Chapter 3

Performance of bench scale Moving Bed Biofilm Reactor (MBBR) with the consortium of the selected biofilm forming denitrifying bacteria and its evaluation

3.1 Introduction

Activated sludge process is the most widely used biological wastewater treatment process that uses aeration and microbial organisms. However, usage of biofilm reactors has increased over activated sludge processes due to its advantages such as less space requirement, flexibility in operation, short HRT, flexibility to changes in the environment, high biomass, resistance to dehydration, enhanced ability to degrade recalcitrants and low sludge production (Bassin and Dezotti, 2008; Wilderer and McSwain, 2004). MBBR has shown great success in reducing pollution, and its use in wastewater treatment has increased over the years (Rodgers and Zhan, 2003). Due to advancements in their designs and operation, MBBRs have become excellent alternatives for wastewater treatment because they are reliable and compact systems with reduced footprints, significantly lower suspended solid production, and consistent ability to produce high quality and reusable water, and minimal waste disposal. The MBBR has a high load fluctuation tolerance, and provides high effluent quality therefore has been widely used for nitrogen removal in recent years (Rusten et al., 1995, 2006; Martina et al., 2010). In spite of its advantages, slow start-up and poor performance are the major drawbacks of MBBR. One of the promising approaches to overcome this problem is bioaugmentation of specific microorganisms in the MBBR. In a recent report, bioaugmentation of *Pseudomonas* sp. SZF15 in MBBR efficiently removed nitrate (Su et al., 2019). Bioaugmentation of *Corynebacterium pollutisoli* SPH6 in the A/O-MBBR system showed potential in nitrogen removal (Liu et al., 2018). *Acinetobacter* sp. CN86 augmentation showed promising approach for simultaneous removal of nitrate, Cd^{2+} and Ca^{2+} in the MBBR process (Su et al., 2019). Zhang et al., (2020b) developed MBBR with heterotrophic nitrifying and aerobic denitrifying (HN-AD) bacteria which shortened the start-up time and improved TN removal performance in livestock and poultry breeding wastewater.

From this perspective, the studies in this chapter entail bioaugmentation of dMBBR with consortium DC5 for removal of nitrate from high nitrate containing synthetic effluent composed of acetate as carbon source, nitrate as nitrogen source and the other nutrients optimized in chapter 2. Performance of MBBR was enhanced by optimizing various parameters such as carrier filling ratio, HRT, C/N ratio, surface area of carriers (Aygun et

al., 2008; Daija et al., 2015; Wang et al., 2016; Jaafari et al., 2017). Thus various factors affecting the performance of dMBBR for nitrate removal were optimized by one factor at a time (OFAT) approach. Further, studies were conducted with (i) suspended growth reactor (without carriers), (ii) control reactor (MBBR without consortium DC5 inoculum) and (iii) dMBBR inoculated with activated sludge in order to compare their performance with that of dMBBR developed with consortium DC5. The applicative potential of consortium DC5 for the treatment of various industrial effluents was also studied.

3.2 Materials and Methods

3.2.1 Preparation of consortium DC5

Consortium DC5 was developed by growing all the selected isolates in PNB for 24 h individually; then the absorbance of 0.5 OD_{600nm} was set and 400 µl of each isolates were pooled to make the consortium of 2 ml final volume. The cell pellet obtained after centrifugation at 8000 rpm for 7 min was washed twice with PBS and resuspended in the same volume of PBS. Then 1 ml of this suspension of the consortium was then added in 100 ml of MM2 medium and incubated at 37 °C under the static condition for 24 h to be used as inoculum.

3.2.2 Bench-scale dMBBR developed with consortium DC5

A schematic representation of the dMBBR used in this study is shown in Fig.3.1. 10 L reactor was constructed from the polyacrylic material with 45 cm height and 16 cm width. A submersible pump fixed at the center of the reactor facilitated the movement of the carriers inside the reactor. The reactor was housed in a room where constant temperature of 37 ºC ± 2 was maintained throughout the studies. Synthetic effluent (MM2 medium) was continuously fed from the influent tank into the reactor with a peristaltic pump and treated effluent was simultaneously collected in the effluent tank. Each experimental parameter was investigated in 10 L synthetic effluent three times and each run was considered as one slot. Aliquot from the inlet and outlet sample of each slot was collected and analyzed for the nitrate, nitrite, ammonia, COD levels, dissolved oxygen, turbidity and pH.

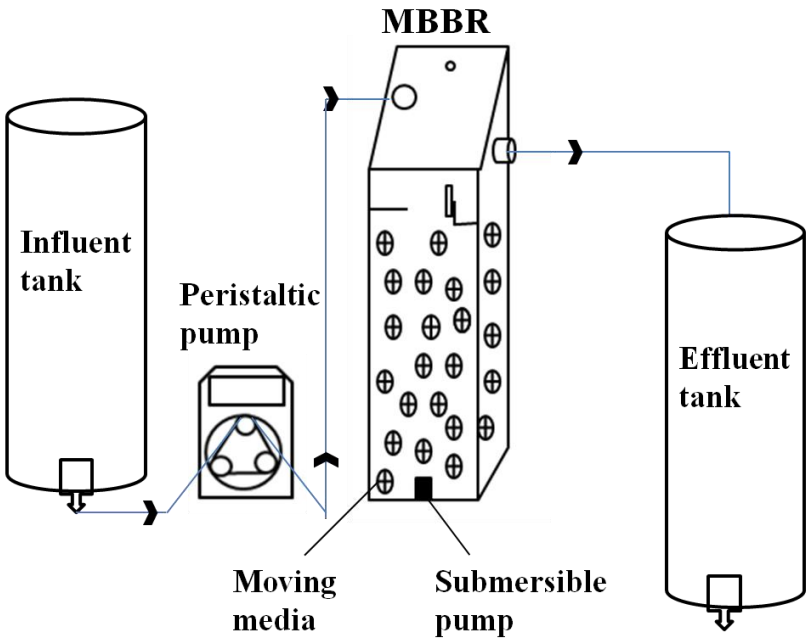

Figure 3.1 Experimental setup of dMBBR

3.2.3 Continuous dMBBR studies with consortium DC5

Synthetic effluent was continuously fed from the inlet tank to the reactor with the peristaltic pump (MasterflexR). pH 8, DO-0.1-0.8 mg L^{-1} and 15-350 NTU turbidity was maintained throughout the operation of dMBBR.100 ml inoculum prepared as mentioned in section 3.2.1 was added in the 10 L reactor containing carriers after which the biofilm was allowed to form for 10 days in dMBBR. For continuous reactor studies, synthetic effluent was continuously fed from the inlet tank into the reactor with the peristaltic pump and treated effluent was collected in the outlet tank and assayed for nitrate, nitrite, ammonia, pH, turbidity, biomass and DO. Various operational parameters to gauge the performance of dMBBR were investigated three times.

3.2.3.1 C/N ratio

C/N ratio is the amount of available carbon source consumed to the amount of nitrogen compounds reduced. Different concentration of C/N ratio 0.7, 0.4, 0.3 and 0.2 were taken where sodium acetate was used as carbon source and potassium nitrate was used as a nitrate source. Aliquots from the inlet and outlet of each slot was collected and analyzed for the nitrate, nitrite, ammonia, COD levels, dissolved oxygen, turbidity and pH.

3.2.3.2 HRT

Hydraulic retention time (HRT) is the time spent by the influent inside the reactor. 8, 6, 3 and 2 h HRT was set by maintaining the flow rate with the help of peristaltic pump and continuous reactor studies were carried out with an initial nitrate concentration of 620 mg L^{-1} where C/N ratio was set at 0.3. Aliquots were drawn from the inlet and outlet of each slot and analyzed for the nitrate, nitrite, ammonia, COD levels, dissolved oxygen, turbidity and pH.

3.2.3.3 Nitrate loading (mg L^{-1})

Nitrate loading is the concentration of influent nitrate. Here, 620, 744, 930, 1116, 1500, and 2400 mg L^{-1} nitrate loading was taken and continuous reactor studies were carried out in dMBBR with optimized C/N ratio 0.3 and HRT 3 h. Aliquots from inlet and outlet of each slot were collected and analyzed for the nitrate, nitrite, ammonia, COD levels, dissolved oxygen, turbidity and pH of the reactor was measured.

