

Nitrogen and carbon removal efficiency of a polyvinyl alcohol gel based moving bed biofilm reactor system

Khalid Muzamil Gani, Jasdeep Singh, Nitin Kumar Singh, Muntjeer Ali, Vipin Rose and A. A. Kazmi

ABSTRACT

In this study, the effectiveness of polyvinyl alcohol (PVA) gel beads in treating domestic wastewater was investigated: a moving bed biofilm reactor (MBBR) configuration (oxic-anoxic and oxic) with 10% filling fraction of biomass carriers was operated in a continuously fed regime at temperatures of 25, 20, 15 and 6 °C with hydraulic retention times (HRTs) of 32 h, 18 h, 12 h and 9 h, respectively. Influent loadings were in the range of 0.22–1.22 kg N m⁻³ d⁻¹ (total nitrogen (TN)), 1.48–7.82 kg chemical oxygen demand (COD) m⁻³ d⁻¹ (organic) and 0.12–0.89 kg NH₄⁺-N m⁻³ d⁻¹ (ammonia nitrogen). MBBR performance resulted in the maximum TN removal rate of 1.22 kg N m⁻³ d⁻¹ when the temperature and HRT were 6 °C and 9 h, respectively. The carbon removal rate at this temperature and HRT was 6.82 kg COD m⁻³ d⁻¹. Ammonium removal rates ranged from 0.13 to 0.75 kg NH₄⁺-N m⁻³ d⁻¹ during the study. Total phosphorus and suspended solid removal efficiency ranged from 84 to 98% and 85 to 94% at an influent concentration of 3.3–7.1 mg/L and 74–356 mg/L, respectively. The sludge wasted from the MBBR exhibited light weight features characterized by sludge volume index value of 185 mL/g. Experimental data obtained can be useful in further developing the concept of PVA gel based wastewater treatment systems.

Key words | efficiency, low temperature, moving bed biofilm reactor, nitrogen mass balance, PVA gel

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INTRODUCTION

Many systems have been developed for the treatment of domestic wastewater, and the moving bed biofilm reactor (MBBR) has emerged as one of the promising biofilm based technologies (Odegaard 2006). MBBR systems are gaining good interest among designers and treatment facility engineers because of the low space requirements (low cost factor), ample concentration of relevant microorganisms at desired treatment point and greater biomass liquid separation responsible for a simple and cheaper final separation technique. The immobilization maintains the high biomass concentration, leading to better rates of nitrification and organic removal (Rostron *et al.* 2001). Apart from the factors like pH (Jaroszynski *et al.* 2011), temperature and loading rates (Zhang *et al.* 2014), the treatment process in these systems depends upon the properties of the media used as biomass carriers in the process (Bengtsson *et al.* 2008). Large specific weight of media requires more energy to remain in suspension and lower specific volume of media leads to more available space for wastewater in the reactor. Numerous biomass carriers are available on the market, and until now the most

commonly used is Kaldness (K1). Recently, polyvinyl alcohol (PVA) gel has been proven effective in growth and higher enrichment of bacteria (Kuraray 2005). The PVA gel is a 4 mm spherical bead with a network of 10–20 micron pores crossing through the bead, which allows cultivation of bacteria in a sheltered mode and thus reduces sloughing of biomass (Kuraray 2005; Hoa *et al.* 2006; Gani *et al.* 2014; Singh *et al.* 2016). Due to its better fluidity, minimum energy is consumed for mixing. The larger porosity of gel beads favors better supply of oxygen and carbon to residing bacteria, resulting in stable treatment under variable loading. Volumetric packing ratios of the beads are typically 5–15%, which is much less compared with other carriers (50–70% usually) (Levstek *et al.* 2010). PVA gel displayed better performance as an immobilization medium by providing higher nitrification rates, with less bulk, thereby occupying little space in the reactor (Rostron *et al.* 2001).

