Role of Moving Bed Bioreactor (MBBR) in Dye Removal

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Abstract Dye containing effluents disposed from various industries include several toxic chemicals and have adverse health effects on human as well as derogatory impacts on ecosystem. Among various dyes, chromophores with azo bonds are most abundant and are investigated to be a potential carcinogen and mutagen. Dyes in wastewater are difficult to degrade as they are made to be stable under the action of external factors including temperature, microorganisms and chemicals including bleaches. Analysing the strengths and weaknesses of various treatment technologies available, biodecolourization is often economically and environmentally favoured. Moving Bed Bioreactors (MBBRs) are one of the advanced biological systems that allow degradation of a wide range of recalcitrant compounds with notable advantages over other treatments and have been modified as well as coupled with several other technologies to obtain complete mineralisation of the dyes leaving non-toxic by-products. The present work reviews the aspects of dye removal from effluents in MBBR with critical outlook on various investigations undertaken in the reactor. The major points include the following (1) Dye degradation takes place mostly by azo bond cleavage (bacterial biomass) or enzymatic action (fungi), along with biosorption (10–50% at most) and bioaccumulation. (2) Under identical experimental conditions, dye removal in MBBR is comparatively higher (51.6%) than in activated sludge process (26.8%). (3) Anaerobic–aerobic MBBR results in 85% colour removal whereas complete decolourization was recorded in MB-SBBR and SBR-MBBR combination. (4) Around 95% colour removal was obtained in case of MBBR combined with coagulation, ozonation and membrane filtration. (5) All these experimental results are highly influenced by a number of parameters and efficiency also includes economical aspect of every process. Generally, initial dye concentration, pH, HRT and biocarrier concentration has a threshold value which yields maximum removal, above or below which removal efficiency decreases.

Keywords Moving Bed Bioreactor (MBBR) · Dye removal · Anaerobic · Aerobic · Combined physico-chemical and biological processes · Biocarriers · Biofilm

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1 Introduction

At present, dyeing process comprises of more than 8000 chemical compounds with structural, compositional and behavioural variations that produce up to 1,00,000 different dyes [15, 21, 67, 77]. These dyes are majorly used in textile industries along with substantial application in paper, rubber, electroplating, food, printing, leather tanning, cosmetic and pharmaceutical sectors [2, 21, 30, 52, 132]. The contribution of major industrial sectors towards the production of dye containing effluents is shown in Fig. 1 [41, 134]. For more stability of colour on different products manufactured in these industries, dyes are constantly upgraded and new compounds are combined that results in more resistance to degradation by water, sunlight, temperature, detergents or any washing substances [5, 163]. Dye wastewater is considered one of the most toxic industrial effluents [39]. Around 17–20% of pollution caused by textile industry is due to various dyeing mechanisms [67] which contains a total of 72 toxic chemicals, only 30 of which can be treated by conventional treatment processes [31]. Dye wastewater often exhibits highly fluctuating pH, generally towards the higher range [159], high temperature and high COD concentration along with hazardous and xenobiotic compounds [74, 144].

Based on origin, dyes can be organic or synthetic. Synthetic dyes have more complex structure and are made to be more resistant to chemical action and fading thus making them less susceptible to biodegradation [141]. Synthetic dyes include acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal complex dyes [15], among which, the most toxic group of dyes includes basic and direct diazo dyes [136]. Azo dyes, which constitute more than 50% of the dyes [91], are characterized by –N = N– (azo) bonds and are often xenobiotic in nature [141]. Azo groups are present as chromophores in anionic and non-ionic dyes which makes them the most abundant type of dyes in wastewater. Dyes can be broadly classified as anionic, cationic and non-ionic dyes [89, 102]. According to Wang et al. [161] and

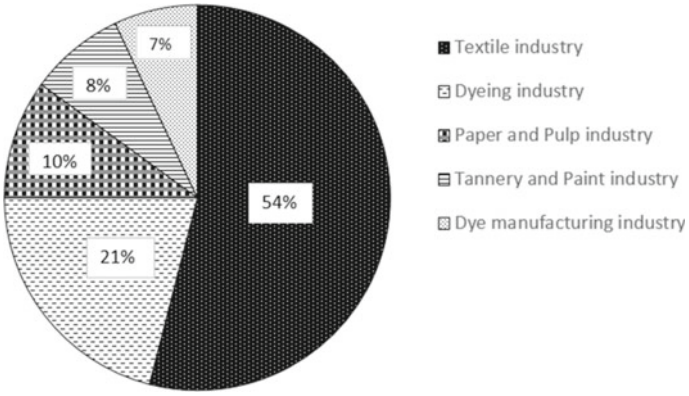
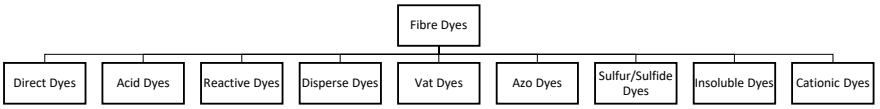


Fig. 1 Contribution of different industrial sectors towards production dye wastewater [41, 134]

Katheresan et al. [77], fibre dyes can be produced by one or combination of more than one of the following groups:



Considering water quality, one of the major concerns of untreated dye wastewater is its elevated chemical oxygen demand (COD) along with the presence of organics that are not easily biodegradable. Dye effluents are often dark coloured and thus block sunlight when disposed into any water body affecting the aquatic ecosystem of that place [12, 57, 159]. Dye effluents having a high pH can alter the pH in disposed of waterbody along with its dissolved oxygen [72]. Synthetic dyes often react with other chemicals in the environment to form more recalcitrant compounds [21]. Presence of organic chemical-based dye fixing agents such as formaldehyde, softeners having hydrocarbons, chlorinated stain removers also found in dyeing wastewater have carcinogenic effects [3]. Several dyes have been seen to cause bioaccumulation in biotic species due to its stable nature and resistant to biodegradation. Metal-based dyes when eliminated into water system may release the metals (like chromium) which have adverse health effects on animals in the surrounding ecosystem [15]. Adverse human health effects associated with residual dye effluents include irritation, permanent eye injuries when in contact with eyes, respiratory problems, reproductive failures, effects on immune system along with genetic mutation and carcinogenic effects [72, 136].

Besides aesthetic concerns, one of the major concerns of adverse health effect on humans is the potential carcinogenicity of several dyes [99, 149]. Among the diversified categories of dyes, the azo groups constitute the largest, contributing to about 70% by weight of total dyes in the effluent [34], being most common and most toxic commercial dyes [37, 139]. Azo dyes are reduced to toxic, mutagenic and/or carcinogenic intermediate by-products [60] in anaerobic conditions of sediments [112, 132, 163] and intestines of humans [35, 141]. As many as 46 different strains of gut bacteria have been isolated that can reduce a number of azo dyes [36]. Human skin bacteria are also reported to reduce the dye Direct Blue 14 to amines that are carcinogenic [126]. These dyes are directly linked with bladder cancer, mutation of chromosomes and splenic sarcomas [133]. It has been observed that the faecal anaerobic bacteria in human digestive tract degrade tetrazine, an azo dye to form amines like benzidine and 4-aminoaniline which are carcinogenic in nature [25, 112]. Benzidine based azo dyes are shown to produce various aromatic amines including N-acetylated derivative that is tumorigenic in the urine of sample mammals [131]. Even after treatment of azo dye containing wastewater, the effluents are often found to have toxic effects along with several azo dyes being completely unaltered [110].

Treating wastewater economically and effectively is often considered as one of the challenging issues due to the presence of harmful recalcitrant substances [124]. At

present, effluents released from dye utilizing industries must comply with the standards as prescribed by The International Dye Industry Wastewater Discharge Quality Standards [46]. The methods developed for treatment of dye wastewater are not individually employed because of incomplete and inadequate treatment along with associate disadvantage for each treatment method [76, 119]. Generally, a biological process is selected along with a chemical pretreatment process for effective treatment of dyestuff effluent [53]. Physical and chemical treatment methods often do not necessarily eliminate complex contaminants of dye wastewater [21]. Effective physicochemical processes are often coupled with high cost treatment plants and operational expenses, intensive energy consumption, excess amount of chemical requirement and thus sludge production, which necessitates the cost of sludge handling [50, 53, 71]. These treatment methods are also sensitive to a variable wastewater input, which is often experienced in industries [15, 144]. Chemical coagulation alone uses a lot of coagulants, producing a large amount of sludge with comparative low efficiency in treatment [80]. Electrochemical oxidation produces a number of intermediary pollutants which needs to be treated in associative treatment systems, thus increasing plant and operational costs [80].

Microbial degradation of dyes or biodecolourization is often considered as a cost effective and ecologically safe method for treatment of dye wastewater [15, 52, 169]. Dyes are often toxic to microbial species and may lead to failure of conventional biological treatment system [109] although it has been observed that microorganisms growing in vicinity of areas where there is regular disposal of dye effluent can utilize the dyes as their nutrient sources [72]. Dye wastewater is generally characterized by very low BOD to COD ratio, even around 0.1 [40, 147] which often necessitates the use of a pretreatment process to make it more biodegradable [53]. The effectiveness of using multi-staged reactor comprising of anaerobic process followed by aerobic treatment system is thoroughly studied throughout the past years [105]. The mechanism for biodecolourization of azo dyes by bacteria may involve both degradation of azo bond cleavage by reductase enzymes as well as cell adsorption [112]. The cleavage of azo bond in absence of oxygen is brought about by non-specific enzymes [172] which is the main reason behind easy anaerobic degradation compared to aerobic degradation where the enzymes are dye specific [177]. Decolourization by fungi is brought about by non-specific enzymes as well as adsorption by dead fungal cells [54].