3.2.3.4 Carriers design and filling ratio

Different carrier designs viz. Pall ring with surface area (275 m^2/m^3), Kaldnes K1 (500 m^2/m^3) and Fluidized biomedia (400 m^2/m^3) were used for continuous reactor studies. Similarly, filling ratio (amount of carriers added in the reactor in %) 20 %, 30 % and 40 % were set and continuous reactor studies were carried out in 10 L dMBBR. Aliquots from the inlet and outlet samples of each slot were collected and analyzed for the nitrate, nitrite, ammonia, COD levels, dissolved oxygen, turbidity and pH.

3.2.3.5 Suspended reactor

Consortium DC5 was prepared according to section 3.2.1. Prepared inoculum was inoculated in 10 L dMBBR. No carriers were added in the reactor hence biomass was in suspended growth condition as in activated sludge system. Continuous reactor studies were carried out in suspended reactor with nitrate concentration of 620, 744, 930, 1116, 1500 and 2400 mg L^{-1}. Aliquots from inlet and outlet of each slot were collected and analyzed for the nitrate, nitrite, ammonia, COD levels, dissolved oxygen, turbidity and pH of the reactor.

3.2.3.6 dMBBR inoculated with Activated sludge

Activated sludge was collected from the domestic wastewater treatment plant. 1 % sludge was inoculated in 100 ml MM2 media and incubated at 37 °C for 24 h. After 24 h enriched sample was inoculated in the 10 L MBBR. After adaptation and biofilm formation continuous reactor studies were carried out in the dMBBR with optimized 3 h of HRT and C/N ratio 0.3 with filling ratio 20 % at nitrate concentration of 620, 744, 930, 1116, 1500 and 2400 mg L^{-1}. Aliquots from inlet and outlet of each slot were collected and analyzed for the nitrate, nitrite, ammonia, COD levels, dissolved oxygen, turbidity and pH of the reactor.

3.2.3.7 Control dMBBR

10 L dMBBR was developed without addition of any inoculum. The reactor was allowed to develop on its own. Continuous reactor studies were carried out with optimized 3 h of HRT and C/N ratio 0.3 with filling ratio 20 % at nitrate loading 620, 744, 930, 1116, 1500, and 2400 mg L^{-1} dMBBR. Aliquots from inlet and outlet of each slot were collected and analyzed for the nitrate, nitrite, ammonia, COD levels, dissolved oxygen, turbidity and pH of the reactor.

3.2.3.8 Analytical Methods

Methods for Nitrate, Nitrite and Ammonia analysis were performed according to APHA1998 (section 2.2.13). DO was measured using a DO probe (Thermo Scientific) and the turbidity was checked using turbidity meter (Hanna instruments).

3.2.3.9 Analysis of biofilm composition

Biofilm biomass was scraped from the surface of carriers and suspended in 100 ml of PBS at a concentration of 0.1 g wet weight. For EPS extraction cation exchange resin (CER) method was used (Hong et al., 2020). 30 ml of biofilm suspension was centrifuged (8000 rpm, 15 min, 4 °C) and biomass from biofilm sample was washed twice with PBS (pH ~ 7.0) and resuspended to a volume of 30 ml with CER (70 g g^{-1} dry cells). Centrifuge tubes were shaken at 250 rpm for 2 h at 4 °C and then kept static for 5 min to settle the CER. The biofilm suspensions were centrifuged (10,000×g, 15 min, 4 °C) and

filtered using a 0.45 µm filter membrane. Carbohydrate, protein and lipid content from extracted biofilm suspension were quantified.

3.2.3.9.1 Protein estimation by Folin Lowry assay

200 µl of biofilm suspension was added to 800 µl D/W to make a system of 1 ml.4.5 ml of reagent I was added to the system and incubated for 10 min. Then 0.5 ml of reagent II was added and system was incubated for 30 min. Absorbance of the developed color was measured at $OD_{660\ nm}$ (Lowry et al., 1951).Composition of all the reagents is given in Table 3.1.

Table 3.1 Composition of reagents for Folin Lowry method

Reagents	Composition
Reagent A	2 % Na_2CO_3 in 0.1 N NaOH
Reagent B	1 % NaK Tartrate in H_2O
Reagent C	0.5 % $CuSO_4.5\ H_2O$ in H_2O
Reagent I	48 ml of A, 1 ml of B, 1 ml
Reagent II	1 part Folin-Phenol reagent [2 N]: 1 part D/W.

3.2.3.9.2 Carbohydrate estimation by Phenol Sulfuric acid method

150 µl of concentrated H_2SO_4 was added to the 50 µl of biofilm suspension and incubated for 10 min. Then 5 % phenol was added to it and incubated further for 10 min. Absorbance of the developed color was measured at $OD_{490\ nm}$ (Masuko et al., 2005).

3.2.3.9.3 Lipid estimation by Phospho-vaniline method

2 ml of concentrated H_2SO_4 was added to 0.10 ml of biofilm suspension and heated for 10 min, in a boiling water bath and cooled in water bath for about 5 min. 0.10 ml of aliquot of the mixture was taken and 0.10 ml of concentrated H_2SO_4 was added to it. Further 5 ml of Phospho-vanillin reagent was added and incubated at 37 °C for 15 min. Absorbance of developed color was measured at $OD_{540\ nm}$ (Frings et al., 1970).

Biomass from the carriers was quantified by drying carrier material at 105 °C for 1 h.

3.2.4 Treatability of industrial effluents by consortium DC5

Inoculum of consortium DC5 was prepared as mentioned in section 3.2.1. 100 ml of the inoculum was added in the 10 L reactor containing carriers upon which the biofilm was allowed to form for 10 days. For treatability studies in dMBBR 10 L industrial effluent was continuously fed from the inlet tank to the reactor with the peristaltic pump and treated effluent was collected in the outlet tank and assayed for nitrate, nitrite, ammonia, pH, turbidity, biomass and DO.

3.2.5 Statistical Analysis

All the experiments were performed in triplicate. Statistical significance was analyzed using the one way ANOVA analysis. Error bars represent standard deviations in GraphPad Prism 6.0 (San Diegao, CA, USA).

3. 3 Results and Discussion

3.3.1 Denitrification studies in continuous dMBBR developed with consortium DC5

Biotreatment processes comprise mixed microbial cultures, which are important for their efficient operation. Unlike microbially mediated production processes, microbial mediated environmental protection and restoration processes involve microbial cultures comprising microbial consortia. Uses of microbial consortia are important for the efficient operation of biotreatment processes (Hamer, 1997). Various microbial consortia have been used for the treatment of nitrate containing wastewater. Bioaugmentation of functional bacteria has been widely used to improve ammonia tolerance and TN removal efficiency in reactor inoculated with activated sludge (Zhang et al., 2017b). Tannery effluent treatment with specially isolated strains of *Brachymonas denitrificans* improved biological nitrogen removal (Leta et al., 2005). Commercially available bacterial consortium B350, which comprised of 28 naturally occurring microorganisms showed promising results for the treatment of nitrate micro-polluted water (Gan et al., 2019).Microbial consortium BM-S-1 effectively removed COD, nitrogen and phosphorus from tannery wastewater (Kim et al.,2013; Kim et al.,2014). Therefore, dMBBR studies were carried out with a specially developed seed of consortium of denitrifying bacteria in

order to understand the performance enhancement upon bioaugmentation of specially selected bacteria.

The dMBBR setup was as depicted in Fig. 3.2. The reactor was run continuously for 300 days during the investigations where the consortium DC5 inoculum was seeded only once at the start of the reactor. Each parameter viz. C/N ratio, HRT, nitrate loading, carrier design and filling ratio that was optimized by OFAT approach was carried forward in the next experiment.

Figure 3.2 Bench scale dMBBR setup developed with consortium DC5 used in denitrification studies.

3.3.1.1 dMBBR performance at different C/N ratio

MBBR is very suitable for the nitrate and COD removal of wastewater(Yuan et al., 2015). Supplementation of exogenous carbon source is the most important factor for the denitrification process. Balance between electron donor and electron acceptor plays an important role in biological denitrification. In heterotrophic denitrification studies low C/N ratio generally limits the electron supply and thus it leads to accumulation of denitrification intermediates (NO_2^-, NO and N_2O), which is harmful to the environment and human health. On the contrary, use of excess electron donor results in wastage of expensive electron source and increases the effluent COD (Mohan et al., 2016). Therefore, optimization of C/N ratio should be done in such a way so that it does not cause secondary contamination in synthetic effluent and shows complete denitrification. As shown in Fig.3.3a at different C/N ratios 0.7, 0.4 and 0.3 nitrate removal was above 95 % with an initial nitrate concentration of 620 mg L^{-1}. However, COD was above permissible range (i.e. 250 mg L^{-1}) at C/N ratio 0.7, whereas C/N ratio 0.4, 0.3, 0.2 showed 96 %, 100 % and 78 % nitrate removal, respectively and COD below permissible range (Fig. 3.3a). Overall results as depicted in Fig.3.3a revealed that higher C/N ratio of 0.7 increased COD concentration in wastewater on the other hand, lower C/N ratio of 0.2 decreased nitrate removal efficiency. Hence, 0.3 C/N ratio was selected for further studies as the reactor run at this ratio was able to remove 100 % of nitrate (620 mg L^{-1}) at the same time reducing the COD below permissible range of 250 mg L^{-1}.