Until now, numerous studies have been done for investigating the effect of temperature and hydraulic loading on performance of MBBRs generally having K1 or similar media

as biomass carrier (Dulkadiroglu *et al.* 2005; Bassin *et al.* 2012; Zhang *et al.* 2014). Therefore, the aim of this study was performance evaluation of a MBBR having PVA gel as biomass carrier with a 10% filling fraction, treating real municipal wastewater at different hydraulic retention times (HRTs) and temperatures. The performance was assessed in terms of removal of organic matter, nitrogen, phosphorus and suspended solids. Morphology of the biomass present in the sludge was identified by using scanning electron microscopy (SEM). Settling features of the sludge produced from the treatment process were also identified.

MATERIALS AND METHODS

Reactor configuration and operating conditions

A pilot-scale MBBR reactor (Figure 1) consisting of three cuboidal tanks of 10 L effective capacity, connected in series, followed by a sedimentation tank of 5 L volume was installed at the municipal sewage treatment plant, Habak, Srinagar, India. Complete mixing in the tanks was ensured by means of a central, four-blade single stirrer of 30 cm diameter with blades placed near the bottom. The treatment scheme included a nitrification tank (dissolved oxygen (DO) = 6.5–7 mg/L), an anoxic tank, and a secondary aerobic tank (DO = 5–6 mg/L) followed by a sedimentation tank. Methanol (2.88 mL/day) was added as an external carbon source in the anoxic tank because of the relatively lower biological oxygen demand (BOD) of wastewater prior to sedimentation. Poly aluminum chloride (PACl) was added (6 g/day) for phosphorus precipitation. The fraction of gel beads used in all three tanks was 10% (1 litre). Suspension of media was carried out by aeration in the first and third reactors and by a two-blade mixer in the anoxic tank. The temperature in the reactors was controlled by automatic heaters installed in each reactor. Maximum and minimum ambient temperatures at the study location were 15 °C and –3 °C, which made it

feasible to carry out the batch study at desired temperatures with automatic heaters. The period of operation of the pilot plant was from 25 October 2013 to 25 January 2014.

Analysis

DO and temperature were measured in reactors by a portable DO meter (Hach 110Q multimeter, Hach, USA). pH was measured with a portable pH meter (HQ11d pH Meter, Hach). Flow was measured daily by visual measurement with cylinder and timer. Grab samples of 1 L were taken from the raw wastewater tank and at the outlet of the sedimentation tank. The grab samples were analyzed for chemical oxygen demand (COD), BOD, total suspended solids (TSS), ammonia, nitrate and phosphorus according to *Standard Methods* (APHA 1998).

Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) of the wasted sludge were investigated according to *Standard Methods* (APHA 1998).

NITROGEN MASS BALANCE

The equations mentioned in this section were used to calculate the nitrogen mass balance of the system. Equation (1) was used to calculate mass of nitrogen in the influent per day (M_{inTN}). Influent total nitrogen (TN) in each phase was calculated on the basis of flow (Q_{in}) and content of nitrogen in influent (TN_{in}). The TN in influent was calculated as the sum of all individual forms of nitrogen, which were total Kjeldahl nitrogen (TKN) and nitrate.

$$\text{Input TN } (M_{inTN}) = Q_{in} TN_{in} \dots \dots \dots (1)$$

The fate of the influent nitrogen in the system was that some portion of nitrogen ($M_{DEN TN}$) was removed in the denitrification process and some portion was contained in wasted

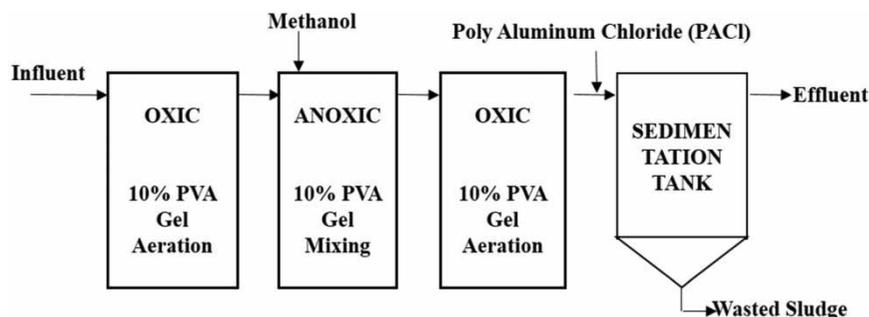


Figure 1 | Schematic of the pilot plant used in the study.

