CHAPTER 1

Introduction

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1.1. Introduction

Industrialization is one of the key aspects of the growth and economic development of any country (Asri et al., 2018; Varjani and Upasani, 2017a). The industries such as petrochemicals, refineries, textiles, pulp and papers, pharmaceuticals, leathers, etc., have enormous economic significance due to their involvement in vibrant production and employment generation (Sonwani et al., 2019a; Vikrant et al., 2018; Xiao et al., 2019). These industries consume a large amount of water for the production of different products and discharge huge amount of wastewater, which contains a wide range of pollutants (Hassard et al., 2014; Vikrant et al., 2018; Yaseen et al., 2019). One of the sectors causing excessive environmental concerns is the petroleum and textile industries since they release toxic pollutants, namely chlorobenzene, aromatic hydrocarbons (AHCs), dyes, heavy metals, etc., in water bodies (Talha et al., 2018; Sonwani et al., 2019a). Among these pollutants, most of the dyes and AHCs have been listed as precarious pollutants by various international environmental legislations (Demeter et al., 2017; Silva et al., 2009).

1.2. Dyes: sources and their adverse effects

The dyes are the organic substance having property to impart their colour to other materials such as fabrics, papers, drugs, foods, etc. (Yagub et al., 2014). They are mainly composed of two groups, i.e. chromophore and auxochrome, and can absorb the light in the visible range (380-700 nm). The chromophore groups such as -N=N-, -C=C-, -C=N-, $-NO_2$, -C=O are responsible for imparting the colour, whereas auxochrome groups, namely $–NH_3$, $-SO_3H$, -COOH, and -OH can enhance the intensity of chromophore (Benkhaya et al., 2020). Most of the dye molecules can withstand degradation when exposed to either strong light or extreme heat or oxidizing chemicals (Ayed et al., 2010; Katheresan et al., 2018). The commercially available dyes can be classified in terms of colour, chemical structure or chromophore group, and colour index. The classification of dyes on the basis of the chemical structure and their properties is summarized in **Table 1.1**. Industries such as textile, carpet, pulp and paper, printing, polymer, and cosmetics discharge large amounts of dye contaminated wastewater, which adversely affects the environment (Hameed and Ismail, 2018; Padmanaban et al., 2016; Bankole et al., 2019). The wastewater from these industries is categorized by highly coloured with the high level of chemical oxygen demand (COD) and biochemical oxygen demand (BOD). The textile industry is reported as one of the world's most polluting industry (Islam et al., 2019; Lellis et al., 2019). According to the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry, more than 90% of dyes and colours used in fabrics have LD50 (lethal dose, 50%) value ≥ 2000 mg/kg (Mishra et al., 2020). More than 10,000 types of pigments and dyes are used in various industries, and about 7.0 lakh tons per annum are produced globally (Ogugbue and Sawidis, 2011; Robinson et al., 2001). It is also reported that about 200 L of water needed to make 1 Kg. of textile product, and around 10-15% of dye used are lost in the dyeing operations and are discharged finally in the water bodies (Yaseen and Scholz, 2019). Further, about 2.8 Lakh tons per annum of dyes contaminated wastewater are discharged in water bodies worldwide (Sonwani et al., 2020a). According to Talha et al. (2020), amongst the various classes of dyes, azo dyes are widely used in various unit operations and unit processes and contribute about 60% of the total consumption of dyes.

The discharge of wastewater from dyes industries can cause an adverse impact on both aquatic organisms and human beings. The large concentration of dye can reduce the sunlight penetration in water and disrupts the photosynthetic activity of the aquatic plants (Kumar et al., 2012; Mishra et al., 2020). The survival of aquatic species is threatened by low dissolved oxygen (DO) in wastewater (Sonwani et al., 2020a). The existence of various dye molecules in water bodies leads to toxic, mutagenic, and carcinogenic effects on human health and aquatic life (Hassan and Carr, 2018; Mittal et al., 2009). Further, the bio-accumulation of dye and its associated molecules can occur in sediments and soil, which might be transferred to the public water supply (Xiang et al., 2016). The bio-magnification of dye molecule into aquatic organisms is another challenging aspect of dye contaminated wastewater followed by the cross-transfer food chain. The acute toxicity to dyes is mainly caused by oral ingestion and inhalation. The workers who handle the unit operation and process have direct contact with reactive dyes, which may lead to dermatitis, conjunctivitis, asthma, or other allergic issues (Lellis et al., 2019). Katheresan et al. (2018) have reported that the dye contained wastewater with high pH (acidic) and temperature are released in water bodies and lead to the depletion of DO and self-purification process.

Table 1.1 A summary of dyes and their properties.

Dye name	Type	Molecular formula	Molecular mass (g/mol)	CAS no.	Maximum absorption (nm)
Acid red 114	Azo	$C_{37}H_{28}N_4Na_2O_{10}S_3$	830.81	6459-94-5	514
Acid orange 7	Azo	$C_{16}H_{11}N_2NaO_4S$	350.32	633-96-5	483
Congo Red	Azo	$C_{32}H_{22}N_6Na_2O_6S_2$	696.66	573-58-0	497
Para red	Azo	$C_{16}H_{11}N_3O_3$	293.28	6410-10-2	489
Methyl orange	Azo	$C_{14}H_{14}N_3NaO_3S$	327.34	547-58-0	464
Reactive blue 4	Anthraquinone	$C_{23}H_{14}C_{12}N_6O_8S_2$	637.4	13324-20-4	595
Alizarin red S	Anthraquinone	$C_{14}H_7NaO_7S$	342.26	130-22-3	556
Indigo Carmine	Indigoid	$C_{16}H_8N_2Na_2O_8S_2$	466.4	860-22-0	555
Naphthol yellow S	Nitro	$C_{10}H_4N_2Na_2O_8S$	358.19	846-70-8	414
Acid blue 7	Triarylmethane	$C_{37}H_{35}N_2NaO_6S_2$	690.8	3486-30-4	625
Malachite green	Triarylmethane	$C_{23}H_{25}ClN_2$	364.9	569-64-2	618
Bromocresol green	Triarylmethane	$C_{21}H_{14}Br_4O_5S$	698	76-60-8	423

1.3. Aromatic hydrocarbons (AHCs): sources and their adverse effects

AHCs are ringed hydrocarbon compounds and are broadly classified as: (a) monocyclic aromatic hydrocarbons (MAHs: e.g., benzene, toluene, ethylbenzene, and xylene) and (b) polycyclic aromatic hydrocarbons (PAHs: e.g., naphthalene, fluorene, pyrene, etc.) (Varjani and Upasani, 2017a). In general, MAHs contain one aromatic benzene ring (e.g., benzene). Whereas, PAHs possess two or more fused aromatic benzene rings with their molecules organized in linear and angular arrays (e.g., naphthalene, fluorene, and pyrene) (Patowary et al., 2015; Wu et al., 2019). Physical and chemical possessions of AHCs depends on conjugated π-electron systems. In this work, the details PAHs are mainly discussed and included in the thesis.