3.3.1.2 dMBBR performance at different HRT

HRT is another important parameter affecting nitrate removal in MBBR. HRT is the contact time of the influent wastewater with the microbial biomass inside the reactor (Ji et al., 2016). An appropriate extension of HRT improves the nitrate reduction (Wang et al., 2009). If HRT is too long it may waste treatment capacity and consume high energy (Wang et al., 2009). The data shown in Fig. 3.3b indicates that at HRT 8, 6 and 3 h, nitrate removal was 100 % with initial nitrate loading of 620 mg L^{-1} keeping the COD below permissible range. This means that at HRT of 3 h and above the wastewater had sufficient contact time with the bacteria in the reactor to achieve complete nitrate reduction. Whereas at 2 h HRT nitrate removal efficiency decreased from 100 % to 70 %, as the contact time was insufficient to achieve complete nitrate removal. Here, HRT 3 h

is the contact time with maximum nitrate removal efficiency. Increasing HRT above 3 h did not influence the nitrate removal. Hence, 3 h was selected as an optimum HRT for the subsequent reactor studies. At 3 h HRT, the developed MBBR showed 100 % nitrate removal efficiency at initial loading of 620 mg L^{-1} nitrate concentration. This is the shortest HRT (3 h) reported compared to other reports in the literature that required more time to remove nitrate. MBBR developed with *Pseudomonas* sp. SZF15 reported HRT of 11.96 h for removal of 47.64 mg L^{-1} nitrate with 79.78 % removal efficiency (Su et al., 2016). Chen et al., (2018) reported 8 h as optimum HRT for efficient treatment performance and nitrogen removal in biofilm reactor. Zhang et al., (2016a) reported 3.5 h HRT for 99.23 % nitrate removal in sponge based MBBR.

3.3.1.3 dMBBR performance at different nitrate loading

The nitrate concentrations in wastewater tend to fluctuate widely even in the wastewater produced by the same industry. Considering this, the effect of input nitrate content was studied by increasing the initial nitrate concentration in synthetic wastewater from 620 to 744, 930, 1116, 1500 and 2400 mg L^{-1} in different individual runs. As shown in Fig.3.3c the denitrification efficiency in the dMBBR was 100 %, 92.25 %, 93.02 %, 80.43 %, 72.23 %, 70.45 % at 620, 744, 930, 1116, 1500 and 2400 mg L^{-1} of nitrate concentration respectively accompanied every time by COD reduction below permissible range i.e. 250 mg L^{-1}. As Fig.3.3 a, b & c suggests that the consortium DC5 was able to reduce nitrate up to 2400 mg L^{-1} at optimized C/N ratio 0.3 and HRT of 3 h. Results obtained in this study also showed highest nitrate removal compared to other reports published previously by specific microorganisms such as *Brevundimonas diminuta* MTCC, *Pseudomonas butanovora*, *Pseudomonas* sp. SZF15 in MBBR (Kavitha et al., 2009; Kesseru et al., 2003; Su et al., 2016).

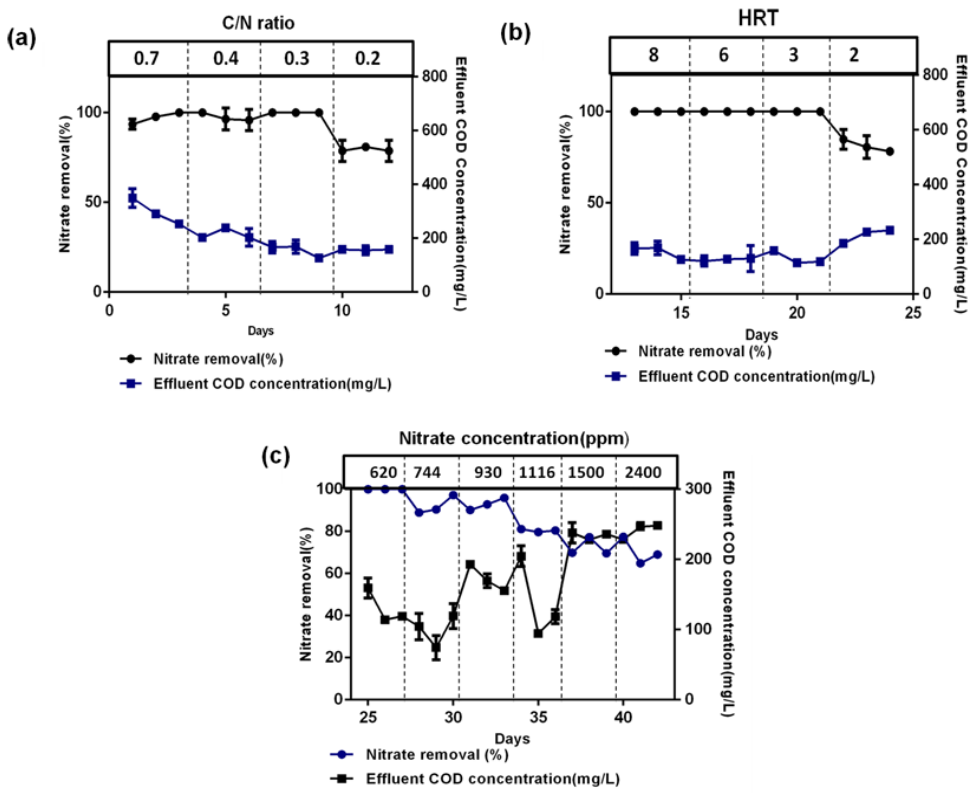

Figure 3.3 Performance of dMBBR inoculated with consortium DC5 at different (a) C/N ratio, (b) HRT and (c) Nitrate loading (n = 3)

3.3.1.4 dMBBR performance with carriers of different designs

Carrier media in MBBR increases cell attachment and biofilm development, which ultimately enhance treatment capacity of the MBBR. Surface area, size and shape of carriers have a profound effect on the biofilm formed on it (Odegaard, 2006). Carrier shape, structure and surface properties provide protection to microbial community developed on the carriers and form thicker biofilm whereas microbial growth on the surface area exposed to abrasions form thin biofilms (Mahendran et al., 2012). These carriers are durable and without need of replacement in the life time of MBBR processes. They are made up of virgin high-density polyethylene (HDPE) and high-density polypropylene (HDPP) (McQuarrie and Boltz, 2011). In the present study, three different types of carrier designs were checked having surface area, 275 m^2/m^3 (Pall ring), 500 m^2/m^3 (Kaldnes K1) polyethylene and 400 m^2/m^3 (Fluidized biomedia) polypropylene (Fig.3.4). Chemical nature of carriers used in this study was polyethylene, polypropylene and polypropylene for Kaldnes K1 carrier, Fluidized biomedia and for Pall ring

respectively. As shown in Fig. 3.5 a & b Pall ring carriers showed the highest nitrate removal efficiency from 620 to 2400 mg L^{-1} nitrate concentration and COD reduction below permissible range, while the biomass quantified from Pall ring (Polypropylene), Kaldnes K1(Polyethylene) and Fluidized biomedia was 35, 11.6 and 12 mg/carrier, respectively (Table 3.2). Maximum biomass was developed on Pall ring carriers. Low biomass content on the carriers with Kaldnes K1 and Fluidized biomedia must have been possibly due to shedding of biomass upon collision of carriers with each other as the shape of carriers supported more biomass on the outer surface of the carriers (Fig. 3.4). In EPS component analysis proteins were found to be most abundantly present in the biofilms obtained from all the carriers. Here, Pall ring carriers developed biofilm with highest EPS components (Table 3.2). The protein content in the biofilm samples was higher than carbohydrate which is similar to the results of Hong et al., (2020). Pall ring carriers have more surface area inside the carriers, which prevented the biofilm biomass from sloughing off. On the contrary Fluidized carriers and Kaldnes K1 carriers have more area on the outer surface, which facilitated sloughing off during collision between carriers, as a result causing reduction in overall biomass associated with the carriers.