MBBR is often regarded as one of the most effective and promising water treatment technology due to its capability to degrade a wide range of wastewater [144]. It comprises a homogenously mixed reactor vessel where biomass remains attached to carriers in fluidized condition [108]. In aerobic condition, the carriers remain suspended with the help of aeration supplied by diffusers at the bottom of the tank whereas in anaerobic condition, it is done using mechanical stirrers. A schematic representation of MBBR is shown in Fig. 2 which illustrates the reactor operating in aerobic and anaerobic mode. Biocarriers are characterized by density close to that of water which helps to remain in suspended condition and integrity when kept in water for a long period [23]. MBBR has proven to treat a large volume of wastewater at once thus reducing reactor volume [169]. Sustainability of more biomass

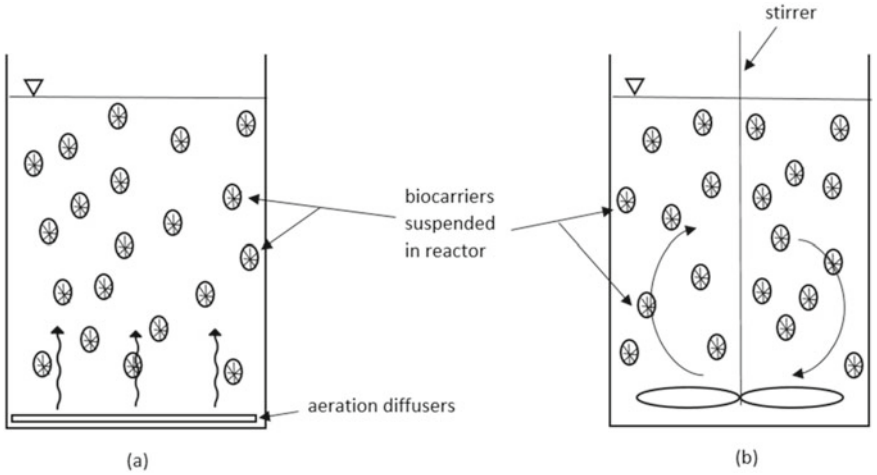


Fig. 2 Schematic representation of moving bed bioreactor in **a** aerobic condition and **b** anaerobic condition. Note that both homogenous mixing of water as well as fluidization of carriers in aerobic reactor is caused by upward aeration whereas that in anaerobic reactor is caused by mechanical stirrer

in the reactor along with maintaining a stable concentration of microorganism is one of the basic reasons behind the increasing acceptance of MBBR as a versatile treatment technology [108]. It provides comparatively larger solid retention times (SRT) where older cells with diminished supplementary nutrient supply exhibit better degradability [166]. Fluidization of biocarriers increases the contact opportunity of wastewater with the biomass thereby removing the desired pollutants more effectively [121]. Since there is no need for separate sedimentation tank, the constructional and operational cost as well as reactor footprint is drastically reduced [80].

Being attached to a support medium, biofilm cells are observed to be more toxicity resistant than suspended biomass [49] due to the extrapolymeric substances (EPS) formed by the biofilm that restricts the diffusion speed of hazardous substances [144] and acts as a buffer [103]. Wastewater including dye effluent often has elevated temperatures and has been notably treated in MBBR using thermotolerant bacteria [95]. Anaerobic MBBRs are found to be highly resistant against shock loads which are often experienced in dye related industries [62]. Other advantages of using MBBR include high rate of nitrification, uniform oxygen transfer, stable operation and increased surface area for biological activities [142, 173]. Comparing all aspects of dye effluent treatment in activated sludge reactor, MBR and MBBR, it was observed that with same operational cost as MBR, capital expenditure is 68.4% lower in case of MBBR along with comparatively less environmental impacts as observed by Life Cycle Assessment (LCA) analysis. It was also confirmed that the water treated by MBBR can be reused in industrial sector [170].

Albeit all these advantages, a basic drawback for MBBR is lower sludge settle ability in comparison to that of a suspended reactor which can be solved by adding

coagulants if necessary [169]. High fluid flow rate is also not desirable in MBBR as it reduces HRT and increases the risk of biomass washout [142]. In view of increasing efficiency of dye removal and decrease the load on MBBR, several other technologies are associated with the process. The present review focusses on various perspectives of dye removal in an MBBR system with or without additional treatment processes, various parameters to be considered during the operations and biological aspects of this treatment process. In this regard, the present chapter discusses the mechanism of dye degradation under various operational conditions in both bacterial and fungal MBBR, followed by the different instances of using MBBR as dye treatment unit coupled with other treatment technologies and the effect of different microorganisms and influencing parameters for dye degradation in MBBR and areas for further investigation. Over the years, several experiments have been conducted but little effort has been made to recapitulate the investigations which is attempted in the present work, thereby pointing out the research gaps in this field.

2 Mechanism of Dye Degradation in MBBR

Considering the different microorganisms that have been attached with carriers, biomass in MBBR can be grouped into bacteria-based carriers and fungi-based carriers. Both of these microbes have different enzymes responsible for decolourization, thereby has different approaches towards it. For example, bacterial enzymes are more substrate specific, whereas that in case of fungi is non-substrate specific which makes it easier to degrade a wide range of dyes. The approach towards degradation by bacteria is usually by breaking the azo bond in azo dyes by reductases whereas in fungi cellulase and peroxidases comes into action. The basic advantage of using MBBR over suspended systems reflects in removal efficiencies. Under identical experimental conditions, it is established that the high concentration of active biomass in carriers contribute to higher COD and colour removal efficiencies in MBBR (61.2% and 51.6%, respectively) as compared to those in activated sludge system (34.1% and 26.8%, respectively) in considerably lower HRT [144].

2.1 Bacterial Dye Degradation

Different microorganisms in a consortium responsible for dye degradation often require different environmental conditions for optimum performance and thus, the need for understanding the mechanism of dye degradation under different conditions is extremely necessary. Studies show that anaerobic–aerobic system of biodegradation of dyes is often more effective than aerobic treatment [90]. Azo dye reduction may occur in three probable mechanisms: azo bond cleavage, bioadsorption [113, 174] and hepatic microsomal reduction [51] although dye degradation following the latter is almost negligible [100]. Bioadsorption also contributes to a low as 14%

of decolourization [113]. In general, the azo bond cleavage takes place in anaerobic conditions and is almost impossible to occur in aerobic environment. Azo bonds are characterized by strong electron withdrawing nature which supports easily cleavage in oxygen deprived environment [113]. Azoreductase enzymes are usually responsible for initiating this degradation of azo dyes including Orange II [176].

A number of researches have in conducted confirming the efficiency of using anaerobic/aerobic profile in dye removal in MBBR [112]. The main reductase enzyme needed for the azo bond cleavage is functional in anaerobic environment [70] in the presence of NADH, NADPH and FADH [157]. The transition of anaerobic to aerobic environment is necessary for complete degradation of dyes because the intermediates formed as a result of azo bond cleavage are recalcitrant in absence of oxygen [29]. Thus, the colour in dye wastewater is removed in anaerobic stage, whereas a large proportion of COD is removed in aerobic stage [1, 64, 85, 112, 143]. However, COD removal does take place in anaerobic chambers, although it is often within the range of 1–40% in a single staged reactor. In case of double staged reactor, the removal is quite high, measuring up to 70%. It was experimentally determined that a small proportion of decolourization may take place in the aerobic phase, as low as 20% [13]. In a study conducted by Dong et al. [44] using anaerobic and aerobic MBBRs showed, 20–35% COD being reduced in anaerobic phase. The COD reduced in aerobic phase are contributed by the anaerobically recalcitrant amines formed in absence of oxygen [62]. Complete degradation of azo dyes takes place in two steps: (as shown in Fig. 3).

Step1: Cleavage of azo bond under anaerobic conditions.

After the cleavage of azo bond, the nitro groups react with protons to form aromatic amines [51]. Experimental studies confirm that a large proportion of the dyes are biodegraded rather than being mineralised at this stage [62].

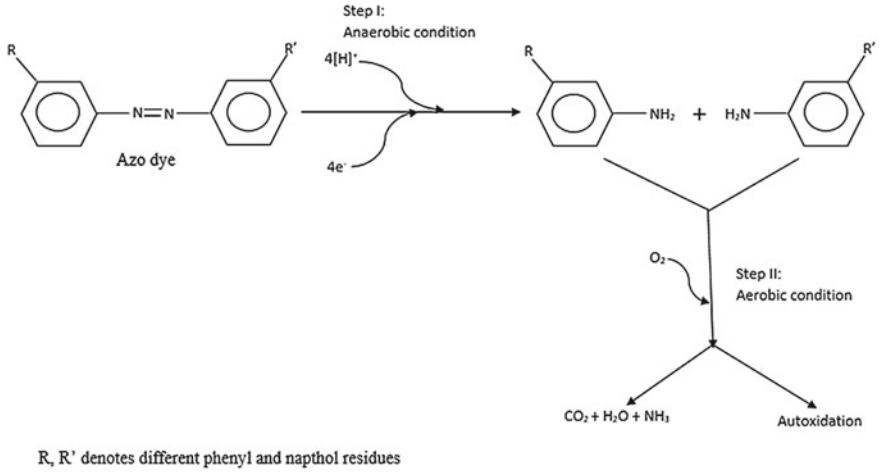


Fig. 3 Schematic representation showing bacterial azo dye degradation via anaerobic/aerobic pathway [159]

Step2: The intermediate amines are degraded via hydroxylation and ring cleavage in aerobic conditions.