PAHs enter into the soil, water, air, and sediments via natural and anthropogenic activities (Haritash and Kaushik, 2009). The natural activities include forest fires, volcanic activity, and natural oil deposition, while anthropogenic activities such as crude oil transportation, biomass burning, coal, asphalt, leakage from the petrochemical industries, and incomplete combustion of organic materials (Liu et al., 2014; Varjani et al., 2015; Vane et al., 2014). Zheng et al. (2016) reported that PAHs could contaminate the aquatic environment by either deposition or release of fuel or oil spills. The water of the river and canals in Bangkok are enormously contaminated with PAHs (Boonyatumanond et al., 2006). Moreover, the soil adulterated with PAHs could be responsible for groundwater contamination via leaching (Liang et al., 2017). Gases emitted from petroleum industries also contain some amount of PAHs which enter in the atmosphere via evaporation (Lamichhane et al., 2016). Due to positive interactions between the partition coefficient (K_d) of PAHs and organic compounds, PAHs are also get adsorbed onto organic sediments (Patrolecco et al., 2010). In soil, PAHs enter by either physical and chemical adsorption or solubilization into organic or particulate matter (Nguyen et al., 2014). Moreover, the soil contaminated with PAHs may pollute the water bodies via leaching and enhance toxicity to plants and animals (Liang et al., 2017).

The body exposes to these pollutants mainly via three routes: (a) food ingestion (e.g., vegetables, fruits, and meats), (b) dermal contact or skin penetration, and (c) inhalation through respiration. Most of the PAHs are considered as persistent organic pollutants (POPs) and cause several health issues, such as kidney, lung, and liver damage (Long et al., 2013). These PAHs are toxic, carcinogenic, and mutagenic to human health, aquatic life, and microorganisms (Desforges et al., 2016). The high exposure to PAHs causes birth defect and decreases body weight during pregnancy (Kim et al., 2009). **Table 1.2** lists the toxicity of PAHs reported by various environmental agencies, namely, the Unite State Environmental Agency (USEPA), International Agency for Research on Cancer (IARC), and Agency for Toxic Substances and Disease Registry (ATSDR). **Figure 1.1** demonstrates the chemical structures of 16 PAHs.

The harmful concern of dyes and aromatic hydrocarbons motivated the researchers to develop cost-effective, environmentally benign, and efficient treatment technologies to ensure the sustainability of the environment to future generations.

Table 1.2 Toxicity of polycyclic aromatic hydrocarbons reported by various environmental agencies.

Compound	Molecular formula	Molecular mass (g/mol)	CAS no.	Toxicology EPA[a]	IARC[b]	Toxicity rank ATSDR[c]
Naphthalene	$C_{10}H_8$	128.17	91-20-3	C	2B	80
Acenaphthene	$C_{12}H_{10}$	154	83-32-9	D	3	168
Acenaphthylene	$C_{12}H_8$	152	208-96-8	D	n.c	-
Fluorene	$C_{13}H_{10}$	166.22	86-73-7	D	3	-
Phenanthrene	$C_{14}H_{10}$	178.23	85-01-8	D	3	246
Anthracene	$C_{14}H_{10}$	178.23	120-12-7	D	3	-
Fluoranthene	$C_{16}H_{10}$	202.26	206-44-0	D	3	138
Pyrene	$C_{16}H_{10}$	202.25	129-00-0	D	3	-
Benzo[a]anthracene	$C_{18}H_{12}$	228	56-55-3	B1	2B	37
Chrysene	$C_{18}H_{12}$	228	218-01-9	B2	2B	141
Benzo[k] Fluoranthene	$C_{20}H_{12}$	252	207-08-9	B2	2B	61
Benzo[b] Fluoranthene	$C_{20}H_{12}$	252	205-99-2	B2	2B	10
Benzo[a]pyrene	$C_{20}H_{12}$	252	50-32-8	B2	1	8
Benzo [g,h,i]perylene	$C_{22}H_{12}$	276	191-24-2	D	3	-
Dibenzo[a,h] Anthracene	$C_{22}H_{14}$	278	53-70-3	B2	2A	15
Indenol[1,2,3-cd]pyrene	$C_{22}H_{12}$	276	193-39-5	B2	2B	-

[a]EPA carcinogenic classification; A= human carcinogenic; B1 and B2= probable human carcinogenic; C= possible human carcinogenic; D= not Classifiable as to human carcinogenicity; E= evidence of non-carcinogenicity for humans; [b]IARC; 1= carcinogenic to humans; 2A= probably carcinogenic to humans; 2B= possibly carcinogenic to humans; 3= not classifiable as carcinogenic to humans; n.c= not classified; [c]ATSDR, NPI-Australia (2013). EPA= Environmental protection agency; ATSDR= Agency for toxic substances and disease registry; IARC= International Agency for Research on Cancer (Ghosal et al., 2016; Biswas et al., 2015).

