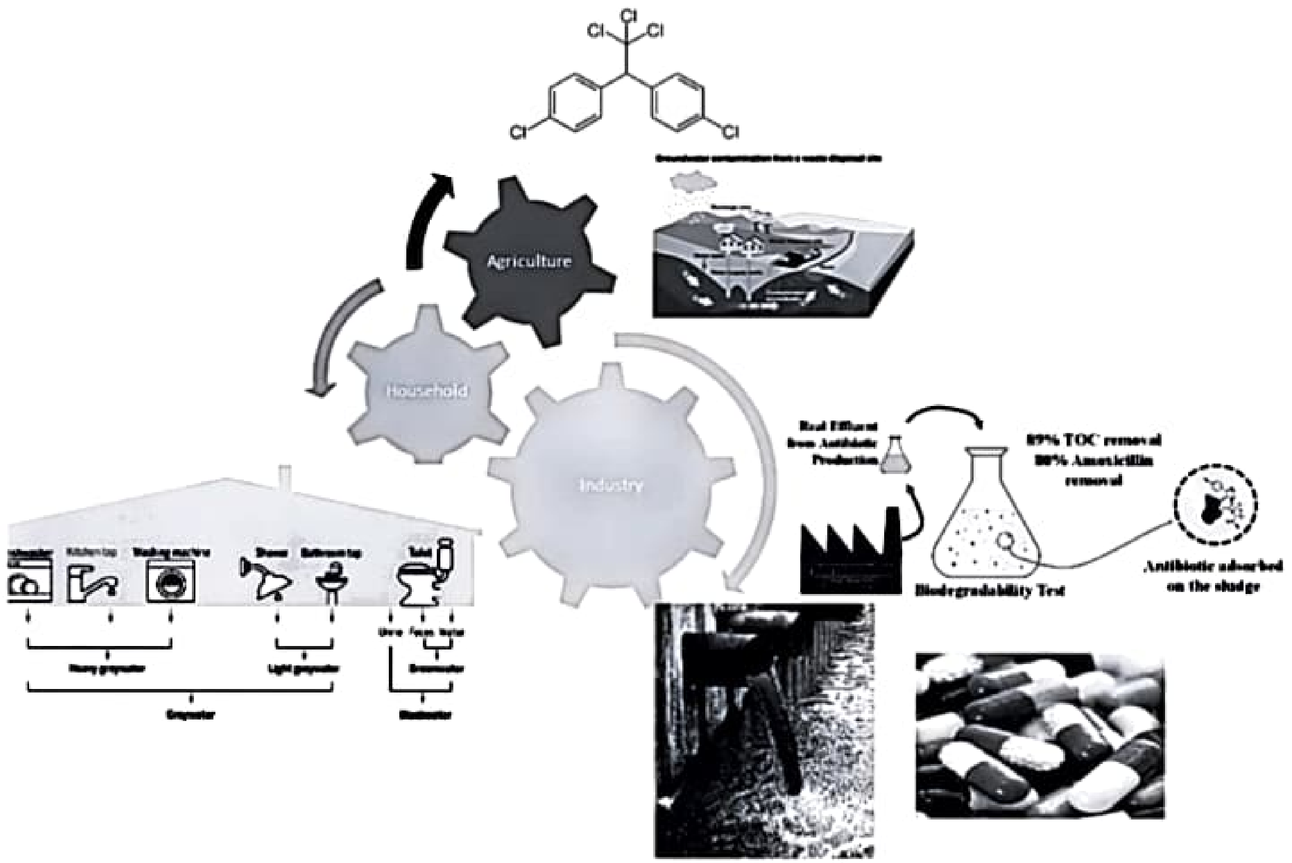

FIGURE 3.1 Types of pollutants in various sectors.

conditions new advanced techniques are used for wastewater treatment, such as biological treatment, which involves use of microorganisms to break down compounds that should not discharge in waterbodies. In this process, mix population of microbes are use so as to achieve best results. Wastewater treatment with biofilm systems has several advantages as compared to suspended growth systems.