This step is necessary for transformation of amines to CO_2, H_2O and organic acids [8]. Another possibility of aromatic amine transformation, especially when ortho-substituted hydroxyl groups are present, is autoxidation in aerobic environment [88, 158]. This autoxidation is one of the main reasons behind recalcitrant nature of aromatic amines [159]. Even though it is theoretically considered that anaerobically formed amines are easily degraded in aerobic conditions, it has been observed aromatic amines containing sulfonates such as naphthyl amine sulfonate and aminobenzene sulfonate are not mineralized in conventional aerobic suspended growth systems due to hindrance in transport across cell membrane [120]. Considering the stratification of biofilms in MBBR carriers, anoxic and aerobic environment simultaneously exists in a single reactor that will facilitate both the processes at different biofilm depths [26, 87, 113].

2.2 Fungal Dye Degradation

Unlike bacterial species, fungi are capable of degrading a wide range of organic pollutants and are not dye specific [135] due to non-specific nature of enzymes that aid in dye degradation. Enzymes like lignin and manganese peroxidases and laccase catalyse oxidation of even complex azo dyes which are both phenolic and nonphenolic [58, 140]. Interaction and relative contribution of these enzymes vary with fungal species. Similar to bacterial dye degradation pathways, biodegradation, biosorption and bioaccumulation are the noted mechanisms for fungal biodecolourization by both live and dead cells [78] however bioadsorption is limited to a maximum dye removal of 50% [83]. In case of white rot fungi *Phanerochaete chrysosporium*, Lignin Modifying Enzyme (LME) is responsible for reacting with dissolved oxygen producing H_2O_2 [122] and does not require Lignin Peroxidase (LiP) enzyme to degrade dyes including azo, heterocyclic and triphenyl methane dyes [111]. In the case of *Trametes versicolor*, another fungal species studied in MBBR, Manganese Peroxidase (MnP) does not participate in decolourization, which is brought about by oxidation catalysed by ligninase and laccase for majority of the studied dyes including azo and anthraquinone dyes [164, 171]. Laccases that belong to the group of oxidase enzymes catalyse the oxidation of aromatic compounds to their corresponding radicles ultimately reducing to oxygen and water [79]. Adsorption by *T. versicolor* is observed to be only 5–10% [19].

3 MBBR as a Treatment Unit

A comparison between MBBR with other suspended growth processes in the context of dye degradation revealed that the former is far more efficient for removing both

COD and colour from dye wastewater [13, 121, 144]. Comparative studies between moving bed systems and SBR show the difference in COD removal was higher by almost 20% within a HRT of 24 h [13]. MBBR as a sole biological treatment setup for satisfactory degradation of dyes is not frequently studied due to the requirement of multi-staged setup for complete degradation, low BOD: COD ratio along with presence and/or formation of hardly degrading substances. A study involving three MBBR units—two anaerobic followed by an aerobic reactor demonstrated a total removal of 86% COD and 50% colour. Almost all the colour removal took place in the anaerobic reactors, the aerobic unit accounted for mere 1% removal. COD removal efficiency in the two anaerobic reactors was found to be unsatisfactory but was increased to 86% after treatment in aerobic chamber [121]. Anaerobic MBBR operated at different HRTs and temperatures to study the effect of these parameters on removal efficiency showed that under optimum conditions of 48 h HRT and 30 ± 5 °C, rate of decolourization and COD removal was 85% and 78.3%, respectively. Decrease in HRT up to 12 h decreased the COD reduction rate to 45% and temperature decrease also resulted in drastic change for dye decolourization [139]. It is evaluated that up to a certain temperature, there is proportional relation with dye degradation [10]. Dye degradation in MB-SBBR resulted in 100% decolourization when initial dye concentration is increased stepwise. Inducing shock load, decreased the removal efficiency by 40%. Degradation of aromatic intermediates, formed during anaerobic phase, was efficiently completed in the aerobic period along with 10% decolourization [113]. Experimental study confirmed high temperature dye effluent treatment using aerobic MBBR and substantial COD removal was achieved in the single system [95]. Albeit these performances, a number of other treatment technologies coupled with MBBR are also experimented, which are discussed in detail in the following sections (Table 1).

3.1 SBR Along with MBBR Setup

To facilitate the aerobic degradation of dye intermediates formed anaerobically, aerobic MBBR setups can be installed in sequential mode for better performances that aid in complete mineralisation by alternating anaerobic and aerobic phase. Further improvement can be investigated by applying an anaerobic SBR prior to MBSBBR. For an instance, it has been observed that up to an initial acid dye (AR18) concentration of 100 mg/L, 100% removal of dye intermediate 1-Napthylamine 4-sulfonate can be achieved in aerobic moving bed sequential biofilm reactor (MB-SBBR) with corresponding COD removal of at least 71.5%. Increasing dye concentration even up to 1000 mg/L, the removal of 1-N 4-S was never less than 83.9% with an HRT of 2.75 d which conforms that aerobic moving bed reactors can efficiently degrade dye intermediates which are otherwise challenging. Using this setup, 98% colour removal was obtained with initial dye concentration ranging between 100 and 1000 mg/L [85].