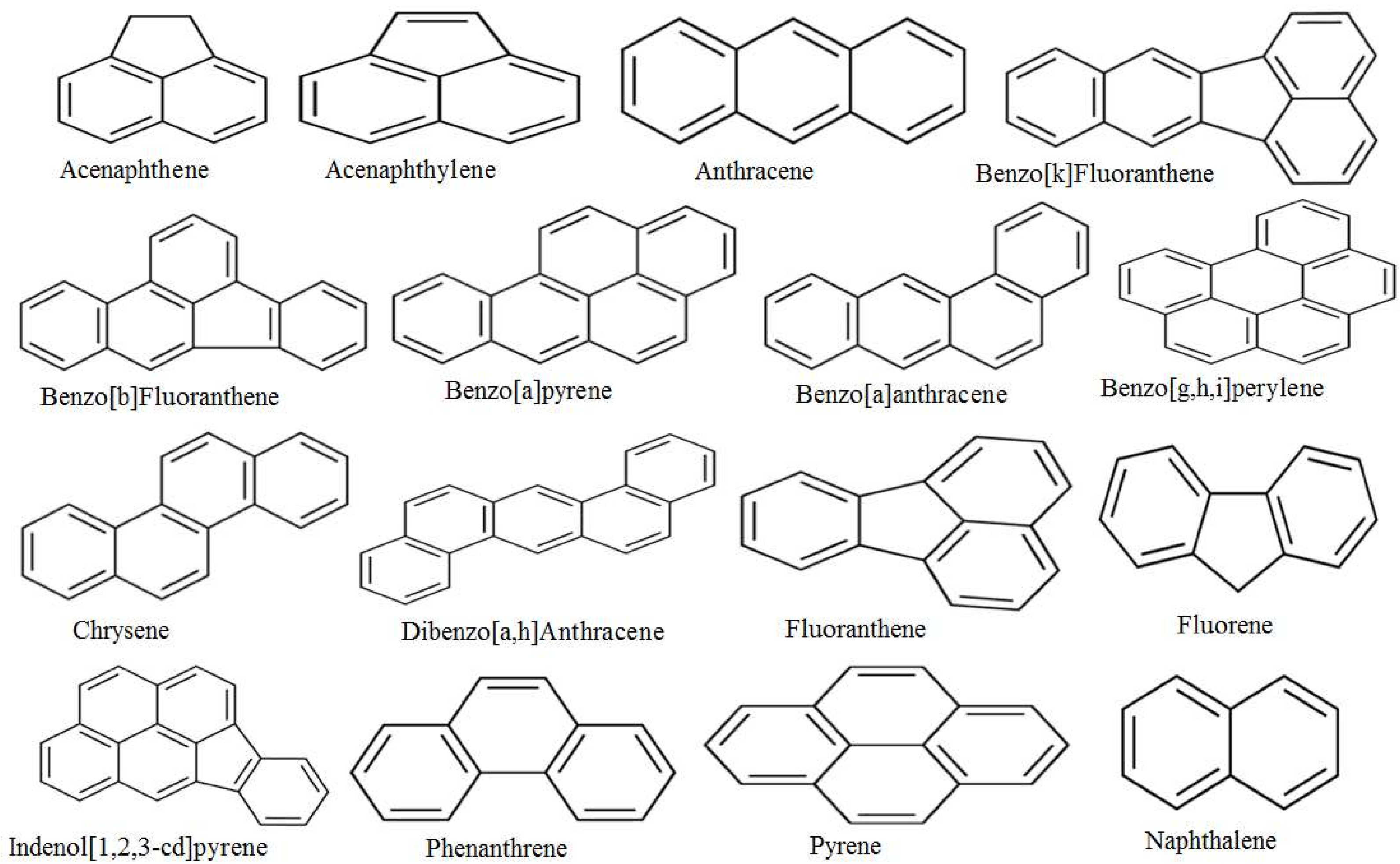

Figure 1.1. Chemical structures of 16 polycyclic aromatic hydrocarbons.

1.4. Treatment methods

Various physicochemical and biological methods have been developed to remediate/treat the dyes and PAHs. The physicochemical techniques, namely adsorption, filtration, ozonation, advanced oxidation, UV-Fenton, and photolysis have been widely applied for the treatment of dyes and PAHs (Kumar et al., 2019; Nzila et al., 2018; Sonwani et al., 2019b; Varjani et al., 2017b; Zhou et al., 2013). However, these techniques often suffer from limitations such as high operating and maintenance cost, sludge generation, and harmful by-products formation (Ukiwe et al., 2013). Also, the conventional methods do not eliminate the pollutants entirely but transfer them from one phase to another (e.g., adsorption). As compared to physicochemical methods, the treatment of wastewater *via* biological method (i.e., biodegradation) is considered as an effective method because of its cost-effective and environmentally sound nature (Sonwani et al., 2019a; Vikrant et al., 2018). Considering the merits of biological method, the present study is mainly focused on the treatment of dyes and PAHs *via* biodegradation.

1.5. Biological method

In the last few decades, microorganisms are widely used in the biodegradation of dyes and PAHs. In biodegradation, microorganisms, such as bacteria, fungi, and algae, act on organic pollutants and metabolize them as their food and energy source (Biswas et al., 2015; Varjani et al., 2015). The pollutants can be degraded by microorganisms either by aerobic (i.e., presence of oxygen) or anaerobic (i.e., absence of oxygen) process. In the aerobic process, oxygen acts as a final electron acceptor to achieve hydroxylation of the aromatic ring, whereas anaerobic biodegradation employs diverse approaches mainly based on reductive reactions to break down aromatic rings (Ghosal et al., 2016). Based on the applicability of the process, biodegradation can be classified as *in-situ* (on-site treatment) and *ex-situ* process (off-site treatment).

1.5.1. *In-situ* biodegradation

In-situ biodegradation involves the treatment of the pollutants on-site. It can be classified as (i) *bioattenuation*, (ii) *biostimulation*, and (iii) *bioaugmentation*. *Bioattenuation* is the natural way of pollutant degradation by microorganisms present in the contaminated site. In this technique, the biodegradation rate mainly depends on the soil/water characteristic and site condition (pH, temperature, humidity, availability of microorganisms, etc.). It is considered to be an economical approach because of less manpower and equipment requirements (Megharaj et al., 2011). However, this technique is less attractive due to the slow biodegradation rate. In *Biostimulation*, the nutrients (e.g., nitrogen and phosphorous), water, and electron acceptors or donors are added to the contaminated site to enhance the rate of biodegradation. The composition of adding nutrients, water, and electron acceptors or donors depends on the characteristic of contaminated sites. *Bioaugmentation* is a process in which the biodegradation rate is improved by adding specific microbial cultures to pollutant sites. The efficacy of *in situ* biodegradation mainly depends on the composition and area of the polluted site, environmental conditions, solubility, oxygen transfer, volatility of the pollutant (s), and availability of microbial populations (Farhadian et al., 2008).

1.5.2. *Ex-situ* biodegradation

Ex-situ biodegradation refers to the excavation of pollutants from the contaminated site, and they are treated elsewhere under the control condition (Nikolopoulou et al., 2013). In the last few decades, *ex-situ* biodegradation or engineered biodegradation has been designed and employed to treat the different pollutants (Varjani et al., 2015). Generally, bioreactor (e.g., packed bed bioreactor (PBBR), activated sludge process) and enzyme-based degradation processes come under this category (Varjani et al., 2017b; Vikrant et al., 2018). The enrichment of microorganisms, process conditions (such as pH, temperature, dissolved oxygen, and salinity), types of the bioreactor, and pollutant composition are some important factors that affect the extent of *ex-situ* biodegradation. Application of enriched cultures, either pure or consortia, is very common in *ex-situ* biodegradation. However, the real application of *ex-situ* biodegradation may often be limited by a number of factors, including extraction of samples from the subsurface, mass transfer resistance, improper monitoring of process conditions, and expensive equipment (Farhadian et al., 2008; Meghraj et al., 2011).