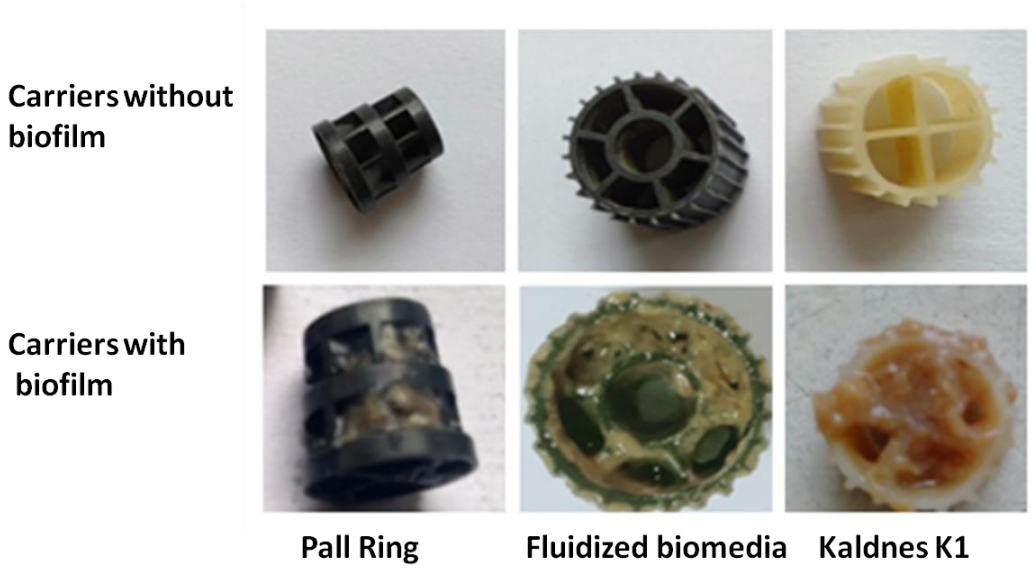

Figure 3.4 Carriers with different design and surface area used for dMBBR studies

Table 3.2 EPS components analysis of biofilm developed on different carrier types

Components	Pall ring media	Fluidized bio media	Kaldnes K1 media
Carbohydrate (mg L^{-1})	5.69	4.35	5.42
Protein (mg L^{-1})	183	20	35.3
Lipid (mg L^{-1})	9.3	0	2.7
Biomass (mg/carrier)	35	12	11.6

3.3.1.5 dMBBR performance at different filling ratio

Filling ratio of carriers is the volume of carriers added to the reactor. It should be below 70 % for the movement of the carriers freely in suspension. High filling ratio increases collisions between the carriers, leading to the selection of those bacteria that can grow on the carrier under particular reactor conditions and make the system more efficient (Wang et al., 2005). Wang et al., (2005) reported that biofilm thickness on each carrier decreased with the increase in filling ratio, which facilitated the mass exchange and biofilm renewal in the dMBBR and resulted in higher denitrification rate. At high filling ratio i.e. greater than 50 % anoxic zone on the carrier surface was found to be reduced due to formation of thinner biofilm. Thinner biofilm was found to increase aerobic microorganisms which cause carbon competition with the denitrifying bacteria and decrease denitrification efficiency (Hansson and Gunnarson, 1990). The biomass quantity was declined from 21 mg to 8 mg and removal rates of the COD, phenol and thiocyanate also decreased when carrier filling ratio was increased from 20 % to 50 % (Gu et al., 2014).

As represented in Fig. 3.5 c & d, at 20 % filling ratio highest denitrification efficiency was achieved in the dMBBR. However, filling ratio did not influence COD reduction (Fig. 3.5d). As filling ratio increased from 20 % to 40 % denitrification efficiency was decreased and concomitantly biomass on the carrier material too decreased from 35 to 12 mg/carrier respectively. It was also observed that 20 % filling ratio allowed proper circulation of the carriers and uniform biofilm formation, while at 30 % and 40 % filling ratio biofilm development was scanty. Moreover, a higher filling ratio of Pall ring carriers in the dMBBR showed reduction in nitrate removal possibly due to the

insufficient area for circulation of the carriers, which rendered biofilm formation, increased particle-particle collision and enhanced the shear stress on the biofilm. Similarly in this study also reduction in biomass at higher filling ratio can be attributed to the reasons by Wang et al., (2005). Zhang et al., (2016a) have also reported that at a 20 % filling ratio sponge carrier achieved maximum biomass amount per gram of sponge.

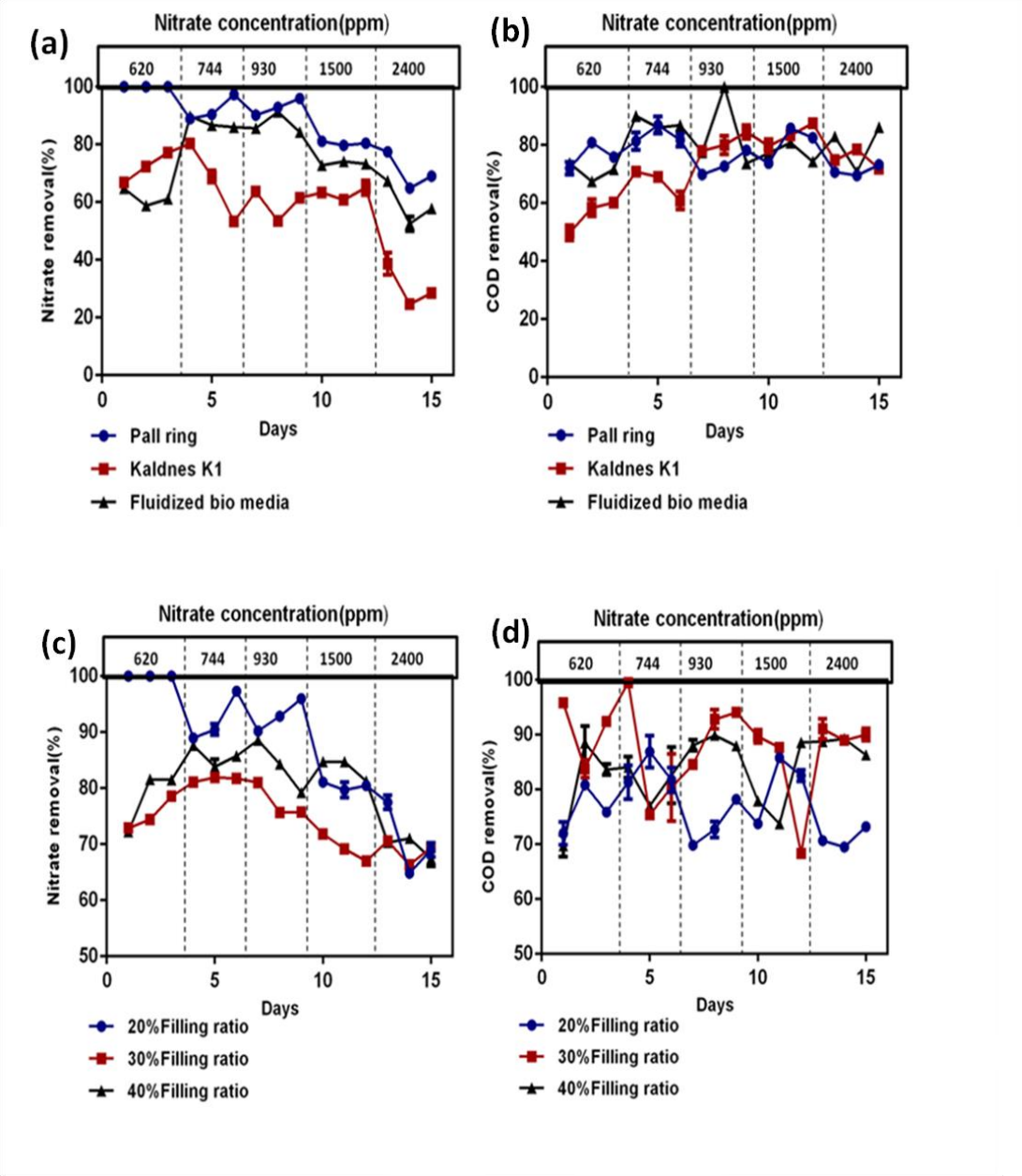

Figure 3.5 dMBBR studies with different carrier design and filling ratio.