Table 1 Studies demonstrating the role of MBBR as dye effluent treatment unit

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
Coagulation with alum + Polyurethane-based fluidized reactor	* Carrier: polyurethane media * CFR[a]: 15% (v/v) * Attached biomass: 2800 mg/L * Suspended biomass: 2000 mg/L	* COD: 654–1092 mg/L * SS: 46–152 mg/L * pH: 12.3 * temp: 32.6 ±2.1 °C	Not specific (dye effluent from the polyester deweighted process)	Bacteria (Mixed culture)	* 92% COD removal	* Coagulation followed by biological treatment performed better than biological pretreatment followed by coagulation * Alum dose required (600 mg/L) was less in the former trial with less sludge production	Park and Lee [123]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
MBBR + chemical coagulation with $FeCl_3.6H_2O$ + electrochemical oxidation using NaCl	* Carrier: hexahedronal media * CFR[a]:10% (v/v) * HRT: 48 h * DO: 3–4 mg/L * Attached biomass: 570 mg/L	* COD: 870 mg/L * colour: 1340 Pt–Co unit * pH: 13	Not specified (synthetic textile dyeing factory wastewater)	Bacteria (*Aeromonas salmonicida* and *Pseudomonas vesicularis)*	* 68.8% COD removal * 54.5% colour removal	* 3.25×10^{-3} mol/L $FeCl_3.6H_2O$ used as coagulant * 25 mM NaCl was used for oxidation with a current density of 2.1 mA/cm^2 * 95.4% overall COD removal * 98.5% overall colour removal	Kim et al. [80]
3 MBBR reactors (anaerobic, aerobic 1, aerobic 2) + coagulation with $FeCl_2$	* Carrier: polyurethane activated carbon * CFR[a]: 20% (v/v) * HRT: 44 h	* COD: 900 mg/L * colour: 3200 Pt–Co unit * pH: 13 (adjusted to 7) * temp: 42 °C	Not specified (synthetic textile dyeing factory wastewater)	Bacteria (Mixed culture)	* 85% COD removal * 70% colour removal	* 14% $FeCl_2$ used for coagulation * 95% COD removal obtained after total treatment * 97% overall colour removal efficiency	Shin et al. [144]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
3 MBBR reactors (anaerobic 1, anaerobic 2, aerobic)	* Carrier: polyurethane activated carbon * CFR[a]:20% (v/v) * HRT: 44 h * Attached biomass: 3000 mg/L	* COD: 608 mg/L * colour: 553 Pt–Co unit * T-N: 33 mg/L *T-P: 3.5 mg/L * pH: 12.5 * temp: 40 °C	Not specified (sample collected from dyeing wastewater treatment plant)	Bacteria (Mixed culture)	* 86% COD removal * 50% colour removal	* COD degradation rate in aerobic reactor was higher than that in anaerobic reactors * colour removal in aerobic reactor was 1%	Park et al. [121]
SBR (anaerobic) + MB-SBBR (aerobic)	* Carrier: polyethylene carriers * CFR[a]: 50% (v/v) * DO > 3 mg/L * HRT: 2.75 d * Attached biomass: 1318–1614 mg/L	* dye conc: 100, 5000, 1000 mg/L * COD: 3040–3620 mg/L * Temp: 22 ± 2 °C	Acid Red 18	Bacteria (collected from municipal wastewater treatment plant)	* 65–72% total anaerobically formed dye metabolites were degraded * more than 80% 1-napthylamine 4-sulfonate was degraded	* Glucose and lactose as co-substrate * 98% dye decolorization and above 80% COD removal accoutred in anaerobic SBR	Koupaie et al. [85]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
2 MBBRs (both aerobic) + chemical coagulation by alum or $FeCl_2$	* Carrier: Polyurethane-dyeing sludge carbonaceous material (PU-DSCM) foam * CFR[a]: 10–30% (v/v) * Attached biomass: 2900 mg/L * HRT: 48 h * DO: 2.3 and 5.2 mg/L	* COD: 539 mg/L * colour: 622 Pt–Co unit * pH: 12.5 * Temp: 40 °C *T-N: 33 mg/L *T-P: 3.5 mg/L	Not specified (effluent collected from synthetic textile dyeing factory)	White rot fungus (*Phanerochaete chrysosporium)*	* 79% COD removal * 54% colour removal	* Both alum and $FeCl_2$ was tested for coagulation * Optimum alum dose: 1.55 mg alum/mg COD * $FeCl_2$ dose: 11 mmol/L * 95.7% COD removal * 73.4% colour removal * COD and colour removal was much higher in the first reactor in compared to the second	Park et al. [122]
2 MBBRs (anaerobic, aerobic) + membrane filtration	* Carrier: polypropylene cylindrical carriers * CFR[a]: 37% (v/v) * HRT (anaerobic): 11 h (aerobic): 5 h	* COD: 500 mg/L * SS: 310 mg/L * dye conc.: 400 Pt–Co * pH: 11	Azo dye: Reactive Brilliant Red X-3B	Bacteria (Mixed culture)	* 90% colour removal * 85% COD removal * 94% SS removal	* Hollow fibre PVDF membrane of pore size 0.02 μm was used for membrane filtration	Dong et al. [44]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
Photocatalytic oxidation with TiO_2 + MBBR (aerobic)	* Carrier: LECA (special clay granules) * CFR[a]: 50% (v/v) * HRT: 8–20 h	* COD: 1650 mg/L * BOD: 390 mg/L * pH: 11 * colour: 0.81 A_{335} * Temp: 22–26 °C	Not specified (textile plant effluent)	Bacteria (mixed culture)	* 64% COD removal * 72% colour removal	* TiO_2 Degussa P-25 was used as photocatalyst (conc: 0.125 and 0.25 g/L) * maximum removal at 20 h HRT * 79% COD removal * 87% colour removal	Ahmadi et al. [4]
2 MBBR reactors (aerobic)	* Carrier: Anox Kaldnes K1 carriers * HRT: 24 h * SRT: 15 d * DO: 2.0 ± 0.4 mg/L * MLSS: 12.5 g/L	* COD: 650 ± 80 mg/L * NH_3-N: 18 ± 2.2 mg/L * Temp: 30–55 °C	Not specified (effluent from dyeing industry)	Thermotolerant bacteria (genus *Caldilinea, Rubellimicrobium* and *Pseudoxanthomonas)*	* 70.1% COD removal * 39.1% NH_3 removal	* Maximum COD removal was at 40 and 50 °C * Maximum ammonia removal was at 30 and 40 °C	Li et al. [95]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
Fluidized bed Fenton oxidation + MBBR	* Carrier: corrugated PVC cylinders * CFR[a]: 40–80% (v/v) * contact time: 1–3 d	* COD: 600–800 mg/L * BOD: 180–240 mg/L * dye conc.: 100 mg/L * T-N: 33 mg/L * T-P: 3.5 mg/L *pH: 12.5 * temp: 40 °C	Reactive sulphur dye: Chemistar Turq Blue	Bacteria *(Microbacterium marinilacus)*	* 86% COD removal * 81.5% BOD removal	* Conditions for oxidation: pH: 3, Fe^{+2}: 3 mg/L, H_2O_2: 5 mM * Optimum removal efficiency was obtained at a pH of 7.33, HRT 2.25 d and carrier filling ratio of 67.07%	Francis and Sosamony [53]
2 MBBR (anaerobic, aerobic) + ozonation + MBBR (aerobic)	* Carrier: Polyethylene carriers * CFR[a]: 60% (v/v) * HRT: (for first two MBBRs): 14 h (for last MBBR): 10 h * SRT: 10 days * DO (aerobic MBBRs): 2–4 mg/L	* COD: 824 mg/L * SS: 691 mg/L * NH_3: 40 mg/L * colour: 165 degree * pH: 7.9–8.5	Not specified (sample collected from textile dyeing factory)	Bacteria (Mixed culture)	Anaerobic unit: * COD: 23.1% * colour: 72.6% First aerobic MBBR: * COD: 68.9% * colour: 54%	* 14 min of ozonation of 1 mg/L * 94.3% COD, 97.8% SS, 85.3% ammonia and 96% colour was removed during treatment	Gong [62]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
Ozonation + MBBR (aerobic)	* Carrier: Anox Kaldnes K1 * CFR[a]: 40% (v/v) * HRT: 6 h	* COD: 400 mg/L * Dye conc.: 25–100 mg/L *NH_3: 30 mg/L	Azo dye: Reactive Orange 16	Bacteria (mixed culture sludge collected from municipal sewage treatment plant)	* 90 ± 1% COD removal * 97 ± 2% ammonium removal	* 5 min ozonation with dose 51.09 ± 0.76 mg/L * More than 97% of dye removal with 93 ± 1% COD and 97 ± 2% ammonium removal * Glucose used as co-substrate	Castro et al. [28]
Ozonation + MBBR (aerobic)	* Carrier: Anox Kaldnes K1 * CFR[a]: 20% (v/v)	* COD: 1000 mg/L * Dye conc.: 100 mg/L	Azo dye: Remazol Black 5	Bacteria (mixed culture isolated from textile wastewater)	* 81.21% colour removal * 83.63% COD removal	* Ozonation: 0.4 gO_3/h, determine time 120 min * With ozonation as pretreatment, colour removal increased up to 86.74% at 1 h batch time	Pratiwi et al. [129]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
MBBR (anaerobic)	* Carrier: Anox Kaldnes K1 * CFR[a]: 50% (v/v) * HRT: 12, 14, and 48 h	* COD: 2347 mg/L * pH: 7 * Temp: 30 ±5 °C and 21 ±2 °C	Azo dye: Direct Red 75	Bacteria from rice husks	* Optimum performance at 48 h HRT and 30 ±5 °C * 85% colour removal * 78.3% COD removal	* Sensitive to HRT changes * Decrease in HRT resulted in reduction of efficiencies * Bacteria were more effective at temperature around 30 °C	Santos Pereira et al. [138]
Granular activated carbon (GAC) + MBBR (aerobic)	* Carrier: Polypropylene type * CFR[a]: 67.07% (v/v) * HRT: 4–10 h *pH: 7.33	* COD: 1682 mg/L * BOD: 399 mg/L * Colour: 1813 Co–Pt	Not specified (effluent from dye treatment plant)	Bacteria (culture developed from dairy animal faeces and sludge of sewage treatment plant)	* 87.22% COD removal * 80% BOD removal	* Total COD removal 90% * BOD removal 95% * 20 cm GAC bed used	Vaidhegi et al. [155]
Ozonation + MBBR	* Carriers: Anox Kaldnes K1 * CFR[a]: 40% (v/v) * DO: 5 mg/L * HRT: 6 h * Attached biomass: 1300–2100 mg/L	* COD: 375 mg/L * NH_4-N: 40 mg/L * dye conc.: 50 mg/ * Temp: 25 ±2 °C	Reactive Red 239	Bacteria (Mixed culture)	* 88% COD removal * 41% ammonium removal	* Glucose used as co-substrate * 12 min ozonation time of 50 mg/L dose * No colour removal in MBBR	Dias et al. [43]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
MB-SBBR (anaerobic) + MBR (aerobic)	* Carrier: Polyurethane foam * * CFR[a]: 30% (v/v) * HRT: 48 h	* COD: 900 ± 325 mg/L * colour: 300 ± 90SU * TDS: 9500 ± 1500 mg/L	Black B, Black WNN, Red 3BS	Bacteria (mixed culture from poultry slaughterhouse wastewater)	* 78% dye removal * 70.5 ± 5% COD removal	* Total COD removal 77.1 ± 7.9% * Total colour removal 79.9 ± 1.5% * Two-staged system had better performance than single staged	Azimi et al. [13]
Ozonation + 2 MBBR (both aerobic)	* Carriers: Anox Kaldnes K1 * CFR[a]: 40% (v/v) * DO: 5 mg/L * HRT: 3 h	* COD: 400 mg/L * NH_4-N: 40 mg/L * pH: 7 * Temp: 20 ±2 °C	Reactive Red 239	Bacteria (Mixed culture)	* 94% COD removal in total * 40% ammonium removal	* 12 min and 20 min ozonation time of 20 mg/L dose * Formation of 5 identifiable oxidation resistant intermediates after ozonation * Nitrification was inhibited by 4-amino 6-chloro 1,3,5-triazine 2-ol	Dias et al. [42]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
MB-MBR (moving bed membrane bioreactor)	* Carriers: hollow cylinders * CFR[a]: 50% (v/v) * DO > 3 mg/L * HRT: 15.25 h, 16.54 h * SRT: 30 d * MLSS: 1120 mg/L	* COD: 857.2 ± 10.5 mg/L * colour: 505.3 ± 3 Pt–Co * pH: 8.07–8.14 * Dye conc: 10 mg/L * Temp: 20 ±1 °C	Reactive Red 390	Bacteria (Mixed culture)	* 89.2% colour removal * 98.5% COD removal	* In terms of COD removal, carrier filling ratio did not have any impact * Decrease in carrier filling ratio to 10% had almost negligible decrease in colour removal	Erkan et al. [48]
MB-SBBR	* Carriers: K1 carriers * CFR[a]: 5 and 10% (v/v) *HRT: 16.8 h and 33.6 h * Biomass wt: 21.12–35.19 g	* Dye conc.: 50–1000 mg/L * COD (dye + sucrose): 649–1398 mg/L * Temp: 24–26 °C	Reactive Orange 16 (RO16)	Bacteria (Mixed culture)	* 100% colour removal and 97% COD removal when dye conc. is increased stepwise	* Shock loading greatly affected both COD and colour removal, dropping the efficiency to 40% * Sucrose was given as co-substrate	Ong et al. [113]
MBBR	* Carriers: Polyurethane foam-polypropylene carriers * CFR[a]: 10–60%	* Dye conc.: 10–100 mg/L * pH: 5–9	Congo Red Dye	Bacteria (*Bacillus sp.*)	* 95.7% dye removal	* Optimum pH, dye concentration and carrier filling ratio was obtained to be 7, 50 mg/L and 45% respectively	Sonwani et al. [148]