1.6. Factors affecting the biodegradation

The operational parameters like pH, temperature, initial pollutant concentration, nutrients, structure of pollutant, and microbial adaptation directly or indirectly affect the biodegradation rate. To study the impact of these parameters on the biodegradation of pollutants are essential to make the process efficient, faster, and more applicable. Additionally, the optimization of these parameters will also greatly help in the designing of the industrial-scale bioreactor.

1.6.1. Effect of pH

The pH of the solution considerably affects the enzymatic activities of microorganisms. Generally, the microorganisms produce the enzymes that have ionic groups on their active site of cells, and these ionic groups should be in the proper state (acidic or basic) to function. Due to the change of pH, the activity of the ionic form of the active site of enzyme is unfavorably affected, which impede the metabolic activity of the enzyme, and consequently reduce the biodegradation rate (Ghosal et al., 2016; You et al., 2013; Khan et al., 2013). The substrate biodegradation rate is high at a specific pH range (6.0 to 8.0), and it is severely decreased at strong acidic or alkaline conditions (Lin et al., 2010). Bouraie et al. (2016) have investigated the removal of Reactive Black 5 by *Aeromonas hydrophila*. According to their results, *Aeromonas hydrophila* revealed the maximum removal of dye under neutral pH, whereas the dye removal efficiency was lowest at highly acidic (pH 3.0) and highly alkaline (pH 11) conditions.

1.6.2. Effect of temperature

Temperature is one of the key process variable that affects the metabolic activity of microorganisms. It also affects bioavailability, solubility, and microbial growth (El-naas et al., 2014). For example, the solubility of aromatic hydrocarbons increases with temperature and therefore enhances the bioavailability (Ghosal et al., 2016). According to Nur et al. (2018), the temperature affects the strength of hydrogen bonds and corresponding growth and thickness of the biofilm onto carrier or support matrix. The literature survey indicates that the biodegradation activities of the microorganisms are increased with increasing temperature up to a certain limit; afterward, the biodegradation activities of the microorganisms decrease due to the denaturing of enzymes (Geed et al., 2017; Sonwani et al. 2019b). Therefore, to achieve optimum performance, a suitable temperature range must be maintained. Most of the bacterial species could grow in the region of 20-45 °C (El-naas et al., 2014). Kureel et al. (2017) have examined the effect of temperature (25-43 °C) against the benzene biodegradation in a packed bed bioreactor. They found that the optimum removal of benzene was achieved under 37 °C. Similarly, Sutar et al. (2019) have studied the biodegradation and detoxification of malachite green by *Photobacterium leiognathi*. According to their results, the maximum removal of dye was found at 30 °C, and the dye removal rate was decreased with increasing temperature.

1.6.3. Effect of concentration

The effect of the initial concentration of dye and PAHs on microbial degradation is well stated in the open literature (Ghosal et al., 2016; Varjani et al., 2017b). The studies suggested that the specific growth rate of microorganisms increased with an increasing substrate concentration up to a certain limit; afterward, the specific growth rate of microorganisms decreased due to the toxic effect of the substrate at very high concentration (Talha et al., 2018; Khan et al., 2014). The specific growth rate of *Bacillus* sp. decreased beyond 300 mg/L of Malathion due to the substrate inhibition (Geed et al., 2017). Gopinath et al. (2009) have studied the biodegradation of Congo red dye by *Bacillus* sp. ACT 1 and *Bacillus* sp. ACT 2, and found that the specific growth rate of microorganisms was decreased above 500 mg/L of Congo red due to the substrate inhibition.

1.6.4. Effect of nutrients

The microorganisms require various macronutrients (e.g., $Na_2HPO_4.12H_2O$, KH_2PO_4, $MgSO_4.7H_2O$, $CaCl_2$, NH_4Cl, and $FeSO_4$) and micronutrients (e.g., $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, and $MnCl_2.4H_2O$) to ensure the effective metabolism and growth rate (Carvajal et al., 2018; Geed et al., 2017). The addition of the organic nitrogen, including peptone and urea, can regenerate NADH, and help in effective biodegradation of dye (Chang and Kuo, 2000). Studies have shown that petroleum hydrocarbons contain a low amount of inorganic nutrients such as nitrogen and phosphorous. The supply of nutrients is essential for the effective biodegradation of petroleum hydrocarbons (Varjani et al., 2017b). Therefore, sufficient nutrients must be maintained in the biodegradation process to achieve better growth of microorganisms.

1.6.5. Microbial adaptation

Recently, numerous microbial species isolated from petroleum-contaminated sites have been employed for the biodegradation of AHCs and dyes (Demeter et al., 2017; Kumar et al., 2018; Sonwani et al., 2019a). Microbial adaptation involves the pre-exposure of microbial species with high doses of pollutants to enhance the biodegradation potential (Haritash et al., 2009). In a number of studies, soil and water samples collected from petroleum-contaminated sites were exposed to the high concentration of AHCs for several weeks, followed by enrichment and isolation of potential microbial species (Derakhshan et al., 2018a; Shao et al., 2015; Zeinali et al., 2008). The adaptation and enrichment of microorganisms in specific organic sources can significantly improve the rate of biodegradation. For example, Yeom et al. (1997) reported that the benzene adopted *Alcaligenes xylosoxidans* Y234 exhibited two-fold enhancement in biodegradation potential for benzene and toluene relative to ones without such a strategy.

1.7. Bioreactors

In the last few decades, it has been observed that the bioreactors play a significant role in the treatment of wastewater (Derakhshan et al., 2018b; Mudliar et al., 2010). The bioreactor is the heart of any biochemical process. It is a kind of vessel in which the biochemical reaction is carried out under controlled environmental conditions to achieve optimal growth of microorganisms for product formation or substrate degradation. The fundamental mechanisms of all bioreactors are almost similar; the difference exists due to the use of microorganisms (either suspended cell or attached cell), presence or absence of oxygen, configuration of the bioreactor, type of packing materials, and fluid contact pattern (either co-current, counter-current, or cross-current). Based on the application of microorganisms, these are broadly classified into suspended-growth and attached-growth bioreactors. The bioreactors with their merits and demerits are summarized in **Table 1.3**.

Table 1.3. Classification of bioreactors with their merits and demerits.