In biofiltration process porous medium are used on which microorganisms are fixed, which helps to break down the pollutants. The microbes grow in small, slimy layers on the surface of the medium and the medium consists of essential nutrients for growth of biofilms. The overall effectiveness of biofilm is completely based on characteristics of support medium. Generally, in biofiltration method biodegradable pollutants are removed (Chaudhary et al., 2003). The concept of biofilm was discovered by Costerton et al. (1978). New technologies appeared around 1985 at the industries, which include biofiltration methods that help to decrease the suspended biodegradable pollutants and also help for oxidation of organic compounds. Biofilm is generally a complex structure of microbes in which one type of microbe's aggregates with each other or else different types of microbe's aggregates with each other. Biofilms are heterogeneous and forms complex communities of microbial cells suspended in matrix (Quintelas et al., 2011). Many problems affects the growth of biofilm such as nutrient availability, microbial community composition pH, temperature, mobility, attachment to support medium, and other environmental factors

(Singh et al., 2006). Biofilms are different than planktonic growth, it was observed that when microbial cells dispersed from biofilms then they undergo planktonic growth. The natural mode of microbial growth in the environment is biofilm pattern, but some of the microbes use planktonic growth pattern. The self-produced matrix of extracellular polymeric substances, which is also referred to as slime, is a polymorphic conglomeration generally composed of extracellular biopolymers in various structural forms (Vert et al., 2012).

In 1893 the first trickling-based biofilm technology was used for wastewater treatment in England (Lohmeyer, 1957). Today's microalgal biofilms are also used because these are easily available in a natural environment. Microbial biofilms have more efficiency to degrade complex biodegradable compounds than microalgal biofilms and planktonic microbial cells. The biodegradable pollutants are removed in the biofiltration method rather than physical straining as in the case in normal filters (Chaudhary et al., 2003). Microbes in biofilm produces extracellular substances that forms complex aggregates together and forms a structural matrix. Different types of bioreactors used for wastewater treatment include trickling filters, high-rate plastic media filter, rotating biological contactors, air lift reactors, membrane immobilized cell reactors, and granular filters (Lazarova and Manem, 2000). Biofilm support medium is based on size, porosity, density, and resistance to erosion. Biofilms are mainly used in industrial wastewater treatment as well as being used for heavy metal degradation (Rodgers and Zhan, 2003; Singh et al., 2006; Guibaud et al., 2006). For better performance, specific bacterial strains are used in biofilm. Genetically engineered bacteria is also used in wastewater treatment, and this was studied by Stephenson (Stephenson and Stephenson, 1992). Various techniques use genetically engineered microbial species or mixed culture of microbes for wastewater treatment. Wastewater treatment includes use of bacteria, yeast, fungi, and algae in biofilm synthesis (Abzazou et al., 2016; Cong et al., 2014; Badia-Fabregat et al., 2017; Hoh et al., 2016).

All these studies are extremely helpful to design and scale-up wastewater treatment. To successfully achieve the best results in wastewater treatment, selection of biofilm (homogeneous or heterogeneous), support systems, and other optimal conditions are very important.

3.1.3 Biofilm formation assay

Biofiltration is a natural phenomenon which can be formed as biological slims in lakes, rivers, rocks, etc. On any material that is subjected to a certain amount of moisture, a biofilm can form; usually it is visible as a slimy layer. Biofilms can discovered in any environmental conditions, it was observed that some biofilms are fibrous in nature. (Boelee, 2013). If light is present, then the biofilm may contain some photosynthetic bacteria or algal species and may have green color. The overall effectiveness of biofilm is completely based on support medium, which includes some important things such as porosity, water retention capacity and the ability to host the microbial populations. On the basis of wastewater the biofilms are selected such as aerobic, anaerobic, facultative, fungi, algae, and protozoa are developed on the surface of support medium (Devinny et al., 1999; Chaudhary et al., 2003). The commonly used support mediums are of different types such as ceramic, seashell, charcoal, plastic material, fire bricks, sand, natural stones like limestone, and rocky aggregates (Silva et al., 2008; Tarjányi-Szikora et al., 2013).