(continued)

Table 1 (continued)

Reactor configuration	Experimental conditions	Wastewater characteristics	Dye(s) considered	Responsible Microorganisms	Removal obtained in MBBR unit(s)	Other remarks	References
MBBR	* Carriers: Plastic Biofill C2 carriers * CFR[a]: 30% * Average attached biomass: 3.5 g/L * DO > 2.2 mg/L * HRT: 1 d	* COD: 2000 mg/L * colour: 700 Pt–Co * BOD: 400 mg/L * pH: adjusted to 8.6 * TSS: 940 mg/L * Temp: 25 °C	Not specified (Sample collected from local textile industry)	Bacteria (aerobic sludge collected from textile industry effluent treatment plant)	* 82% COD removal * 61% colour removal * 78.8% TSS removal * 90% T-N and T-P removal	* 100% of MBBR treated effluent can be reused in dyeing process of Yellow, crimson and Navy Procion HEXL *	Yang et al. [170]

CFR[a]: Carrier Filling Ratio

3.2 Coupling Membranes with MBBR

Membrane filtration when used along with a series of anaerobic and aerobic MBBR reactors exhibits a number of advantages including lower HRT and high loading rates. Membrane fouling is often a problem encountered in membrane bioreactor which Erkan et al. [48] attempted to solve by using a novel moving bed membrane bioreactor (MB-MBR). It was suggested that no cleaning—physical or chemical was required for the process. In comparison to colour and COD removal percentages in MBR (87.1% and 93.1%, respectively), MB-MBR showed slightly better results with respect to both parameters (89.2% and 98.5%, respectively) but the major advantage lies in avoiding membrane fouling. Degradation of Reactive Brilliant Red in such a treatment process with hollow fibre PVDF membranes showed 90% colour removal [44]. A hybrid system employing MBBR along with membrane bioreactor (MBR) is a striking option in terms of reactor footprint and energy consumption for treating dye wastewater [169]. Degradation of dye wastewater containing three different azo dyes using anaerobic MBSBBR—aerobic MBR setup, resulted in a degradation efficiency of around 79.9 $\pm$ 1.5% and 77.1 $\pm$ 7.9% in terms of colour and COD, respectively. The lower colour removal is attributed to autoxidation of dye intermediates formed in the anaerobic phase [13].

3.3 MBBR and Chemical Coagulation

Chemical coagulation is one of the commonly used treatment technologies for dyeing wastewater [20] because the effluent emitted from the related industries often contains a high concentration of solids in suspended as well as colloidal form [22]. The innovation of biological processes made a promising aspect in combining the two technologies to get efficient removal discarding the disadvantages of high sludge production from coagulation process and high organic loading on biological process. Shin et al. [144] studied the efficiency of treating textile wastewater using a three-staged MBBR coupled with coagulation using $FeCl_2$. A maximum removal efficiency of 95% and 68.5% was achieved in terms of COD and colour, respectively, during the combined treatment process, whereas, maximum removal efficiencies for COD and colour was obtained as 55.3% and 56%, respectively, individually in MBBR. The results showed majority of colour removal took place in anaerobic reactor where electrons were provided by the dyes in reducing environment. A combined treatment process was adopted that involved MBBR as biological pretreatment unit, followed by chemical coagulation and electrochemical oxidation. The combined treatment yielded a colour removal of 98.5% along with 95.4% COD removal using 3.25×10^{-3} mol/L $FeCl_3.6H_2O$ along with 25 mM NaCl at a current density of 2.1 mA/cm^2 and a flowrate of 0.7 l/min. The support media resulted in an increase of 19.7% decolourization and 13% COD removal on the overall process. [80].

In an attempt to understand the better reactor configuration with chemical coagulation and biological treatment, studies were performed as biological pretreatment followed by chemical coagulation (FBAS-C) and chemical coagulation followed by biological fluidized bed reactor (C-FBAS). It was observed that biological treatment followed by coagulation with alum performed better with higher COD removal (92%) with lower alum dosage (600 mg/L) and 20% less sludge production. In C-FBAS configuration, a COD removal of 82% was obtained with 1000 mg/L alum dosage [123]. In place of using bacteria as attached microorganisms, Park et al. [122] attempted dye removal in two MBBRs with attached white rot fungus with chemical coagulation as post-treatment in order to improve COD removal. The study investigated the efficiency of alum and $FeCl_2$ as coagulants. COD removal was observed to be higher in case of alum (81%) with an optimum dose of 1.55 mg alum/mg COD as compared to 11 mmol/L $FeCl_2$ (79%). Colour removal was also observed to be higher in alum (42%) than $FeCl_2$ (30%). Almost 96% COD removal was obtained during this treatment with optimized parameters.

3.4 MBBR Coupled with Advanced Oxidation Processes

Advanced oxidation processes can mineralise a wide range of recalcitrant and toxic substances [9] although high cost of this method restricts its solitary application in large industries and is recommended to be applied in case of BOD/COD ratio less than 0.2 [125]. Dye effluents contain a huge proportion of recalcitrant organic compounds that characterizes the low BOD: COD ratio of the wastewater making it hard to biodegrade completely. To increase the biodegradability, the effluents are often subjected to chemical oxidation that improves BOD: COD ratio [55]. Oxidative pretreatment of textile wastewater using Fenton oxidation resulted in the increased BOD to COD ratio from 0.25 to 0.52 [53]. However, dose and contact time of most of these oxidation processes must be optimized as they may form by-products toxic to microorganisms undergoing further biological treatment [94, 130].

3.4.1 Oxidation with Ozone

Dissolved ozone is able to decompose refractory materials into easily biodegradable substances thereby facilitating its biological mineralisation [97, 153]. The negative charge density prevailing around the azo bond attracts ozone to get oxidized [155]. Dye decolourization was studied in a four staged reactor comprising of an aerobic MBBR followed by aerobic MBBR, ozonation and aerobic MBBR where each reactor had its specific function towards the treatment process. The anaerobic chamber facilitated dye degradation with 76.8% colour removal whereas the first aerobic MBBR reduced its intermediates where BOD: COD ratio dropped from 0.33 to 0.14. The residual recalcitrant intermediates were ozonized reviving the BOD: COD ratio of 0.43 which were easily degraded in the last aerobic chamber [62]. A

similar study conducted by Pratiwi et al. [129] showed that while degrading Remazol Black (RB5), an azo dye, ozonation time of 120 min with a dosage of 0.4 g O_3/h resulted in an increase of BOD: COD ratio from 0.23 to 0.42. It was observed that with a mere retention time of 1 h, dye removal increased from 68.6 to 86.74% with ozone treatment. Allowing a 24 h batch period, optimum colour and COD removal efficiencies were obtained as 96.9% and 89.13%, respectively.

A study was conducted by Dias et al. [43] which involved the application of ozone followed by biological treatment in aerobic MBBR to treat Reactive Red (RR239). The removal efficiencies of COD and ammonium were obtained as 88% and 41%, respectively. It was observed that nitrification was partially inhibited which resulted in accumulation of nitrite in the reactor. The cause of this inhibition was attributed to triazine and benzofuran. To make the process more efficient, ozonation was further followed by two-staged aerobic MBBRs as bio-treatment which proved to be highly efficient for removing RR239 although high ozone dose produced an intermediate 4-amino-6-chloro-1,3,5-triazine-2-ol which still inhibited nitrification in the reactor, but to a lesser extent. In this study, ozone dosage was reduced from 50 to 20 mg/L. With lower ozonation dosage, triazine in the dye could not be oxidized. Longer ozonation time (20 min in place of 12 min) resulted in formation of intermediates that were recalcitrant and toxic for biomass and thus COD removal efficiency dropped from 94 to 90% in the reactors along with nitrification inhibition [42]. However, a similar study undertaken by Castro et al. [28] involving the removal of Reactive Orange 16 using ozone and aerobic MBBR did not show any inhibition towards removal of ammonium, where a maximum of 94% COD, 97% colour and 99% ammonium was removed where ozone was applied at a dosage of 51.09 ± 0.76 mg/L for 5 min. Thus, from the previous works it can be concluded that increase in ozonation time produces more recalcitrant compounds that might inhibit nitrification in aerobic MBBR.