S.N.	Reactor type		Merits	Demerits	References
1	Suspended-growth	CSTBR[a]	-Continuous mixing of fluid, -Ample transfer of oxygen -Easy temperature control -Low operating costs -Easy scale-up	-High energy requirements -High shear to bacterial culture -Concerns about motor size	(El-naas et al., 2014; Vikrant et al., 2018)
2		MBR[b]	-High biomass concentration -Long sludge retention time -High-quality effluent	-Membrane fouling -High initial/replacement cost -Long-term operational stability	(Fatone et al., 2010; Mohan and Nagalakshmi, 2020)
3		UASB[c]	-High biomass concentration - Absence of support media -Energy recovery	-Sludge washout - Long start-up period	(Adhikari and Lohani, 2019; Saravanan et al., 2006)
4	Attached-growth	PBBR[d]	-High biomass concentration -Tolerance to high loading -Reusability of enzymes -High mass transfer -Effective process control	-High pressure drop across column -Internal and external mass transfer resistance -Channeling	(Geed et al., 2017; Kureel et al., 2017)
5		FBBR[e]	-High biomass density -Negligible channeling of flow -High mass transfer due to effective mixing -Low HRT	-High energy consumption - Long start-up period	(Shalini and Setty, 2019)

6	MBBR[f]	-Adequate mixing of fluid -Small foot-print -Effective mass transfer	-High energy cost -Special carriers designed	(Sonwani et al., 2019b)
7	RBC[g]	-High bacterial density developed on disc -Low space requirement -Low HRT	-High energy cost -Design complexity -Dead zone	(Mikesková et al., 2012)

[a]Continuous stirred tank bioreactor; [b]Membrane bioreactor; [c]Up-flow anaerobic sludge blanket [d]Packed bed Bioreactor; [e]Fluidized bed bioreactor; [f]Moving bed biofilm reactor; [g]Rotating biological contactor.

1.7.1 Continuous stirred tank bioreactor (CSTBR)

CSTBRs are completely mixed suspended-growth bioreactors. CSTBRs are extensively used for the treatment of pollutants, such as AHCs and dyes in wastewater (EI-Nass et al., 2014; Vikrant et al., 2018). It is assumed that the exit composition of the sample is similar to the sample present inside the bioreactor. The height to diameter ratio of the bioreactor can be varied between 2:1 and 6:1 and the stirrer may be at the top or at bottom driven. A schematic diagram of a CSTBR is presented in **Figure 1.2**. CSTBR has several merits such as continuous mixing of fluid, ample oxygen transfer to maintain the adequate DO level, effective temperature control, low operating costs, and easy scale-up (Pugazhendi et al., 2017). Despite the merits, it exhibits several limitations, such as high energy requirements due to continuous mixing, high shear to bacterial culture, and anxieties about motor size limitations.

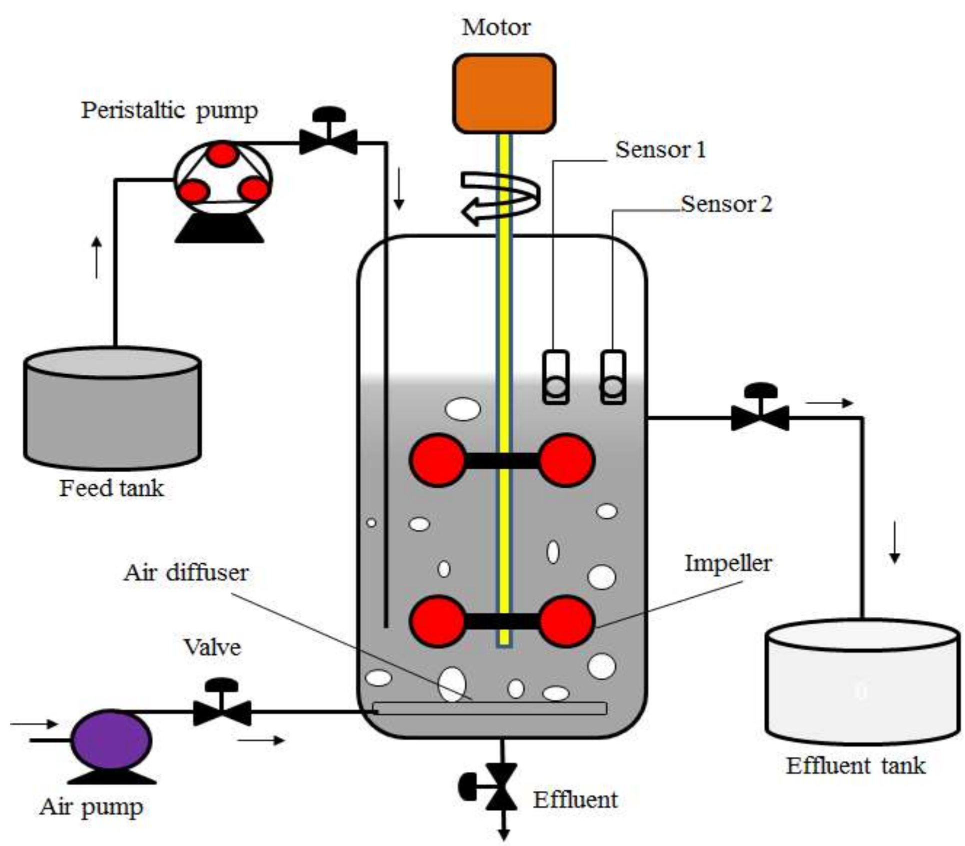

Figure 1.2. Schematic diagram of a stirred tank bioreactor.

1.7.2. Up-flow anaerobic sludge blanket (UASB)

Up-flow anaerobic sludge blanket (UASB) bioreactor is the developed form of an anaerobic digester, which is widely used for the wastewater treatment. It was developed in the late 1970s. The process is based on the formation of highly dense sludge granules (1-4 mm), i.e., small agglomerations of microorganisms by the self-immobilization of the anaerobic microorganisms (Saravanan et al., 2006). In this type of bioreactor, any support material is not used for the immobilization of microorganisms. A schematic diagram of a UASB bioreactor has presented in **Figure 1.3**. The UASB is contained the following zone; dense sludge bed (granulated bed), sludge blanket (zone consist of finely suspended particles), clarifier (solid-liquid-gas separator), and gas collection zone. The influent is fed from the bottom of the UASB bioreactor through the inlet liquid distribution system and moves upwards through the biomass of sludge bed. The organic pollutants are biodegraded by microorganisms present in the sludge bed, resulting gases (methane and carbon dioxide) are produced and moved upwards due to low density.