Immobilization of cells to support medium is an important part of biofilm formation. The physical confinement or localization of viable microbial cells to a certain defined region of space in such a way as to limit their free migration and exhibit hydrodynamic characteristic, which differ from those of the surrounding environment while retaining their catalytic activities for repeated and continuous use (Martins, 2013; Dervakos and Webb, 1991; Freeman and Lilly, 1998; Covizzi et al., 2007; Amim et al., 2010). The selection of support medium is one of the most important part of biofiltration method because support medium should support the following criteria such as insoluble, nontoxic, nonbiodegradable, light weight, flexible, simple immobilization procedure, high biomass retention, less expensive, easily available, and minimal attachment of unwanted bacteria (Leenen et al., 1996; Zacheus et al., 2000). In fact rough and porous surfaces are most favorable for cell adhesion and growth (Hoh et al., 2016). Immobilization of microbial cells to support medium can be a natural process or may be artificial; this was studied by mallick (Mallick, 2002). When microbial biofilms are used, then granular activated carbon is used as a support material (Quintelas et al., 2010; Muhamad et al., 2013). Microorganisms are an important part of biofiltration because they are responsible or biodegradation of contaminants. Hence, the concentration of such organisms in biofilm should be high (Cohen, 2001).

Biofilm formation can be divided into different phases such as attachment of microbes to support medium, formation of microcolony, microcolony grow as a biofilm and colonization of cells. Bacteria has a wide variety of structures that help for adhesion mechanism. The initial surface adhesion of bacterial cells is mainly depends on structures such as flagella, pili or capsule. When cells attaches to the surface, then they produce extracellular polymeric substances, which helps for irreversible attachment (Dunne, 2002). During this primary attachment, various forces involves which help for adhesion. Various studies say that this attachment is very fast, and it takes few seconds or minutes (Palmer et al., 2007). After the attachment, the maturation of microbes takes place which is further considered as a biofilm. If optimal conditions are present then bulk growth of microbes will occur, and further biofilm formation takes place. In most of the cases heterogeneous communities are present in biofilms. A mature biofilm is a complex structure which require several days and some studies find some cell to cell communication (Stoodley et al., 2002; Kolter and Greenberg, 2006; Dunne, 2002; De Lancey, 2001). The composition of extracellular polymeric substances varies as per the type of microbes present in biofilm and the environmental conditions (Kolter and Greenberg, 2006). It was observed that EPS components helps as a nutrient source, and mature biofilm is present in this EPS layer. The concentration of bacteria found in the top layer is high than the concentration of bacteria's present in other layers. It was observed that the EPS produced by most of bacteria in biofilm is completely different than EPS produce in planktonic form by the same species (Celmer et al., 2008; Kives et al., 2006). In some cases it was observed that because of some biological, chemical, and physical activities, detachment of biofilm takes place. Absence of sufficient nutrients, high water flow, pressure, sloughing, and erosion can cause detachment. In this detachment the net forces between the cells of biofilms weakened simultaneously some carbohydrate degrading enzymes are released resulting detachment of cells, which further undergoes planktonic lifestyle (Stoodley et al., 2002; Garrett et al., 2008).

Advanced techniques are used for wastewater treatment, which involves genetically engineered bacteria and artificial support mediums which have all the optimal conditions for biofilms, such techniques are effective, easy for handling, and less expensive (Dave and Das, 2020, 2021).

3.1.4 Types of biofiltration techniques

Biological wastewater treatment is a wide concept that involves two main techniques: biofilm-based and freely suspended microbial systems (activated sludge treatment) and also divided into two parts such as aerobic and anaerobic. In aerobic processes, removal of biodegradable organic compounds is more as compared to anaerobic treatment while for industrial effluents anaerobic methods are very useful because the COD of industrial effluent is high (Seghezzo et al., 1998; Chan et al., 2009). There are some advantages and disadvantages of these both methods so to overcome with these problems many industries and Municipal Corporation used anaerobic–aerobic based systems (Frostell, 1983; Cervantes et al., 2006).