3.4.2 Fenton Oxidation

Fluidized Fenton oxidation process is supposed to be advantageous over other processes in the fact that iron crystallization and precipitation results in production of less sludge [104]. The quantity of hydroxyl radicals produced during the process is enough to effectively oxidize most of the compounds present in textile effluent but a major disadvantage of this process is the production of ferric hydroxide sludge which needs further handling and disposal. Further, it has been observed that impact of variation in influent wastewater characteristics can be reduced with Fenton oxidation process [82]. Using immobilized *Microbacterium marinilacus* isolated from textile effluents, fluidized bed Fenton process followed by MBBR was capable of removing 87.22% COD at 67.07% filling ratio. Using Box-Behnken statistics, the optimum pH and HRT were suggested as 2.25d and 7.33, respectively. Sludge production combining the two processes resulted in considerable decrease in sludge production thus being more economical [53].

3.4.3 Photocatalytic Oxidation

Photocatalysis with TiO_2 is a non-toxic, easily available chemical with robust optical properties, large surface area and its use in treatment operations is cost effective as compared to several other AOPs [59, 66]. Using polyaniline-TiO_2 nanocomposites immobilized in polystyrene cubes, 89% of degradation of Acid Yellow 17 was obtained which shows the efficacy of photocatalytic action of TiO_2 alone for dye decolourization [106]. Combination of photocatalysis with biological process helps in efficient treatment of colour using TiO_2 and biological removal of COD. The removal efficiency using TiO_2 is comparable with that using Fenton oxidation process and within 48 h of biological treatment nearly 98% of colour removal could be achieved even using suspended growth reactors [38]. With all these advantages and various studies involving photocatalysis to degrade dyes, an experimental study was undertaken to degrade textile wastewater with a BOD: COD ratio of 0.23 using a combination of photocatalytic oxidation using TiO_2 and aerobic MBBR [4]. The researchers compared the removal efficiencies between activated sludge process, MBBR and MBBR coupled with photocatalysis. Oxidation with photocatalyst proved to be the most effective treatment process with considerably better removal efficiencies in terms of both COD and colour than the other two reactors. Modified Stover-Kincannon model was used to determine the COD removal kinetics for the reactors.

3.5 MBBR Along with Adsorption

Bioadsorption is one of the mechanisms of dye effluent decolourization which has very minimal contribution. However, installing an adsorption medium along with MBBR system decreases the organic load, as also improves decolourization efficiency. Often along with biological action, the biomedia used takes part in adsorption of dyes causing a removal of 96% [87]. Moreover, the presence of high concentration of complex recalcitrant compounds vacillates the use of MBBR without a prior treatment that would decrease the loading on biological system. Granular activated carbon has high retaining capacity along with high surface area suitable for adsorption [167]. A reactor setup comprising of a 20 cm granular activated carbon (GAC) bed and an aerobic MBBR was used to study the performance regarding dye wastewater treatment which showed a maximum of 90%, 95%, and 72% COD, BOD removal and colour removal, respectively. Maximum colour removal occurred in the GAC by adsorption whereas MBBR increased the quality of the effluent by decreasing the organic matters. One important advantage of using GAC is it can be reused after corrosive washing thus minimizing the economic input [155].

4 Microorganisms in MBBR for Dye Removal

Microorganisms including bacteria, fungi, yeast have been recorded to degrade different dyes in various environmental conditions [101]. The selection of microorganisms for specific dye removal is quite challenging for high loading rates as it may often inhibit the activities of respective organisms [148]. Mixed culture is observed to perform better than pure culture bacteria and is also more resistant to toxic effects of recalcitrant dyes and is also easy to maintain in industrial treatment plants [73]. A large number of microbial species have been identified to effectively degrade different dyes [52]. Toxicity of the dyes may have adverse effect on the development of biofilms which is why it is often preferred to carry out the process of biofilm formation on carriers in absence of dyes [69]. Bacterial cultures have been reported to show better results than fungal biomass in the treatment of dye wastewater [128]. Thirteen different diazobenzene dyes have been identified that have antifungal properties [116].

4.1 Bacteria

A number of facultative bacterial species are able to degrade dyestuff wastewater in both aerobic and anaerobic conditions [89]. Bacteria isolated from textile effluents are observed to be capable of decolourising dyestuff effluent over a pH range of 6–10, temperature range of 25–40 °C and an initial dye concentration of 50–200 mg/L [97]. Often, filamentous bacteria are noticed to remain in suspended form in MBBR systems during dye degradation [86]. These filamentous bacteria are also the dominant cultures as observed to be attached to the carriers [68]. Thermotolerant bacteria when used as biomass to treat high temperature dyestuff wastewater showed the abundance of genera *Caldilinea, Rubellimicrobium,* and *Pseudoxanthomonas* at different temperature ranges and a number of species has been identified to successfully treat dye effluent [95]. Details about the pathway of bacterial degradation and consequent conditions are discussed in other sections.

4.2 Fungi

This paper has mainly focused on bacterial degradation of azo dyes. However, fungal immobilization is especially favoured due to its morphologic specificity [125]. Fungal degradation of azo dyes in aerobic condition occurs due to their ligninolytic activity using lignin peroxidase [54]. White rot fungi are the most studied species for biodecolourization [89] among which *Phanerochaete chrysosporium* and *Trametes versicolor* has been reported to exhibit satisfactory dye removal when immobilized [118, 122]. *Phanerochaete chrysosporium* has been observed to remove as high as 96%

COD removal and 73.4% colour removal in MBBR along with several other biofilm-based reactors undergoing dye removal. The ligninolytic enzyme system of this particular species can degrade a number of recalcitrant dyes [111]. Besides these, a number of other species of white rot fungi and several other fungal species are recorded to decolourise various dyes in different reactors [54]. Details about the pathway of fungal biodecolourization and consequent experimental conditions are discussed in the previous sections.

5 Factors Affecting Dye Degradation in MBBR

Biological process in reactors is generally influenced by a number of parameters including pH, temperature, substrate available for biological uptake, retention times, biomass concentration. Along with these, support biocarrier surface, hydrodynamics, flow of water, turbulence also directly affect the formation of biofilms on media [11]. For treatment of recalcitrant compounds like wastewater containing dyes, chemical structure as well as inhibitory concentration of those chemicals [21]. Temperature also has direct effect on biomass community and their growth. It was observed for thermotolerant bacteria, optimum EPD yield was obtained at 45 °C majorly produced from humic acid [95]. Interaction of all these factors influence the optimum removal efficiency of dye wastewater treated in MBBR. To develop a robust process design, all these factors must be considered and a thorough understanding of their effects is required from economical as well as an operation point of consideration.

5.1 Effect of Initial Dye Concentration

Increasing dye concentration often limits removal efficiency as the dyes are often inhibitory to enzymatic activities of microbes at elevated levels [117, 151, 168]. However, too low concentration will limit the enzyme binding capability with the dyes, considered as substrate [56]. Thus, for optimum biodecolourization, knowledge about threshold influent dye concentration is crucial [142]. By varying initial concentration of Reactive Orange (RO16) over a range of 50–1000 mg/L in moving bed sequential biofilm reactor (MB-SBBR), it was observed that increasing concentration beyond a certain limit (300 mg/L dye concentration) significantly decreases the degradation in terms of both colour and COD. The removal efficiencies with initial concentration of 50 mg/L were obtained as 100% and 99.19% for colour and COD, whereas that with dye concentration 1000 mg/L was 26.82% and 36.98%, respectively, under other identical conditions. The study also confirms that shock loads with more than 300 mg/L dye concentration caused considerable effect on the reactor performance due to inhibition by toxic RO16 [113]. Often increasing dye

concentration to an extent cause similar decolourisation but with lower decolourization rates, which is due to the decrease in growth cultures brought about by inhibition [64].

5.2 *Effect of Dye Structure*

The fused aromatic structures of anthraquinone-based dyes make them most resistant to biodegradation over a long period of time. High colour intensity of basic dyes makes them difficult to degrade [15]. Lower potential to decolourize dyes can also be attributed to redox potential of the dye [47, 51]. The presence of azo bonds directly influences degradation efficiency, more the azo bonds present, degradation is harder [56, 156] and so, more dyes are resistant to microbial action [24]. The reason for difficulty in degradation of azo dyes is the presence of NH_2-triazine in the meta position of the compound [147]. Dyes having low molecular weights are easily biodegraded than high molecular weight, complex dyes that often are more recalcitrant [137]. Reactive dyes like Reactive Black 5 (RB5) and Reactive Violet 5 (RV5) often produces intermediates that are extremely hard to biodegrade even in aerobic environment which will produce water unsafe for further use or disposal [88, 96]. Reactive Orange 16 (RO16) has sulfonated group in the second position of naphthol ring which makes it harder to degrade [6]. Presence of sulphonic groups creates an electron deficient condition which makes it even harder to be biodegraded [17], for example, vinyl sulfone groups prevents dye adsorption and substrate fixation in biomass [63]. Azimi et al. [13] compared the removal efficiencies of three different azo dyes with variable number of azo bonds under identical experimental conditions. It was observed that Red 3BS, having 1 azo bonds and 1 triazine had a removal efficiency of 91.4 which in case of Black B (2 azo bonds and 2 sulfato ethyl sulfone groups) was 85.7%. Azo dyes having methyl, sulfo, nitro or methoxy groups are difficult to degrade than those having hydroxyl or amino groups in their molecular structure [107]. Wong and Yu [164] observed decolourization by fungus *T. versicolor* was dependent on dye structure as azo and indigo dyes are not substrates of laccase enzyme. Another fungal species *P. chrysosporium* was able to mineralise compounds with hydroxyl, nitro or amino attached with aromatic ring better than with unsubstituted rings [149].