The efficiency of UASB mainly depends on the active biomass concentration and inlet organic loading rate. The inlet flow rate should be below the threshold limit to keep the sludge granules in suspended form. However, a very low inlet flow rate leads to the channeling issue in the sludge bed. The valuable energy recovery (methane), high active biomass concentration, low space requirement, absence of support media for the immobilization of microorganisms, and handle high organic loading are the merits of the UASB process (Adhikari and Lohani, 2019; Saravanan et al., 2006). The mass transfer limitation, sludge washout, and long start-up period are the disadvantage of this bioreactor.

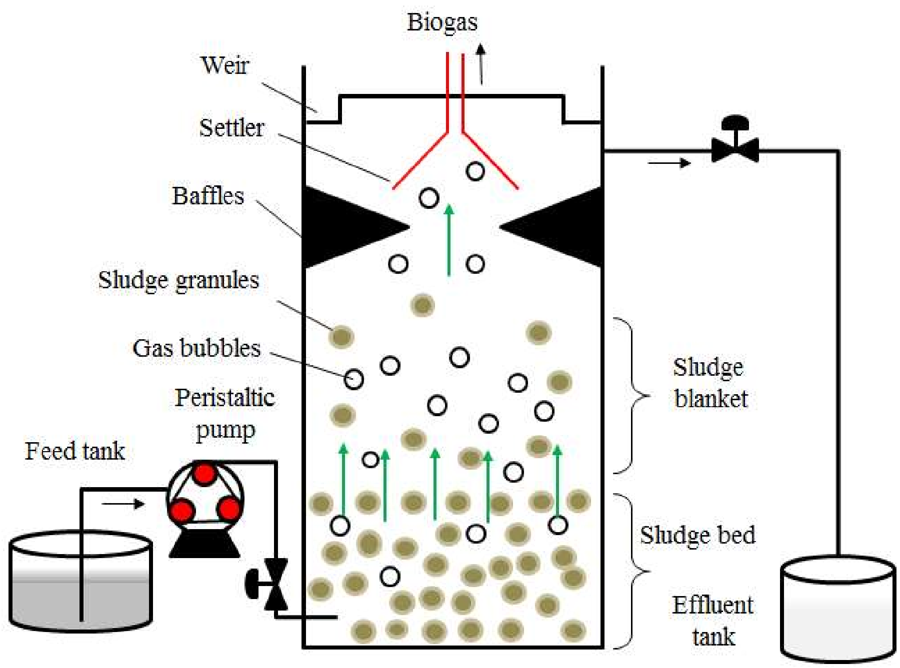

Figure 1.3. Schematic diagram of an Up-flow anaerobic sludge blanket.

1.7.3. Membrane bioreactor (MBR)

MBR is the combination of biological and membrane treatment system to effectively remove the contaminants of concern from municipal and industrial wastewater. It is almost similar to the conventional activated sludge process (ASP) with the exception that the microorganisms responsible for the biodegradation of wastewater are retained within the bioreactor by a membrane (Berube, 2010). MBR process was developed nearly three decades before and further modifications and improvements have been made for the treatment of pollutants present in the wastewater (Xiao et al., 2019). Generally, two types of MBRs; (a) side-stream MBR with external pressure-driven membrane filtration, and (b) submerged MBR with internal vacuum-driven membrane filtration are employed (**Figure 1.4**). The side-stream MBR is usually operated at constant pressure and variable permeate flux, since permeate flux decreases with increasing membrane fouling. The submerged MBR is operated at variable pressure and constant permeate flux because transmembrane pressure increases with increasing membrane fouling (Berube, 2010; Krzeminski et al., 2017). The main highlights of the MBR process are small footprint, production of high-quality effluent, and low sludge generation, whereas high energy consumption and membrane fouling, and its replacement are the major limitations (Meng et al., 2017; Shin and Bae, 2018; Xiao et al., 2019). The biodegradation of real textile wastewater was examined in an anaerobic MBR, and it was effective for the treatment of textile wastewater (Yurtsever et al., 2020).

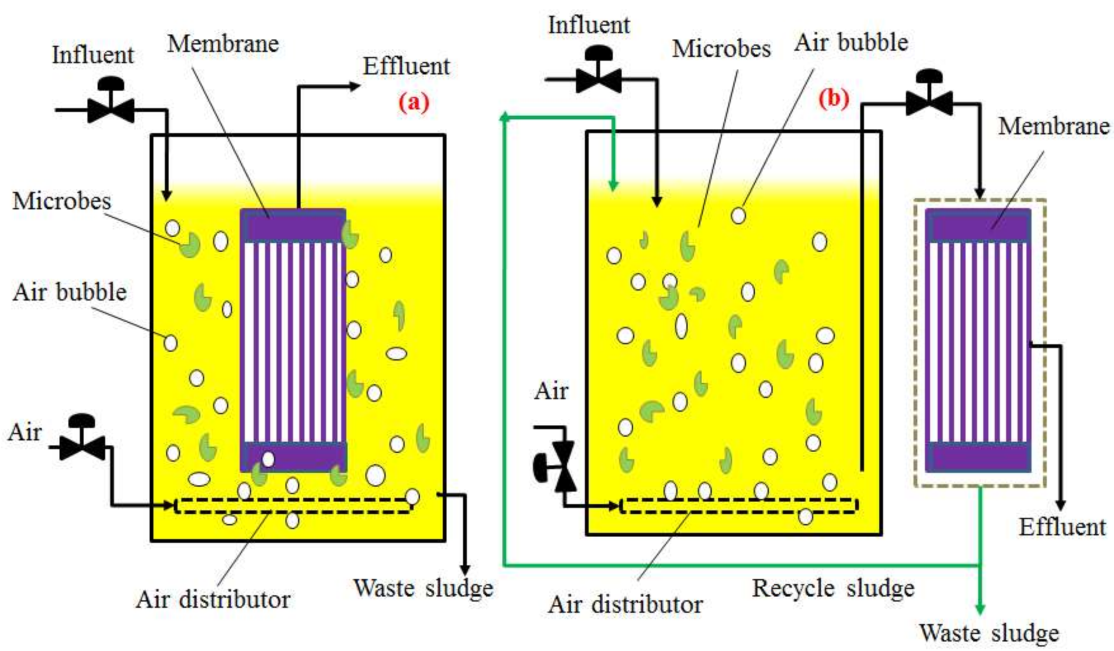

Figure 1.4. A schematic diagram of membrane bioreactor (MBR): (a) submerged MBR; (b) external MBR.