3.2 Types of biofilm based bioreactors

3.2.1 Trickling filter

The first trickling filter was used in 1893 in England. The media used in this process consisted of rocks. After this, due to innovations in the techniques instead of rocks, plastic is used as a support medium; such advanced trickling filters have been used for more than 50 years. Organic compounds are decomposed by biofilms grown on inert support. Facultative bacteria, *Adcaligenes*, *Flavobacterium*, *Nitrobacter*, *Achromobacter*, *Pseudomonas*, and filamentous-like microbes were observed, and some protozoa and fungi are also found in biofilms (Metcalf and Eddy, 2003; Grady et al., 2011). Trickling filter techniques required one important step at the end of the process is liquid–solid separation which involves removal of suspended solid particles. This is done by using rotatory distributor. As compare to rock media small particles such as plastic rings, zeolite, ceramic and sponge are also used so as to enhance the oxygen transfer (Zhang et al., 2016). Roughing filters are used mainly to reduce the organic load to allow more oxidation and nitrification. (Lewandowski and Boltz, 2011).

3.2.2 Rotating biological contactors

This is an slightly advanced technique in which biofilms are grown on disc surfaces of polystyrene, polyethylene, stainless steel, cement, aluminum, glass, PVC, Teflon, or rubber, etc., and these discs are fixed in rotating digesters. The first rotating biological contactors (RBC) was experimented in Germany in 1928 (Tawfik et al., 2005). These techniques used in most of the developing countries because of low cost, simple treatment systems, and highly efficient for oxidation and nitrification (Boller, 1987). Many modifications are done in this system because of some disadvantages, such as odor problems, BOD remains high and it requires oxygen flow so as to avoid anaerobic conditions (Surampalli and Baumann, 1995). To improve the performance of RBC, anaerobic pretreatment methods are used

(Tawfik et al., 2002). The main important feature of RBC is it requires low space and the overall cost is low. There are some disadvantage such as odor problem, sometimes high amount of sludge is produced.

3.2.3 Fluidized bed reactor

Fluidized bed reactor (FBR) are also termed as fluidized bed biofilm reactor (FBBR). These were first introduced in 1980, and this system is a combination of two systems in which biofilm-based systems and activated sludge systems both are used (Burghate and Ingole, 2013). Support medium particles are distributed in a chamber from top to bottom in an increasing-sized gradient pattern. Support medium used for this system may be sand, fly ash, carbon, glass, or calcinated clay particles of small size (Puhakka et al., 1995; Kida et al., 1990). Media particles present on this reactor helps to increase the surface contact between microbial cells and effluents. FBBRs are used more as compared to FBRs because FBBR efficiency for denitrification and removal of oxidized contaminants is higher (McCarty et al., 2005). The microbial population found in FBBR is mixed, complex, and diverse. Different research and development work is going on for improvising FBBR to reduce energy consumption, improve its stability, and reduce the start-up time. FBBR shows promising results in toxic-containing wastewater treatment (Holst et al., 1991). Physical design of anaerobic reactor is similar to aerobic FBR. Such anaerobic treatments are used for hazardous and industrial effluents. Activated carbon is used in many of the anaerobic processes, which increases the cost (Metcalf and Eddy, 2003). Advantage of this method is compact small reactor and low biomass sludge production. This method can be used in three different ways: (1) aerobic, (2) anaerobic, and (3) anoxic (mainly used for denitrification). One more modification was done in this system: inverse fluidized bed reactor (Schugerl, 1997).

3.2.4 Moving bed biofilm reactor

As one of the best and advanced techniques for wastewater treatment, this biofilm-based technique is currently used in more than 50 countries, and it was first developed in Norway in the 1980s and early 1990s (Rusten et al., 2006) various categories of toxins are present in waste water as is shown in Fig. 3.2 generated from various sources. The system contains submerged biofilm reactor and liquid–solid separation units. The liquid–solid separation can be done by various processes. The moving bed biofilm reactor (MBBR) contains plastic as a support medium, which helps for treated effluents to flow toward the next treatment step. This type of bioreactor can be used in aerobic or anaerobic systems (Ødegaard, 2006a, b). In this technique biofilms are grown inside of the plastic carrier and the moving bed reactor helps to decrease the clogging and increase the ability to utilize the whole volume of bioreactor. MBBR provides more surface area for biofilm than other techniques (Ødegaard, 2006a, b). The MBBR process is a continuous process that does not require biofilm thickness control. MBBR, combined with other treatment systems, shows promising results in wastewater treatment because such combinations helps for oxidation and denitrification of organic substances (Gilbert et al., 2014; Malovanyy et al., 2015).

FIGURE 3.2 Toxins from various industries.