5.3 *Effect of Co-Substrate*

Azo dyes being hard to biodegrade substances are often degraded by co-metabolism where the dye is treated as a secondary substrate for microorganisms along with an easier biodegradable primary substrate [141, 152]. During anaerobic degradation, the dyes are decolourized for being electron acceptors for electron transport chain. Thus, an easily degradable carbon source is needed [27]. The simple organic carbon

provides the energy source to catabolize the dyes in biological cycle during anaerobic phase [159]. It has been observed that *Bacillus subtilis* uses glucose as a co-substrate to reduce azo bond by providing essential reducing factors including NADH and FAD [27]. The composition of wastewater to be treated has to be considered in this aspect because effluents from industries like distillery and pulp and paper contain sufficient concentration of carbon whereas those from dyeing and selected chemical plants is characterized by low organic carbon concentration which has to be supplied externally [89].

It has been observed that acetate is a relatively poor electron donor whereas ethanol, glucose and sucrose are used extensively for better dye uptake [113, 156]. In case of fungal dye degradation, glucose, maltose, cellobiose and starch is observed to show better results in comparison to sucrose or lactose [54]. A combination of glucose and lactose when used as co-substrate was able to degrade about 98% of Acid Red 18 even in high concentration up to 1000 mg/L [85, 86]. Sodium benzoate and sodium acetate also yielded satisfactory removal results for Reactive Black (RB5) removal in biofilm reactor which resulted in 94% COD removal and 99% dye removal [114]. Endogenous metabolism also may serve as a source of organic carbon in case of necessities for dye reduction [81, 115]. A study conducted by Ong et al. [115] showed a sharp decrease of MLSS from 7200 mg/L to 4700 mg/L which supplied the organic carbon required for azo bond cleavage thus making complete mineralization of Acid Orange 7 even without external carbon. Similar conclusion could be drawn in case of fungal biomass where dye degradation improved from 5 mg/L/h to 8 mg/L/h with addition of glucose at a concentration of 1000 mg/L for 50 mg/L dye. In suspended biomass, absence of glucose restricted any decolourization whatsoever [145].

5.4 Effect of HRT

Adequate HRTs when provided in the reactor results in effective degradation of a wide range of dyes [16]. Shorter HRTs correspond to the utilization of more dyes as co-substrate along with a primary substrate due to higher biomass activity at high influent dye concentration although total dye removal efficiencies were found to be higher in longer HRTs [141]. Longer retention times in the anaerobic phase is observed to have positive influence on efficiency of dye degradation [84]. Short HRT system requires longer time for biofilm growth in the carriers [68]. In comparison to suspended growth reactors, MBBR can perform similar removal in half of the HRT required for activated sludge process [170]. In the anaerobic MBBR, the recalcitrant compounds formed throughout the process are not completely hydrolyzed at shorter HRTs [61]. However, a much higher HRT in anaerobic reactor decreases acidification efficiency due to the utilization of solubilized volatile fatty acids (VFA) by methanogens [175]. Decrease in HRT from 48 to 12 h resulted in a drop of COD reduction from 78.3 to 54% although the sensitivity of this reduction in HRT is hypothesized to depend on the type of biocarriers [139].

Analysing the effect of HRT on removal, it was observed that increasing flow rates from 25 to 100 mL/h decreased HRT which in turn reduced removal efficiencies from 95.7 to 72.9% [148]. A decrease in HRT from 33 h to 16.8 h resulted in decrease in both COD and colour removal efficiency even though carrier filling ratio was increased from 5 to 10% [113] which is also established in dye degradation studies in other reactors [92]. Optimum HRTs also provide maximum BOD: COD ratio that facilitates optimum aerobic degradation thereby directly effecting removal efficiency [62]. Pratiwi et al. [129] studied the effect of different HRTs varying between 6 and 48 h in the aerobic MBBR and observed that optimum removal efficiencies were obtained at 24 h with maximum removal of both COD and colour. Colour removal efficiency increased linearly from 75.74 to 81.21% from 6 to 24 h HRT and then reduced to 68.6% at 48 h. However, in case of COD removal where efficiency increased from 37.55 to 85.86% linearly with increase in HRT from 6 to 48 h.

5.5 Effect of Biomass Concentration

In case of MBBR, a high removal efficiency can be achieved with a comparatively less biomass concentration thus limiting the cost of waste sludge handling. a conventional suspended growth reactor system usually employs an MLSS concentration of around 3000–4000 mg/L which in case of MBBR systems can be reduced to as low as 570 mg/L [80, 144]. For this reason, while it has been claimed that lower biomass concentration has relative lower decolourization rate due to toxicity, MBBR shows satisfactory removal efficiencies [98, 159]. Since, the majority of biomass concentration is attributed to biofilms attached to media, volumetric filling of biocarriers directly affects the removal efficiency of the process. It is observed that there is an optimum biocarrier filling percentage, below which concentration of active biomass in the reactor could be increased to enhance the removal efficiency. However, increasing carrier filling in the reactor beyond optimum ratio results in decrease in mobility of the carriers thus limiting proper mixing and efficient DO and substrate diffusion in biofilms [18, 148]. The adjustable biocarrier filling ratio is one of the most attracting features of MBBR that helps in keeping desired biomass in the system [146]. Considering the effect of biocarriers in between wide range of filling ratio over 40–80%, the optimum carrier filling ratio was determined to be exact 67.07% which removed maximum COD from the wastewater [53]. Using PU-AC carriers for anaerobic and aerobic reactors for dye wastewater treatment, the optimum filling ratios were identified as 30% and 20%, respectively [121]. Using polyurethane foam-polypropylene carriers (PUF-PP), it was observed that beyond a carrier filling ratio of 35%, almost negligible positive effect on dye removal was noticed [148]. However, in the study of Ong et al. [113], increasing biocarrier filling ratio from 5 to 10%, there was a significant increase in decolourization efficiency. Support media is observed to increase biomass concentration even up to three times as that in suspended biomass systems which might be due to both the increase in active biomass concentration as well as limiting inhibitory effect of toxic substances on biomass [80]. Similar to

bacterial biomass, fungi attached biocarrier filling have similar effect on dye degradation. Increasing filling ratio of Polyurethane-dyeing sludge carbonaceous material (PU-DSCM) foam from 10 to 20% had sharp increase in dye removal efficiency, however the same in case of increasing the ratio to 30% had no notable impact on removal [122].

5.6 *Effect of Biocarrier*

Addition of biocarriers in the reactor ensures the presence of active biomass thereby increasing removal efficiencies in terms of both dye concentration and COD in comparatively short retention times and high influent dye concentration [65]. Polyurethane foam is often used as support materials in dye degradation due to the inward matrices that serve as sites of anaerobic environment and help in the retention of microorganisms [121, 144, 160] and the pores aid in the formation of stable biofilms [117]. Using simple hexahedral carrier, it was observed that biomedia increased COD and colour removal up to 38.2% and 27.4%, respectively. Addition of biocarriers also resulted in higher SRT, almost 3 times as that activated sludge process effects in dye degradation in MBBR [80]. *Shewanella indica* when immobilized on biocarriers showed high tolerance for Reactive Black 5 (RB5) with stability of 90% on an average [174].

Alginate often proves to be a good immobilization media which was studied by a number of researchers. Using *Orchis mascula* as organic biocarriers for immobilization of mixed cells, almost 100% decolourization was obtained for different Reactive azo dyes including Red (RR2), Blue (RB4), and Yellow (RY15) in anaerobic environment [65]. It took a retention time of 40 h to completely degrade 40 mg/L of RR2 and RB4. Other Reactive dyes like Red (RR195), Orange (RO72), Yellow (RY17), and Blue (RB36) were effectively degraded using immobilized *Pseudomonas putida* and *Bacillus licheniformis* immobilized in polyacrylamide and sodium alginate [150]. Sodium alginate along with PVA was used to entrap mixed activated sludge which completely degraded Reactive Blue with the concentration varying between 10 and 40 mg/L. 87–88% COD removal was observed in each case. Cheng et al. [33] immobilized *Burkholderia vietnamiensis* on alginate-PVA-kaolin gel beads for efficient removal of crystal violet. Both starch and sodium alginate entrapped biomass showed similar results towards Reactive Red (RR2) azo dye degradation where complete decolourization was achieved in anaerobic phase [63]. Using polyethylene glycol media to entrap biological cells, 50% of anaerobically formed recalcitrant intermediates were degraded in aerobic environment at an HRT of 2–8 h [14]. Using polyurethane foam to immobilize *Bacillus subtilis* in a novel multi-staged fluidized bed reactor, about 92% of COD was reduced from real textile wastewater containing Congo Red dye with an initial concentration of 100 mg/L, PUF weight 5 g and pH of 8 [142].

In the light of fungal azo dye degradation, a series of cost-effective biological materials were experimented as biocarriers for growing *Trametes versicolor* and it was

observed that jute showed best result with respect to biomass growth, decolourization, and material integrity. Straw however showed better decolourizing property but was easily disintegrated within 2 weeks. Toxicity of amaranth dye towards the fungus was less when immobilized in any carrier which showed the advantage of using attached biomass [145]. *Phanerochaete chrysosporium* or white rot fungus was immobilized on Polyurethane-dyeing sludge carbonaceous material (PU-DSCM) foam carriers which showed impressive removal efficiency [122] (Table 2).