(a) Nitrate removal studies with Pall ring, Kaldnes K1 and Fluidized biomedia carriers, (b) COD removal studies with Pall ring, Kaldnes K1 and Fluidized biomedia carriers, (c) Nitrate removal studies at 20 %, 30 % and 40 % of filling ratio, (d) COD removal studies at 20 %, 30 % and 40 % of filling ratio (n = 3) ($P < 0.05$)

3.3.2 Studies on kinetics of denitrification in dMBBR

Modified Stover-Kincannon model was applied to experimental results from the continuously operated MBBR for removal of nitrate from synthetic effluent and kinetic constants for denitrification were determined (Derakhshan et al., 2018).

The Stover Kincannon model considers the organic substance removal rate as a function of organic loading rate at steady state in Eq. (1).

$$\frac{ds}{dt} = Q/V(Si - Se) \quad \textbf{1}$$

This model for denitrifying MBBR is described as in Eq. (2).

$$\frac{ds}{dt} = \frac{Q(Si - Se)}{V} = \frac{Umax\left(\frac{QSi}{A}\right)}{KB + \left(\frac{QSi}{A}\right)} \quad \textbf{2}$$

Rearrangement of Eq. (2) gives the following relationship

$$\left(\frac{ds}{dt}\right)^{-1} = \frac{V}{Q(Si - Se)} = \frac{KBV}{UmaxQSi} + \frac{1}{Umax} \quad \textbf{3}$$

The model applied to the dMBBR and nitrate as substrate (mg L^{-1}.day) in the Eq. (3), where dS/dt is the substrate removal rate (mg L^{-1}.day), S is the reactor substrate concentration (mg L^{-1}.day); Umax is the maximum removal rate constant (mg L^{-1}.day) and KB is a saturation value constant (mg L^{-1}.day). If $(dS/dt)^{-1}$ is taken as V/Q (Si-Se) which is the inverse of the loading removal rate and this is plotted against the inverse of the total loading rate V/ (QSi), a straight line results. The intercept and slope of the line are 1/Umax and KB/Umax respectively.

The substrate balance for the reactor can be written as follows

$$QSi = QSe + V\left(\frac{ds}{dt}\right) \quad \textbf{4}$$

Relationship (4) for dS/dt can be substituted giving

$$QSi = QSe + \left(\frac{Umax(QSi/V)}{KB(QSi/V}\right)V \quad \textbf{5}$$

This expression can be solved for either the required volume of the denitrifying MBBR reactor or the effluent concentration.

$$V = \frac{QSi}{\left(\frac{UmaxSi}{Si - Se}\right) - KB} \quad \textbf{6}$$

$$Se = Si - \frac{UmaxSi}{KB + (QSi/V)} \quad \textbf{7}$$

Eq. (7) employed to calculate the outlet nitrate concentration at a given nitrate loading rate and influent concentration for the lab scale dMBBR.

Fig.3.6 indicates the relationship between predicted and observed effluent nitrate concentration in the developed dMBBR. There is a linear relationship between observed and predicted effluent nitrate concentrations with R^2 = 0.981 regression coefficient indicating that kinetic constants can be used in predicting effluent nitrate concentration of developed dMBBR. Fig. 3.6b illustrates the results of plotting the model graph i.e., inverse of specific substrate removal rate Q (Si-Se)/V versus inverse of total loading rate QSi/V for different nitrate loading. The kinetic constants KB and Umax can be estimated as 17.10 mg L^{-1}.day and 20.54 mg L^{-1}.day, respectively from Fig. 3.6 b. Experimental data applied at high correlation (R^2 of 0.96) to the model (Fig. 3.6) suggests that optimized dMBBR was efficient for nitrate removal.

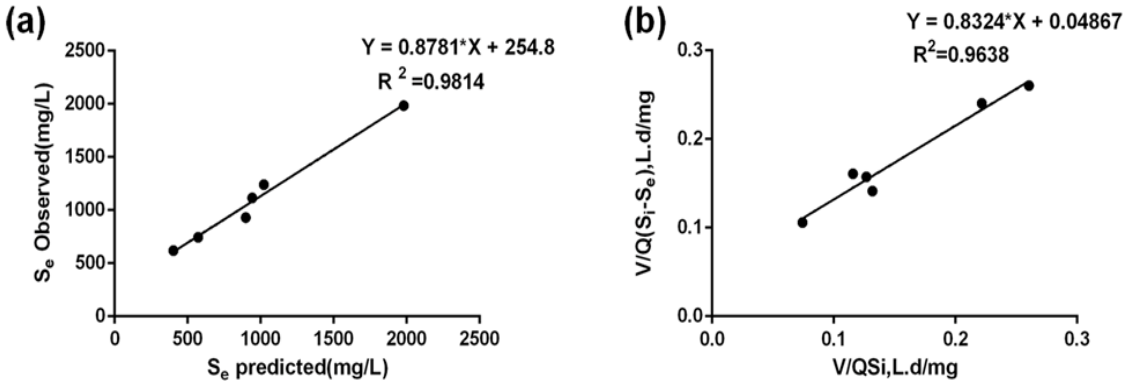

Figure 3.6 Stover-Kincannon model for denitrifying MBBR (a) Predicted, (b) Observed model

3.3.3 Comparative study between suspended growth reactor and dMBBR developed with consortium DC5

Comparative nitrate removal studies between optimized dMBBR developed individually with consortium DC5 and suspended growth reactor system with consortium DC5 as inoculum showed that the dMBBR yielded higher nitrate removal than suspended growth reactor (Fig.3.7). Compared to suspended growth reactor developed with consortium DC5, biofilm reactor developed with consortium DC5 showed better performance due to biomass accumulation and retention thereby giving higher reaction rate as, reported by Nicolella et al.,(2000) too. Biomass washout in suspended growth reactor was a strong reason why its performance was poorer than the dMBBR. The results here are similar to Falas et al., (2012) and Mazioti et al., (2015) who also demonstrated that biomass in moving bed biofilm carriers have a higher pollutant removal capacity potential than biomass in the suspended growth reactor system. Chao et al., (2016) reported that the diversity and abundance of nitrifiers and denitrifiers were more in biofilm reactor than in suspended growth system of activated sludge which also increased nitrogen removal ability.