3.3 Applications of biofilm based reactors

1. Biofilm based reactors mainly help to reduce BOD, COD, heavy metals, toxic pollutants, and biodegradable substances (Chen et al., 2008).
2. Biofiltration methods are used as biosensors and also used in some industries for specific bioconversion of waste to specific products.
3. *Hypomicrobium* spp, *Micrococcus* spp. helps for denitrification and nitrate reduction (Lazarova et al., 1994).
4. Some methanogenic cells produce methane by using wastewater nutrients.
5. Some fungal spp degrades phenolic substances from wastewaters of hospitals and laboratories (Anselmo et al., 1985).
6. Enzymes are extracted from biofilms used in various industries to hydrolyze the glucose, lactose, and cellulose.
7. Cyanide- and chromium-contained wastewater treatment involves use of mycelia which converts cyanide into formamide (Leyva-Ramos et al., 2008).
8. Biofilm-based reactors are now used to convert stored chemical energy into electrical energy (Aelterman et al., 2008).

3.4 Microbial growth and its kinetics

Removal of organic matter is the major objective of biological treatment. Microbes can degrade and decompose organic containment. This property may be specific toward a containment or microbial growth plays major role in this case. Microbial cell division or their kinetics will define the degradation rate of containment. Biological growth is linked to both the synthesis of cellular constituents (biomass) and the multiplication of individual cells to produce offspring. In a growth medium with excess of nutrients and under stable environmental conditions with respect to pH, temperature, and so on, and the time required for cell division and produce new generation.

Wastewater treatment is usually carried out in three stages. Primary treatment: Heavy solids are separated by sedimentation and lighter compounds like oil and grease are retained on the surface. Secondary treatment: Aeration, biofiltration, and biological processes included to remove dissolved and suspended organic compounds like pollutants, toxicants, etc. There are two kinds of clarifiers used in secondary wastewater treatment: primary clarifiers (sludge collected in a sump) and secondary clarifiers (biological floc removed and hence the effluent becomes clearer). Finally, tertiary treatment involves the removal of inorganic compounds and improves wastewater quality before recycled, reused, and discharged into the environment.

T is the secondary treatment where the microorganisms are biologically used to remove toxic contaminants, and the purpose of secondary clarifiers is to separate flocculated biomass, that is, the settleable suspended solids from liquid wastewater. In this stage, the wastewater is treated with activated sludge, bugs, and microorganism mass (mostly bacteria and protozoa). *Bacillus* is best suited to treat fats, oil, and greases (Dave and Das, 2020, 2021).

There are two modes of action specific to bugs: aerobic digestion—microbes digest organic waste by stealing its oxygen, altering the chemical makeup of the material into a less environmentally damaging matter. Anaerobic digestion—biological fermentation of organic matter. Organic materials are converted into biogas, which helps to reduce pollutants and contaminants. Biofilm process is also used to treat wastewater-containing biodegradable substances, which provides resistance against toxic pollutants and capable of retaining biomass under continuous operation. Development of kinetics for an aerobic biofilm reactor has been presented using a new model of biofilm (Hinson and Kocher, 1996), which is formulated from correlation between substrate concentration in the influent and effluent and at the bottom with substrate flux and thickness complying monod growth kinetics.

Due to some advantages like low energy consumption, easy maintenance, better chemical and physical stability, and excellent biomass retention capacity, the importance of biofilm is increasing in the biological treatment of wastewater. There is a limited number of methods available for determining the kinetic coefficient required for the solution of the model of an aerobic biofilm bioreactor. The concept of a biofilm model is based on three basic assumptions, that is (1) nature of kinetics for substrate utilization in suspended growth and attached growth in similar manner; (2) external substrate transport from the bulk liquid to the biofilm via flick law of molecular diffusion; and (3) substrate transport within the biofilm.

3.4.1 Principles of growth kinetics

Generally, growth is associated with either an increase in number or increase in size depending upon its influencing parameters, whereas microbial growth is a rapid and relatively simple process that infers to increase in cell number and not in cell size. The growth mechanism is dependent on nutritional and environmental constituents. Moisture, pH, oxygen level, and pressure are the major physical/environmental factors affecting microbial growth. Under optimum environmental conditions, nutrients play a vital role in cultivation of microbes properly in the laboratory. Microbial nutrition encompasses carbon and energy sources, necessary nutrients specific to microbes, salt levels, and other trace elements. Both the physical and chemical factors equally affect the growth and survival of microorganisms.