1.7.4. Fluidized bed biofilm reactor (FBBR)

Initially, a fluidized bed reactor was developed in 1920s by Winkler F., for the cracking of petroleum (Andalib et al., 2014). Fluidized bed reactor was modified later in 1920s for biochemical process and referred as FBBR. FBBR is characterized by two-phase (fluid and solid) bioreactor in which biofilm immobilized carriers are fluidized by means of downward or upward recirculation using a high liquid or gas flow rate (Ozkaya et al., 2018). The basic mechanism in a fluidized bed involves passing a fluid through the microbial immobilized solid bed. As the fluid velocity increases, the bed particles start to expand and become suspended because buoyancy force balances the drag and gravitational force (Andalib et al., 2014). At minimum fluidization velocity, the pressure drop across the bed becomes equal to the weight of the immobilized bed, resulting in fluidization of the bed (Khan et al., 2014). A typical schematic diagram of FBBR is represented in **Figure 1.5**. The small reactor volume, high biomass density on carriers, high mass transfer due to excellent mixing, and negligible channelling flow, resulting high biodegradation rate, are the merits of FBBR (Andalib et al., 2014; Shalini and Shetty, 2019). The demerits of FBBR include high energy requirements due to the fluidization of carriers in a liquid medium and a long start-up period (Ozkaya et al., 2018). Mustafa et al. (2014) studied the treatment of municipal wastewater in an anaerobic FBBR using zeolite as a carrier media for the development of biofilm. The maximum COD and volatile suspended solid removal efficiencies were found to be 85% and 88%, respectively, under 9.0 days.

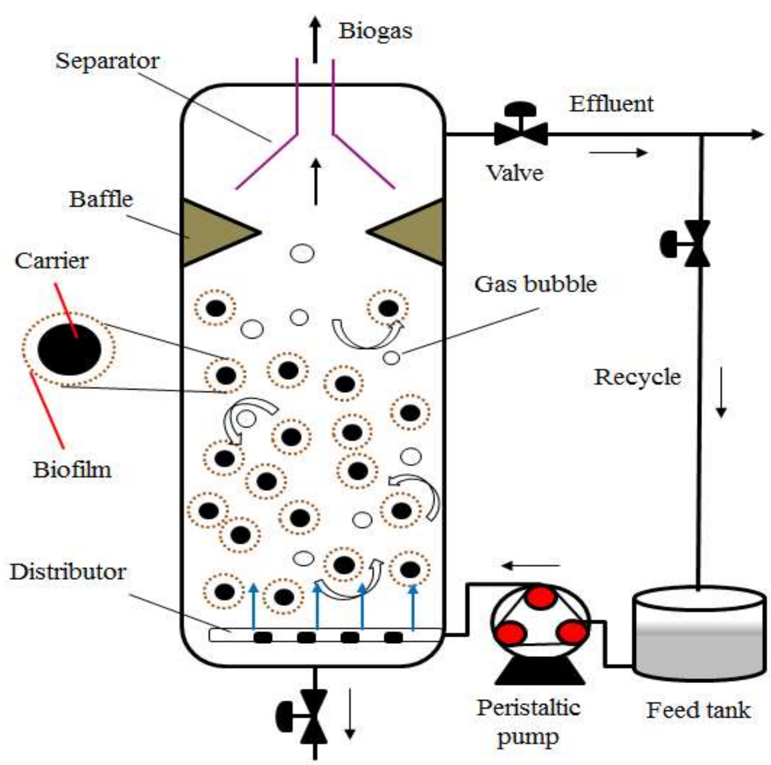

Figure 1.5. Schematic diagram of an anaerobic fluidized bed biofilm reactor.

1.7.5. Packed bed bioreactor (PBBR)

PBBR is a type of bioreactor in which a single or mixed culture of microorganisms are attached to materials such as gravels, plastics, polymers, and activated carbons. PBBRs are generally a fabricated hollow tube or pipe and are filled with attached-growth packing media. The attached-growth biofilm into porous packing media consumes substrate and nutrients for their metabolism (Singh et al., 2010). A schematic diagram of PBBR is shown in **Figure 1.6**. The wastewater is passed over the population of microorganisms developed on the packing bed and substrate diffused through it, resulting the microorganisms consume organic and inorganic substrate for their metabolism. The ideal PBBR is well mixed in the radial direction, while negligible mixing takes place in the axial direction (Sen et al., 2017). PBBR can be operated under three flow modes; upward, downward, and recycle.

Based on the availability of oxygen, PBBR can be broadly classified as an aerobic and anaerobic bioreactor. In aerobic bioreactor, the air diffusor is provided in bed to assure the uniform supply of oxygen and subsequently maintain the desired dissolved oxygen level (3.0 to 6.0 mg/L), whereas the anaerobic process is carried out in the absence of oxygen. The properties of packing materials such as particle size, density, porosity, and surface characteristics can adversely affect the overall bioreactor performance. The high biomass concentration, continuous mode of operation, reuse of enzymes, high mass transfer and reaction rates, and effective control of the process are the advantages of PBBRs over conventional bioreactors (Sen et al., 2017). Despite the merits of PBBR, it has some limitations, such as high pumping requirement due to pressure drop, clogging, and mass transfer resistance (Singh et al., 2010; Sonwani et al., 2019a).

The PBBR filled with bio-char immobilized with *Alcaligenes faecalis* (as the biodegrading medium) was used to degrade the Methylene blue (Bharti et al., 2018). They reported that 87% of Methylene blue removal was found up to 500 mg/L of the initial Methylene blue. The biodegradation of Reactive Red 120 dye was carried out in PBBR using polyurethane foam (PUF) immobilized with *Bacillus cohnii* RAPT1 (Padmanaban et al. 2016). In another work, Kureel et al. (2017) studied the biodegradation of aromatic hydrocarbon (benzene) by *Bacillus* sp. in a continuous PBBR and reported that the maximum RE of 93% was obtained on 38th days of operation.