5.7 Effect of pH

pH of wastewater is a basic factor that is responsible for the transport of dyes through cell membranes of microorganisms thereby facilitating degradation [32]. Raw dyeing wastewater is usually characterized by high pH ranging around 12–13. A very few bacterial species are capable of growing at such a high alkaline condition. For biological treatments, pH should generally be kept close to neutral, however, azo dye degradation is reported to take place within a pH range of 7–10 [12, 127]. Experimental studies showed that at pH 7, COD removal efficiency was higher than that obtained at pH 12 [121]. Optimum pH for the removal of Congo Red dye was also obtained at pH 7 [148]. Francis and Sosamony [53] conducted a study to observe the effect of pH over a range of 6–9. The results showed that maximum removal efficiency was obtained in between 7 and 7.5 because the concerned microorganism showed maximum growth within that pH range. Since biological azo bond cleavage is enzyme mediated and enzyme ionization will be directly affected by pH change [56]. Setty [142] observed that slight alkaline conditions favoured degradation of azo dyes [93] and increasing pH from 7 to 8 resulted in 5% increment in dye removal efficiency. Moreover, anaerobic cleavage of azo bonds results in basic aromatic amines which increases the pH of the effluent if sufficient buffering action of other chemicals is not present [56].

This, however, is not the case for fungal dye degradation. Fungi often grow in acidic environment and have optimum dye degradation around a pH of 4.5 [75]. Raw dye effluent is often characterized by a high pH at which enzymatic activity of fungi is absent. A comparison between fungal activity towards dye degradation at pH of 7 and 12 showed removal at both pH. It was stated that removal at pH 7 was due to enzymatic degradation whereas that at pH 12 was due to biosorption [122].

6 Research Gaps and Scope of Future Work

A number of the experimental works and kinetics are developed using simulated dye wastewater considering the presence and concentration of that dyestuff only. However, real dye effluents contain salts, sulphur compounds, several nutrients, heavy metals including zinc, chromium, copper, arsenic besides organic nutrients

Table 2 Characteristics of different biocarriers and their effects in dye degradation in MBBR

Carrier type	Dimensional specifications	Physical properties	Carrier filling ratio	COD removal efficiency[a]	Colour removal efficiency[a]	References
Polyurethane media	2 × 2 × 2 cm with 1 cm hole through the cubes	Specific gravity: 1.03 Pores/cm: 30–40	15% (v/v)	92%	–	Park and Lee [123]
Regular hexahedron biomedia	1.3 × 1.3 × 1.3 cm	Density: 0.21 g/cm^3 ESA: 8.51 m^2/g	10% (v/v)	68.8	54.5	Kim et al. [80]
Polyurethane activated carbon (PU-AC)	0.5 × 0.5 × 0.5 cm	Porosity: 0.82 Density: 1.064 g/cm^3 Surface area: 7.4 m^3/kg	20% (v/v)	55.3%	56%	Shin et al. [144]
Polyurethane activated carbon (PU-AC)	1 × 1 × 1 cm	Density: 1.064 g/cm^3 ESA: 59.7 m^2/g	20% (v/v)	86%	50%	Park et al. [121]
Polyethylene carriers	1.5 × 1.5 × 1.5 cm	Density: 0.95 g/cm^3 SSA: 415 m^2/m^3 No. of carriers: 900	50% (v/v)	71.5%	98%	Koupaie et al. [8586]
Polyurethane-dyeing sludge carbonaceous material (PU-DSCM) foam	Height: 18 cm Diameter: 11 cm	Density: 0.85–0.93 g/cm^3 ESA: 55.7 to 58.3 m^2/g	10–30% (v/v)	79%	54%	Park et al. [122]
Polypropylene cylindrical carriers	–	Density: 0.95–0.98 g/cm^3	37% (v/v)	85%	90%	Dong et al. [44]
LECA (special clay granules)	Grain size: 4–10 mm	SSA: 525 m^2/m^3	50% (v/v)	79%	87%	Ahmadi et al. [4]
Anox Kaldnes polypropylene K1 carriers	Height: 7.2 mm Diameter: 9.1 mm	Density: 150 kg/m^3 SSA: 500 m^2/m^3	–	70.1%	–	Li et al. [95]

(continued)

Table 2 (continued)

Carrier type	Dimensional specifications	Physical properties	Carrier filling ratio	COD removal efficiency[a]	Colour removal efficiency[a]	References
Poly-vinyl chloride (PVC) corrugated cylinders	Height: 14 mm Diameter: 15 mm	–	40–80% (v/v)	86%	–	Francis and Sosamony [53]
Cylindrical polyethylene carriers	Height: 10 mm Diameter: 10 mm	Density: 0.98 g/cm^3	60% (v/v)	68.9%	76.8%	Gong [62]
Anox Kaldnes K1 carriers	Height: 7.2 mm Diameter: 9.1 mm	Density: 150 kg/m^3 SSA: 500 m^2/m^3	40% (v/v)	94%	–	Castro et al. [28]
Anox Kaldnes K1 carriers	Height: 7.2 mm Diameter: 9.1 mm	Density: 150 kg/m^3 SSA: 500 m^2/m^3	20% (v/v)	89.13%	96.95%	Pratiwi et al. [129]
Anox Kaldnes K1 carriers	Height: 7.2 mm Diameter: 9.1 mm	Density: 150 kg/m^3 SSA: 500 m^2/m^3	50% (v/v)	78.3%	85%	Santos Pereira et al. [138]
Polypropylene carriers	–	–	67.07%	87.22%	72.14%	Vaidhegi et al. [155]
Anox Kaldnes K1 carriers	Height: 7.2 mm Diameter: 9.1 mm	Density: 150 kg/m^3 SSA: 500 m^2/m^3	40% (v/v)	91%	–	Dias et al. [43]
Polyurethane foam (PU)	1 cm x 1 cm x 1 cm	–	30% (v/v)	70.5%	78%	Azimi et al. [13]
Anox Kaldnes K1 carriers	Height: 7.2 mm Diameter: 9.1 mm	Density: 150 kg/m^3 SSA: 500 m^2/m^3	40% (v/v)	94%	–	Dias et al. [42]
Hollow cylinders	Height: 5 mm Diameter: 16.75 mm	Density: 170 kg/m^3 SSA: 1036 m^2/m^3	20% (v/v)	89.2%	98.5%	Erkan et al. [48]
			10% (v/v)	88.9%	98.5%	
Plastic carriers	–	–	5–10% (v/v)	99.19%	100%	Ong et al. [113]
Polyurethane foam-polypropylene (PUF-PP) carriers	–	Average wt: 1.19 ± 0.03 g	45% (v/v)	–	95.7%	Sonwani et al. [148]

(continued)

Table 2 (continued)

Carrier type	Dimensional specifications	Physical properties	Carrier filling ratio	COD removal efficiency[a]	Colour removal efficiency[a]	References
Plastic Biofill C-2 carriers	Diameter: 25 mm	Free volume: 90% Density: <1 kg/m^3 SSA: 590 m^2/m^3	30% (v/v)	82%	61%	Wang et al. [161]

* Efficiency[a]: maximum removal efficiency in one single reactor
SSA: Specific surface area
ESA: Effective surface area

which might affect biological process of dye degradation [165]. This limitation will result in erroneous development of process kinetics and might not always show similar degradation when applied in industrial cases [56]. In case of photocatalytic oxidation, experiments have been conducted using TiO_2, but recently, ZnO has started to take its place due to lower cost and higher removal efficiencies for azo dye degradation [7, 38, 45]. These have been used with activated sludge processes but not investigated in MBBR. Focussing on the advantages of the later over suspended process, it can be hypothesized that more efficient removal can be obtained with lower HRT and may be for higher input concentrations.

From the discussion it is clear that different dyes have variable structural complexities and thus optimum removal cannot be theoretically predicted. More thorough experiments are needed to be done in that case. Moreover, since bacterial enzymes are substrate specific, the strains used in other biological reactors may be immobilized or grown on carriers for better biodecolourization. Developing successful process design requires optimization and quantification of parameters through the development of kinetics. A very few works have been undertaken so far to estimate the rate of reactions taking place in MBBR for dyestuff removal. One of them investigated the various models that fit the experimental data obtained from dye effluent in MBBR and inferred the use of Grau second order and modified Stover-Kincannon kinetics justified the results of COD removal. However, colour removal kinetics was not considered.

7 Conclusion

MBBR is one of the advanced water treatment technologies that are applied in treating municipal as well as a variety of industrial wastewater. Keeping in view of the different results obtained from the studies undertaken in MBBR, it can be established that MBBR as a sole treatment technology may require more than one unit to efficiently biodecolourize as well as remove COD and nutrients. Employing multi-staged reactor is often more welcome. Since the BOD: COD ratio of these effluents is quite low, pretreatments like ozonation processes improve biodegradability. Coagulation and adsorption highly increases both colour and COD removal. The biofilm attached to carriers improves resistant against recalcitrant compounds thereby decreasing the effect of substrate inhibition. Determination of influencing parameters as well as optimizing them to get efficient as well as economical removal of dyes is essential for process design which requires determination of kinetics as well as models to estimate the quality parameters of effluent to be safe for disposal or reuse.

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