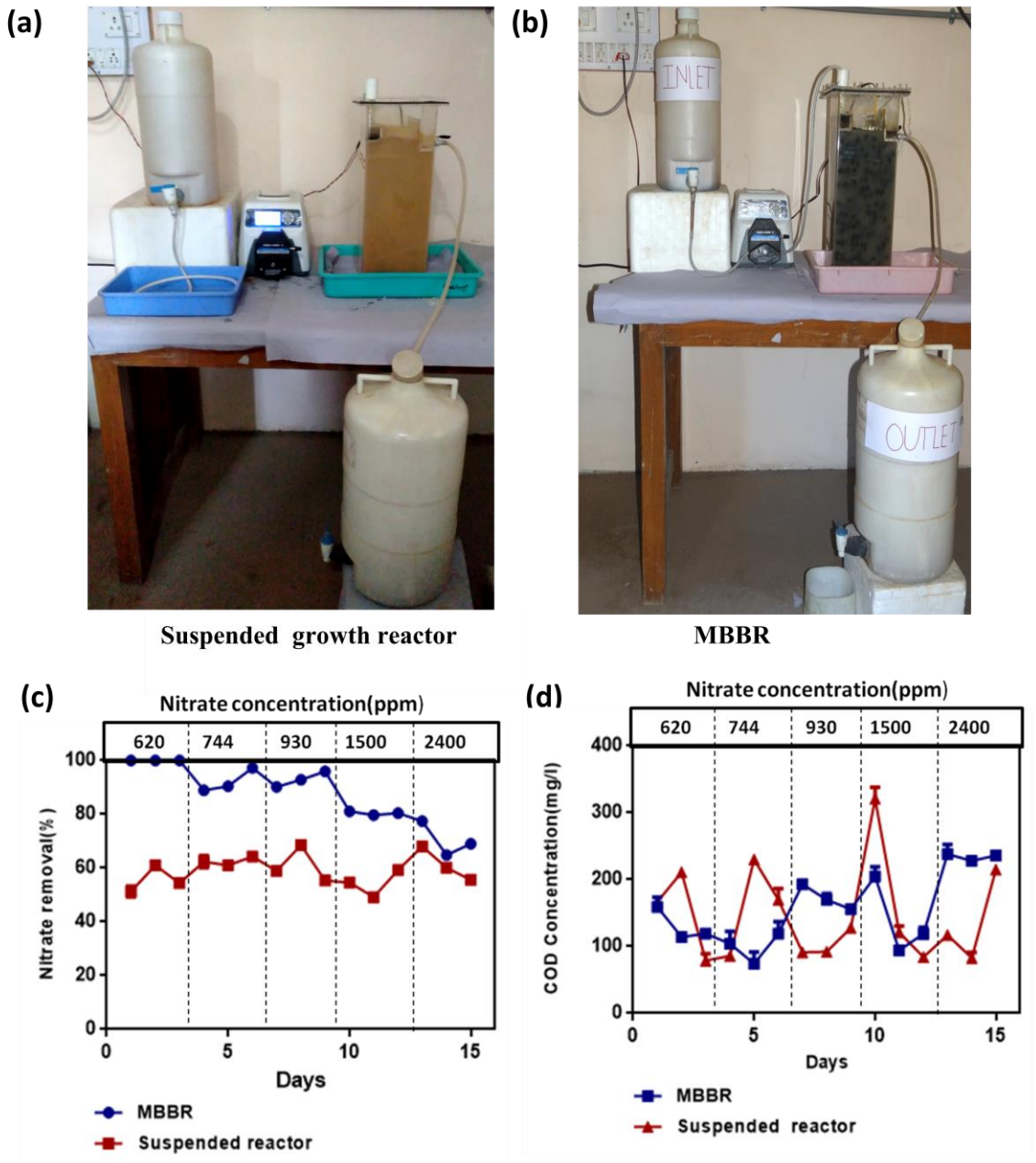

Figure 3.7 Comparative studies between suspended growth reactor and dMBBR
(a) Suspended growth reactor (b) Biofilm reactor (c) Nitrate removal (n = 3) ($P < 0.05$), (d) COD removal (n = 3) ($P > 0.05$)

3.3.4 Comparative studies between activated sludge and dMBBR inoculated with consortium DC5

Comparative studies were carried out between reactor developed (inoculated) with activated sludge sample and reactor developed with consortium DC5 where it was observed that reactor developed with consortium showed denitrification efficiency of 100 %, 92.25 %, 93.02 %, 80.43 %, 72.23 %, 70.45 % whereas dMBBR inoculated with activated sludge 56 %, 70.2 %, 64.1 %, 73 % and 53 % at 620, 744, 930, 1116, 1500 and 2400 mg L^{-1} of NO_3^- respectively. COD was below permissible range in both reactors. Results suggested that dMBBR inoculated with functional bacteria (biofilm forming denitrifying bacteria in the present case) improved the nitrate removal efficiency compared to dMBBR inoculated with activated sludge. Similar results were obtained by Zhang et al., (2020b) who reported that MBBR inoculated with HN-AD bacteria as the inoculum shortened the start-up time and improved TN removal. EPS components of biofilm developed on carriers showed that dMBBR developed with consortium DC5 showed higher EPS compared to dMBBR inoculated with activated sludge (Table 3.3). The activated sludge seed showed biomass of 24.9 mg/carrier whereas consortium DC5 showed high biomass i.e. 35 mg/carrier indicating that biofilm formed on carriers from consortium bacteria had a better biomass building ability possibly due to biofilm forming attribute. Thus functional bacteria had proven to be more effective in effluent treatment due to less loss of biomass.

3.3.5 Comparative studies between control reactor (without inoculum) and dMBBR developed with consortium DC5

In a similar study conducted control reactor performance was attributed to less biomass and lower functional bacterial abundance (Tao and Hamouda, 2020). In the present studies too it may be due to the many competitive bacteria present in the environment competing for the nutrient and having different metabolic capacity than denitrifiers. As a result they do not allow the specific denitrifiers to develop in the biofilm and at the same time they also utilize nutrients.

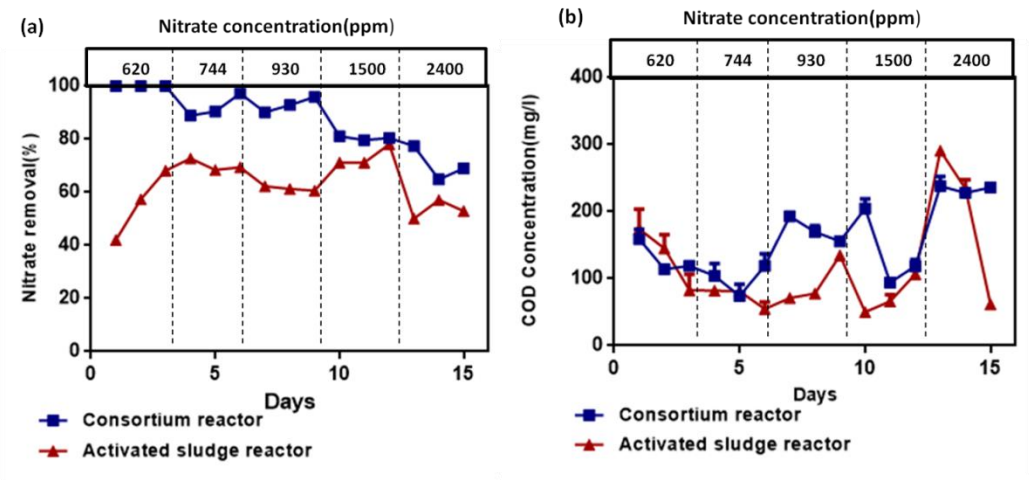

Figure 3.8 Comparative studies between MBBR developed with consortium DC5 and MBBR developed with activated sludge
(a) Nitrate removal (n = 3) ($P < 0.05$), (b) COD removal (n = 3) ($P > 0.05$)

Table 3.3 EPS components analysis

Components	Carriers developed with consortium DC5			Carriers developed with activated sludge		
Carbohydrate (mg L^{-1})	2.84	4.45	2.92	1.0	1	1.5
Protein (mg L^{-1})	180	187	186.5	100	100	105
Lipid (mg L^{-1})	0.13	0.10	0.93	0.10	0.10	0.15
Amyloid (mg L^{-1})	40	45	49	1.2	4.96	8.36
Biomass (mg/carrier)		35			24.9	

Continuous reactor studies carried out without addition of any inoculum under unsterile condition termed as a control reactor and dMBBR developed with consortium DC5 showed differences in performance. dMBBR developed with consortium DC5 was able to reduce nitrate 100 %, 92.25 %, 93.02 %, 80.43 %, 72.23 % and 70.45 % whereas control reactor showed 38 %, 59 %, 60 %, 65 %, 61 % from 620, 744, 930, 1116, 1500 and 2400 mg L^{-1} , respectively.COD level was below permissible range for both the reactors (Fig.3.9). Scanty biofilm formation was observed in the carriers of control reactor but suspended growth of microorganism was observed with turbidity of 30-100 NTU. This suspended growth of organisms might be the reason for the reduction of nitrate and COD

in the control MBBR. Addition of sodium acetate as external organic carbon source and nitrate under the oxygen-limited condition resulted in the sustained growth of organism in the reactor which reduced nitrate and COD in the control MBBR. Overall results of this experiment suggested that addition of denitrifying microorganisms in the MBBR increased biomass and nitrate removal efficiency compared to control reactor under optimized conditions.

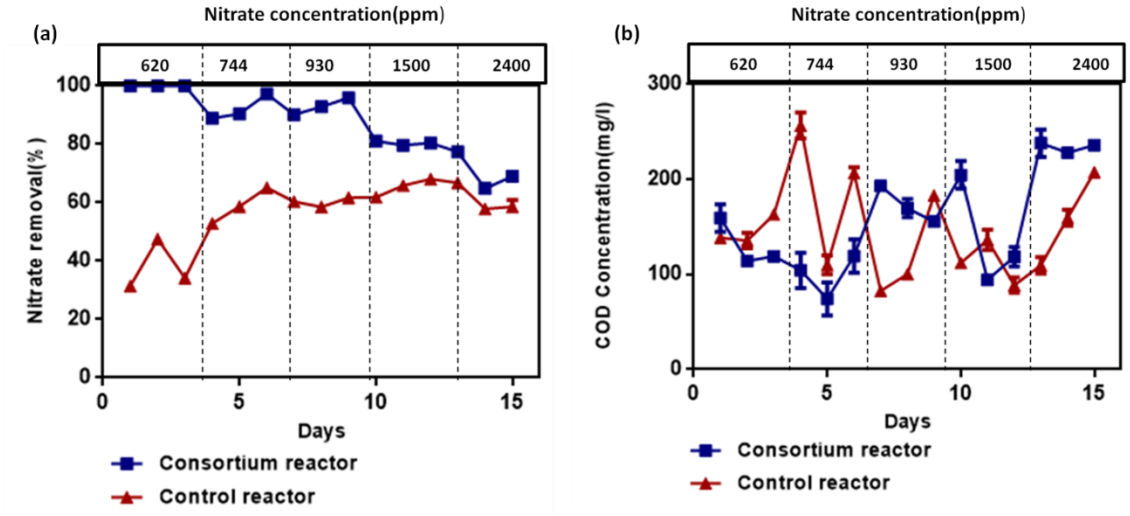

Figure 3.9 Comparative studies between dMBBR developed with consortium DC5 and control dMBBR. (a) Nitrate removal (n = 3) ($P < 0.05$), (b) COD removal (n = 3) ($P > 0.05$).