The use of microbes in biological processing of wastewater achieves fats, oil, and grease (FOG) removal, BOD reduction, COD reduction, total suspended solids reduction, and odor elimination. Being a critical part in the entire purification process, microbial activity is greatly influenced by the kinetic parameters. The maximum elimination of organic components/pollutants is achieved only under optimum conditions of the kinetic factors. Thus the desirable yield of quality water is obtained only when the microbial activity is maximum during exponential (highly active) phase, and this is attained under certain conditions according to the specific microbes.

Microbial growth rate of the biomass utilizing an organic chemical can be related to chemical concentration. Because of low specific rates, at which bacteria grow in the wastewater treatment system, if the culture is grown at high rate cells, the protein-synthesizing system will be fully developed and the cell will contain enzymes, which are required for growth and utilized by substrates. Fully developed bacterial systems have enzymes that increase the growth rate as fast as possible (Grady and Williams, 1975).

3.4.2 Stoichiometry of microbial growth

The quantitative representation of microbial inoculum and the biomass yield or product obtained out of chemical reactions refers to growth stoichiometry. There are two groups of substrates for microbial growth:

1. Catabolic substrates, which are sources of energy; and
2. Anabolic or conserved substrates, which are sources of elements forming cellular material.

Catabolic substrates include H_2 for lithotrophic hydrogen bacteria, and for nitrifying bacteria, S_2 for sulfur-oxidizing bacteria, and oxidizable or fermentable organic substances for heterotrophic bacteria and fungi. The conserved substrates include nearly all the noncarbon sources of biogenic elements (N, P, K, Mg, Fe, and trace elements), CO_2 for autotrophs, and the indispensable amino acids and growth factors.

The overall growth yield coefficient $Y^M_{x/s}$, which is the maximum yield of cell mass per unit mass of substrate consumed when no maintenance is considered. Two other yield and maintenance coefficients of importance are related to ATP consumption and oxygen. The ATP yield coefficient, $Y_{x/ATP}$, represents the amount of biomass synthesized per mole of ATP generated. P′ represents the amount of biomass synthesized per mole of ATP generated. It has been observed that for many substrates and organisms $Y^M_{X/ATP}$ nearly constant at 10 to 11 g dry weight mol^{-1} ATP for heterotrophic growth under anaerobic conditions. The ATP yield for many autotrophic organisms (autotrophic organisms fix CO_2) is $Y^M_{X/ATP} = 6.5$. Under aerobic conditions, the values for $Y^M_{X/ATP}$ are usually greater than 10.5 (Van Hoek et al., 1998).

The prediction of yield coefficients is not exact, because unknown or unaccounted for metabolic pathways and products are present.

Respiratory quotient (RQ) is defined as the moles of CO_2 produced per mole of oxygen consumed. The RQ value provides an indication of metabolic state and can be used in process control. P/O ratio is the ratio of phosphate bonds formed per unit of oxygen consumed (g mole P/g atom O). The P/O ratio indicates the efficiency of conversion of reducing power into high-energy phosphate bonds in the respiratory chain.

The complexity of mass and energy balances for cellular growth can be decreased greatly through the recognition that some parameters are nearly the same irrespective of the species or substrate involved. Microbial growth kinetics of suspended cells have been investigated in the laboratory in batch, continuous-culture, or fed-batch systems. In batch-culture experiments, either the consumption of the growth-controlling substrate or the increase in biomass concentration was monitored as a function of time. Inherent in this system is that the cell's environment and hence the cell's composition and physiological state change.

3.4.3 Variation in growth yield and depending parameters

The operating parameters of the system, such as aeration, agitation, and chemical injection also create quantitative changes between autotrophic and heterotrophic bacteria. In municipal wastewater treatment plants, for example, gram-negative bacteria of the *proteobacteria* type are predominant (21%–65%) of which *Betaproteobacteria* is the most abundant class, largely responsible for the elimination of organic elements and nutrients. The other branches are *Bacteroidetes, Acidobacteria,* and *Chloroflexi.*

Among fungi, *Ascomycetes* are the most common. Then comes the *archaeobacteria,* with *Euryarcheota*. In addition, in presence of ammonia and oxygen, nitrosomonas is very present. Finally, a high sludge age allows protozoa and rotifers to colonize the environment.