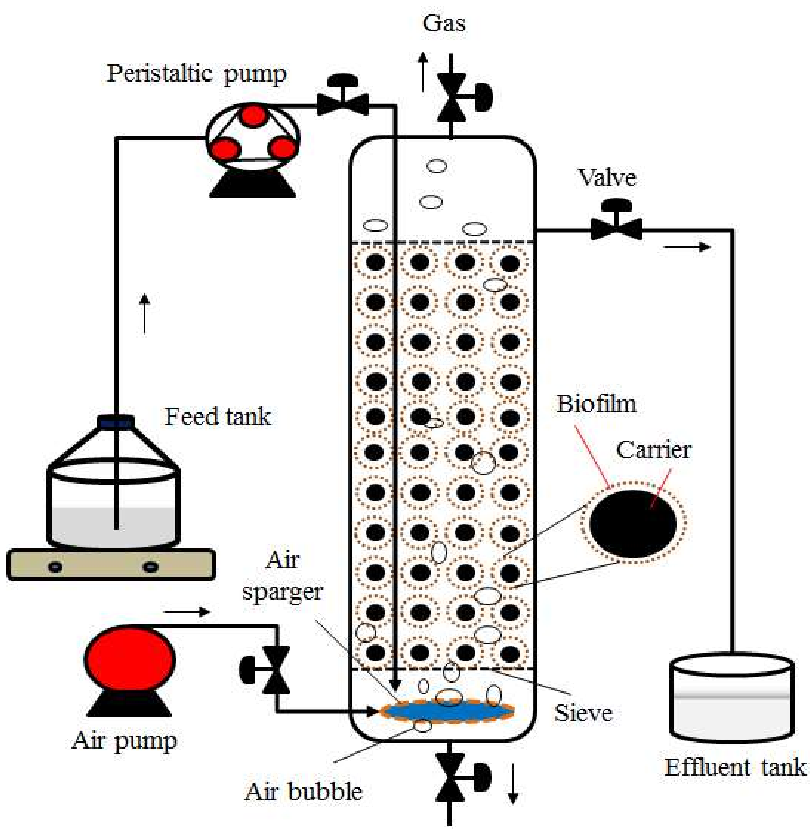

Figure 1.6. A schematic diagram of a packed bed bioreactor.

1.7.6. Moving bed biofilm reactor (MBBR)

MBBR was initially developed in Norway in the late 1980s (Leyva-Díaz et al., 2020). It is a reactor that combines the conventional activated sludge process and FBBR (Nur et al., 2018). It is an attached growth based process and extensively used treat dyes and AHCs (Sonwani et al., 2019b; Swain et al., 2020). A schematic diagram of the MBBR set-up is presented in **Figure 1.7**. In MBBR, the biofilm developed on carriers (e.g., Kaldnes biofilm chip, polypropylene (PP), PUF, etc.) are kept moving within the bioreactor either by aeration (aerobic) or by mechanical stirrers (anoxic/anaerobic) (Leyva-Díaz et al., 2013). The MBBR carriers are generally made of plastic materials and typically designed to have a high surface area per unit volume so that the large surface area could be achieved for the biofilm growth. The void ratio of carrier normally varies from 30-80%, and specific surface area ranges from 350 to 1200 m^2/m^3 (Sonwani et al., 2020a; Leyva-Díaz et al., 2020). A good carrier should have the following characteristic; high porosity, large specific surface area for more biofilm formation, high mechanical strength, inert and reusable (Demeter et al., 2017; Nur et al., 2018). Compared with conventional bioreactors, MBBR offers several benefits, such as adequate mixing of fluid, effective mass transfer, and lower space requirements (Gonzalez-lopez, 2013; Leyva-Díaz et al., 2020). Also, the biomass concentration inside the bioreactor can be easily improved by increasing the number of carriers. Park et al. (2011) used PUF immobilized with the dyeing sludge in MBBR for the biodegradation of dye contaminated wastewater and reported that 79% of COD and 54% of colour removal (initial COD of 539 mg/L and colour of 622 PtCo) were found. Atrazine was successfully removed in the MBBR under nitrate-reducing conditions (Derakhshan et al., 2018b).

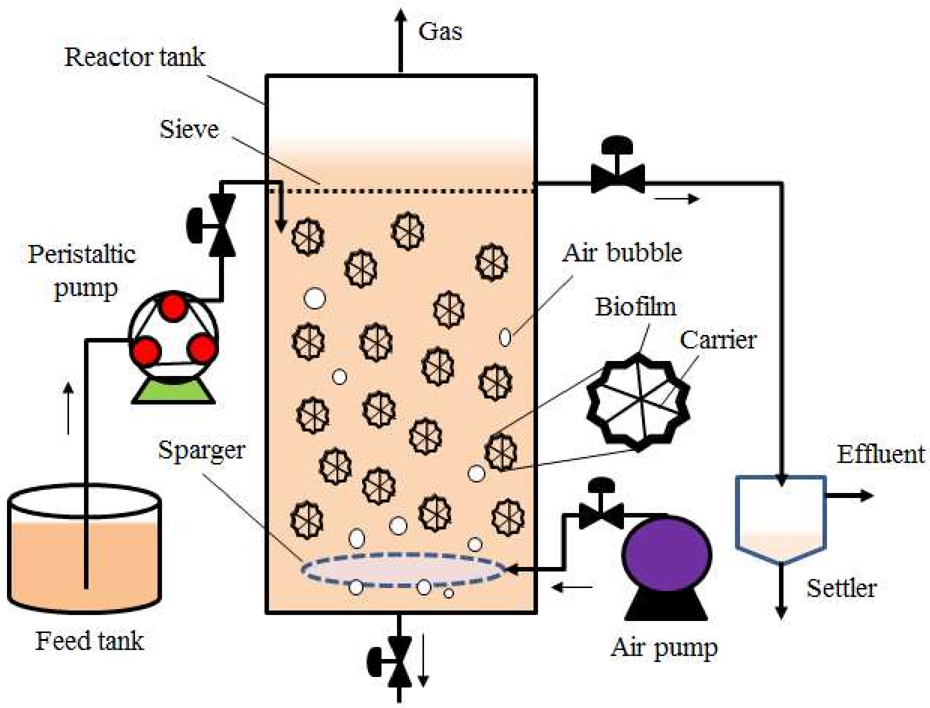

Figure 1.7. A schematic diagram of a moving bed biofilm reactor (MBBR**)** set-up.

1.7.7. Rotating biological contactor (RBC)

RBC is an attached growth type bioreactor that provides an alternate technology of the conventional activated sludge process (ASP). In the early 1920's, the first RBC was made of a cylinder with wooden slats and further significant modifications in media type and reactor configuration were done during 1960's and 1970's (Cortez et al., 2008). RBC is made of a series of rotating discs or plates that are mounted on a horizontal shaft (**Figure 1.8**). The discs of the RBC are partially or completely immersed in wastewater. A mechanical motor continuously rotates the discs mounted on the shaft. The biofilm is mainly developed on the surface of discs or plates. The rotation of the shaft leads to bulk fluid mixing, diffusion of the substrate through biofilm, and subsequent product reverse back in bulk liquid (Rittman, 2018). The substrate biodegradation occurs through the fixed film of microbes (contain active or non-active biomass) (Hassard et al., 2014). RBC is widely employed for the treatment