Comparative studies between dMBBR developed with consortium DC5, suspended growth reactor,dMBBR inoculated with consortium DC5 and control MBBR (i.e. without inoculum) showed that dMBBR inoculated with consortium DC5 showed highest nitrate removal efficiency in biofilm reactor (i.e.100 %) followed by and suspended rector (60 %) in the suspended reactor (Table 3.4). This suggests that bioaugmentation of consortium DC5 increased nitrate removal efficiency in dMBBR compared to control MBBR or MBBR developed with activated sludge.

Table 3.4 Comparative studies between suspended growth reactor, MBBR inoculated with activated sludge and control MBBR (i.e. without inoculum)

Reactor	Nitrate removal (%)	COD
dMBBR with consortium DC5	100 % at 620 mg L^{-1}	Below permissible range (i.e. 250 mg L^{-1})
Suspended growth reactor inoculated with consortium DC5	60 % at 620 mg L^{-1}	Below permissible range (i.e. 250 mg L^{-1})
MBBR developed with activated sludge	56 % at 620 mg L^{-1}	Below permissible range (i.e. 250 mg L^{-1})
Control MBBR	38 % at 620 mg L^{-1}	Below permissible range (i.e. 250 mg L^{-1})

3.3.6 Potential of denitrifying MBBR developed with consortium DC5 in treatment of nitrate containing effluents from different industries

MBBR has proven to be very suitable for the removal of nitrogen and treatment of industrial effluents. Nitrate removal studies from different industrial effluents were carried out with consortium DC5 in dMBBR. Pharmaceutical, Dye and domestic effluents collected from the industries were analysed and the table 3.5 depicts the characteristics of the effluents relevant to the studies.

Table 3.5 Industrial effluents characteristics

Characteristics	Dye industry effluent	Pharma industry 1 effluent	Pharma industry 2 effluent	Domestic wastewater	Pharma industry 3 effluent
Color	Black	Dark brown	Yellow	Colorless	Colorless
pH	13	7.5	8.5	7	8
Turbidity (NTU)	166	11	10	205	542
COD (mg L^{-1})	13,351	7000	870.6	790	800
Nitrate (mg L^{-1})	500	176	106	200	80
Nitrite (mg L^{-1})	0	0.177	0	0	0
Ammonia (mg L^{-1})	0	0	0	0	0

3.3.6.1 Dye industry effluent

Nitrate removal studies were carried out with high COD containing dye industry effluent in continuous dMBBR developed with consortium DC5. It showed 75 % nitrate reduction and 60 % COD reduction within 3h of HRT (Fig. 3.10 a, b). DO was below 1.5 and pH 8.5 was maintained inside the dMBBR. Nitrite accumulated in the dMBBR was 0.5 - 0.8 mg L^{-1} (below permissible range) and no ammonia was detected in the reactor.

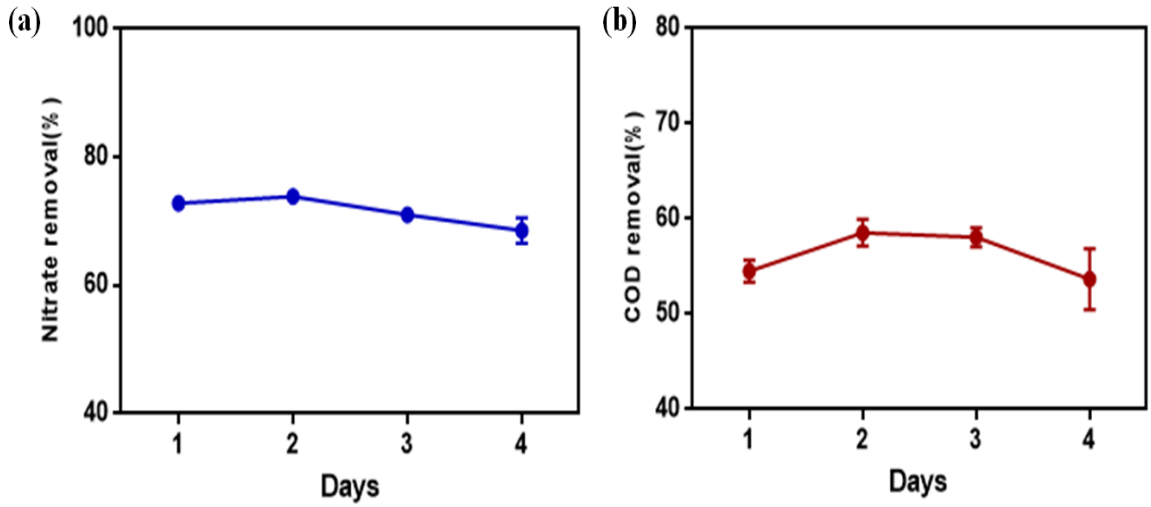

Figure 3.10 (a) Nitrate and (b) COD removal studies from dye industry effluent. Error bars represent standard deviations (n=3).

3.3.6.2 Pharma industry 1 effluent

Treatability of pharma industry 1 effluent which had high COD with consortium DC5 showed 85 % reduction in nitrate and 60 % COD reduction within 3 h of HRT (Fig.3.11a, b). No nitrite and ammonia were accumulated inside the reactor and proper denitrification conditions (DO below 1.5 and pH 8.5) were maintained in the reactor. Even though the initial COD was high the COD reduction was 60 % in both dye and pharma industry I effluent.

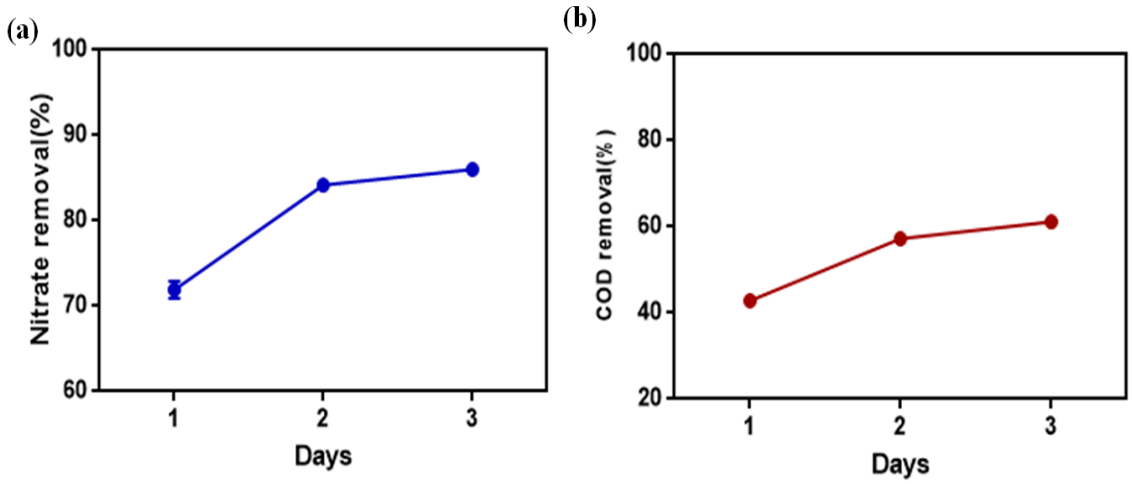

Figure 3.11 (a) Nitrate and (b) COD removal studies from pharma industry 1 effluent. Error bars represent standard deviations (n=3).