Oxygen level: Secondary treatment, oxygen is required as a terminal electron acceptor in organic matter degradation, for example, nitrification by *Nitrosomonas* and *Nitrobacter* species requires dissolved oxygen to occur. Oxygen in secondary treatment is provided manually by pumping oxygen into the sewage continuously, which occurs in an aeration tank.

pH: pH affects the solubility of compounds, which indirectly affect the accessibility by bacteria. Also, bacteria responsible for organic matter degradation are sensitive to the pH of the environment. The pH of sewage treatment is controlled to be around 7. A nitrifier in secondary treatment, *Nitrosomonas* requires a pH between 6 ~ 9 in order to be viable.

Temperature: Bacteria are known to have higher enzymatic activity at higher temperature because of increased thermal energy. For example, when thermophilic sludge treatment is compared to mesophilic treatment, the sludge biodegradability is higher with thermophilic degradation. Hence the temperature has to be controlled precisely to maximize the efficiency of degradation.

The impact of elevated temperature on bacterial community structure and function during aerobic biological wastewater treatment was significantly specific. Continuous cultures, fed a complex growth medium containing gelatin and α-lactose as the principal carbon and energy sources, supported mixed bacterial consortia at temperatures ranging from 25°C–65°C. These temperature and substrate-acclimated organisms were as inocula for treatment purpose in which the kinetics of microbial growth and substrate utilization, efficiency of substrate removal, and mechanism of substrate removal were compared as functions of temperature. The efficiency of substrate removal (pollutants) declined at elevated temperatures. Maximum specific growth rates and the growth yield increased with temperature from 25°C–45°C, but then decreased with further elevations in temperature. Thus maximum specific substrate utilization rates did not vary significantly over the 40°C temperature range (LaPara et al., 2000).

3.4.4 Microscopic approach in growth studies

Cell growth is viewed as a set of transport and intracellular metabolic reactions known for some particular organisms. As a rule, the produced metabolic networks are composed of a combination of true stoichiometric equations for individual metabolites and empirical gross equations.

The microscopic approach has been a successful tool in displaying the microbial processes as a whole with desired accuracy. Depending upon the microbial specificity, the biomass yield or product can be quantified by various approaches after the analysis of target. Microscopic biomass quantification approaches include direct cell counting, colony counting, most probable number, biomass measurement, and light scattering (Mauerhofer et al., 2019).

The rate of biomass growth throughout the cell cycle was hypothesized to be linear, bilinear, exponential, double-exponential, and so on (Cooper and Charles, 1968 and Koch, 1971), Interpretations include: size measurements of individual cells growing in the microculture by use of phase-contrast microscopy to recently developed confocal scanning light microscopy combined with image analysis; pulse-chase labeling of cells with their subsequent separation into different phases of the cell cycle. Most frequently, labeled uracil and leucine are used as precursors of RNA and protein synthesis, respectively; and analysis of the frequency distributions of steady-state populations. However, the resolving power of this approach is rather low because linear and exponential models produce similar patterns. In addition to this, extrachromosomal elements like plasmids, transposons, and insertional elements also contribute to bacterial growth despite the presence of antibiotics in optimum conditions (Panikov, 1995).

Wastewater from industries and other sources has mixtures of pollutants containing organic and inorganic heavy metal impurities. Development of strategies for soil water contamination requires interactions among kinetic substrates. The removal of components may inhibit other components in the mixtures, which can degrade different compounds at different patterns (Alvarez et al., 1991).

Conclusion

It is estimated that the kinetic parameters of microorganisms optimizes the metabolic flux. Depending upon the microbes used for specific pollutants and toxicants digestion, the kinetic parameters are altered and optimized accordingly to achieve the desired biomass yield. The kinetic parameters, together with nutritional requirements particular to microbes, largely influence the substrate utilization or product formation and its quality. A first insight into growth kinetics under more complex conditions is provided by the recent results obtained for the kinetics of growth controlled by more than one carbon source.

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