3.3.6.3 Pharma industry 2 effluent

Treatability of healthcare pharma industry effluent with consortium DC5 showed 100 % reduction in nitrate and 60 % COD reduction within 3h of HRT (Fig. 3.12 a, b). No nitrite and ammonia were accumulated inside the reactor and denitrification conditions (DO below 1.5 and pH 8.5) were maintained in the reactor.

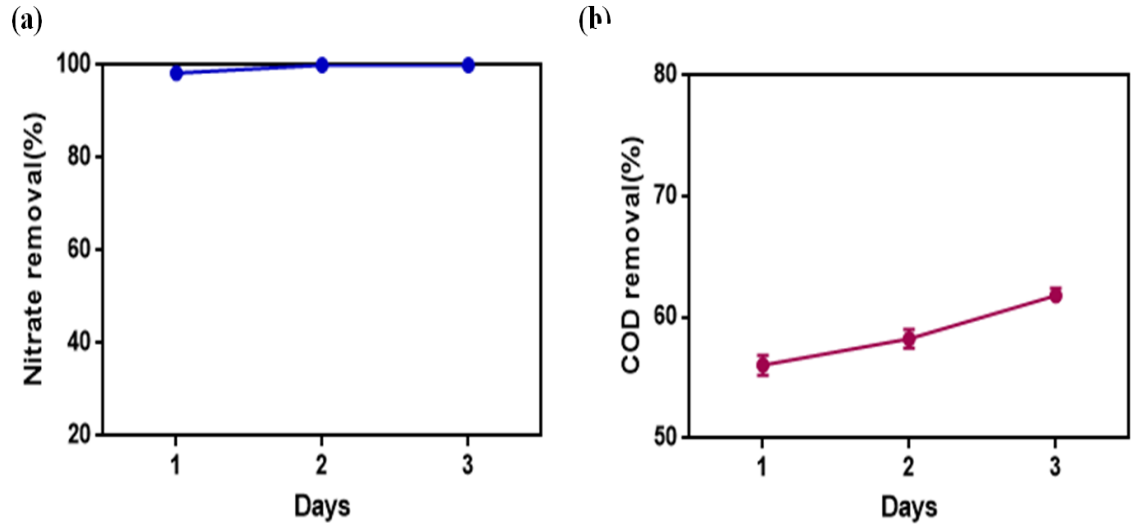

Figure 3.12 (a) Nitrate and (b) COD removal studies from pharma industry 2 effluent. Error bars represent standard deviations (n=3)

3.3.6.4 Pharma industry 3 effluent

Treatability of pharma industry 3 effluent with consortium DC5 showed 76 % reduction in nitrate and 69 % COD reduction within 3 h of HRT (Fig.3.13 a, b). No nitrite and ammonia were accumulated inside the reactor and denitrification conditions (DO below 1.5 and pH 8.5) were maintained in the reactor.

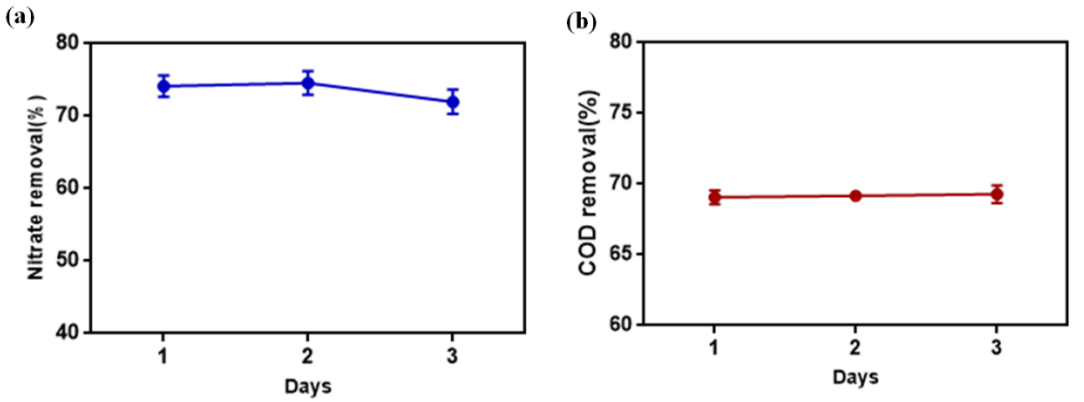

Figure 3.13 (a) Nitrate and (b) COD removal studies from pharma industry 3 effluent. Error bars represent standard deviations (n=3).

3.3.6.5 Domestic wastewater spiked with nitrate

Treatability of domestic wastewater spiked with nitrate with consortium DC5 showed 80 % reduction in nitrate and around 80 % COD reduction within 3h of HRT (Fig.3.14 a, b). No nitrite and ammonia were accumulated inside the reactor and denitrification conditions (DO below 1.5 and pH 8.5) were maintained in the reactor.

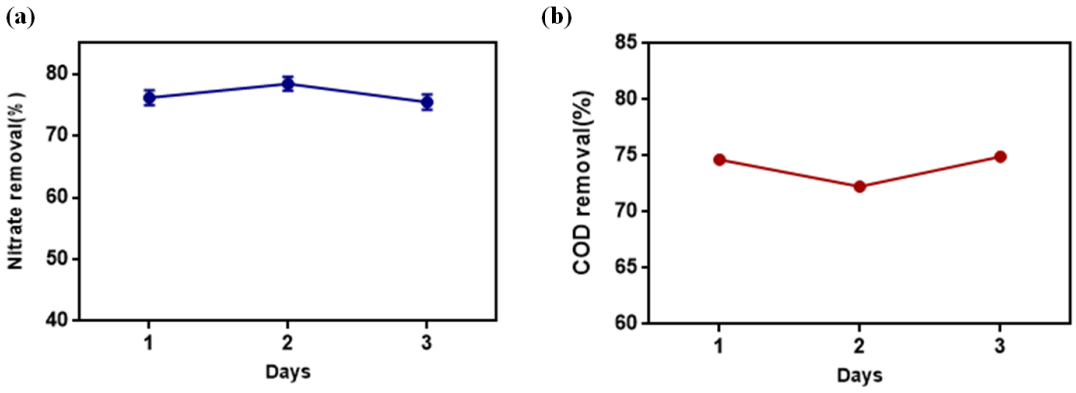

Figure 3.14 (a) Nitrate and (b) COD removal studies from domestic wastewater spiked with nitrate. Error bars represent standard deviations (n=3).

EPS component analysis of biofilm developed inside dMBBR with consortium DC5 showed that protein was the major component in all the biofilms. Biofilm biomass was varying in the biofilm of different effluents. Dye industry biofilm contained highest EPS compared to biofilms developed in other effluents (Table 3.6). However, in case of carrier all the effluents biofilm showed presence of all the EPS components on their carrier suggesting that dMBBR developed with consortium DC5 is suitable for the treatment of different industrial effluents.

Table 3.6 EPS components analysis

EPS Components	Dye industry effluent	Pharma industry	Pharma industry 2	Pharma industry 3	Domestic wastewater
Biomass(mg/carrier)	40	35	29	32	29
Carbohydrate (mg L^{-1})	6	4	3.5	4	6
Protein (mg L^{-1})	177	175	140	130	170
Lipid (mg L^{-1})	6.9	6	6.7	7	4

Table 3.7 Treatment of different industrial effluents with consortium DC5 in dMBBR

Effluents	Nitrate removal (%)	COD removal (%)
Dye industry	75 %	60 %
Pharma industry 1	85 %	60 %
Pharma industry 2	100 %	60 %
Pharma industry 3	76 %	69 %
Domestic wastewater spiked with nitrate	80 %	80 %

Overall, treatability studies of different industrial effluents showed that consortium DC5 was able to remove nitrate from dye, pharma and domestic wastewater in developed dMBBR. Highest nitrate removal was observed in pharma and domestic wastewater compared to dye industry wastewater (Table 3.7). Highest COD removal was achieved in

the domestic wastewater spiked with nitrate as compared to pharma and dye industry wastewater. This might be due to the presence of easily degradable carbon sources in domestic wastewater compared to pharma and dye industry wastewater.

The acetate-fed dMBBR inoculated with specially constructed consortium as seed of biofilm forming denitrifying bacteria performed with high efficiency for 300 days of long term operation. Its performance was enhanced with further optimization of the operational parameters. It gave superior performance in comparative studies with suspended growth reactor and MBBR inoculated with a non-specific seed such as activated sludge and unseeded control reactor. Further performance evaluation of consortium DC5 in treatment of effluents from pharma and dye industries revealed that its versatility and robustness in nitrate removal from effluents of varied composition.