sludge (M_{wasteTN}), and the rest remained in the effluent ($M_{\text{out TN}}$). Therefore, total output nitrogen (output TN) can be estimated as the sum of these three masses (Equation (2)).

$$\text{Output TN} = M_{\text{DEN TN}} + M_{\text{waste TN}} + M_{\text{out TN}} \dots \dots \dots (2)$$

The influent nitrogen present in the form of ammonia was nitrified in the oxic tank I. The portion of ammonia which was not nitrified came out of the system in effluent. This nitrate from nitrified ammonia and nitrate already present in influent was denitrified in the anoxic tank. The remaining amount of nitrate which was not denitrified came out of the system with the effluent. Equation (3) was used to calculate the total amount of nitrogen used in the process. The mass of nitrogen consumed was therefore calculated as the sum of the difference in influent ammonia and nitrate, after assuming nitrite as a byproduct which was produced and converted to nitrate in the mainstream.

$$M_{\text{DEN TN}} = Q_{\text{in}} (\text{NH}_{4\text{in}} - \text{NH}_{4\text{out}}) + Q_{\text{in}} (\text{NO}_{3\text{in}} - \text{NO}_{3\text{out}}) \dots \dots (3)$$

To determine the nitrogen content in wasted sludge, a value for fraction of nitrogen (f) present in sludge was assumed. The recommended value for a series of sludge ages is 0.1 (Lee *et al.* 2007). Using this, the mass of nitrogen in wasted sludge ($M_{\text{waste TN}}$) was estimated according to Equation (4). A similar approach for calculating the mass of nitrogen in wasted sludge was used by Lee *et al.* (2008).

$$M_{\text{wasteTN}} = Q_{\text{waste}} \text{MLVSS } f \text{ TN}_{\text{out}} \dots (4)$$

where MLVSS is the volatile solid content (mg/L) in the wasted sludge.

In effluent, the TN was calculated in the same way as it was calculated for influent.

$$M_{\text{out TN}} = Q_{\text{out}} \text{TN}_{\text{out}} \dots (5)$$

The percentage of nitrogen balance achieved was finally obtained by the ratio of output nitrogen and input nitrogen according to Equation (6).

$$\% \text{Nitrogen balance} = \frac{\text{OUTPUT TN}}{\text{INPUT TN}} \times 100 \dots (6)$$

SEM analysis

For the analysis by SEM, the preparation of samples was done according to Calderon *et al.* (2011) with slight

modifications. Biomass samples were filtered, and the retained content on filter paper was dried and fixed with glutaraldehyde solution (5% v/v), washed and dehydrated with ethanol solution. The samples were coated with gold and analyzed under a scanning electron microscope (LEO 435VP SEM, SEMTECH Solutions, India).

Statistical analysis

In order to analyze the significance of differences in various phases, Kruskal–Wallis test was performed for COD, BOD, ammonia and $\text{PO}_4^{2-}\text{-P}$ using SPSS[®] statistics (version-20.0). The results were presented in the form of Kruskal–Wallis test statistic (χ^2) and p -value < 0.05 .

RESULTS AND DISCUSSION

MBBR performance

Carbon removal (COD and BOD)

Once the pilot plant surpassed the startup phase, experiments were carried out by varying the HRT and temperature. The HRT was changed from 32 h to 9 h and the temperature was changed from 20 °C to 6 °C in subsequent experimental phases (Table 1). The variation in HRT was accompanied with change in COD loading rate. The range of COD loading rate during the study was 1.48 ± 0.62 to 7.82 ± 1.10 kg COD $\text{m}^{-3} \text{d}^{-1}$. The results obtained from the analysis of physico-chemical parameters are summarized in Table 2 and the variation in COD loading during different phases is shown in Figure 2. However, the organic loadings in Table 2 and Figure 2 are with reference to influent COD, because COD due to methanol and its removal was an intermediate process in the pilot plant. Moreover, the calculations of methanol requirement were already consistent with complete removal of COD due to methanol. The first phase was the startup phase operated at 25 ± 0.38 °C for 13 days. The average removal efficiency of COD was lowest ($79.7 \pm 3\%$) in this phase as compared with other phases ($\chi^2 = 28.9$; $P = < 0.01$). This might be due to the obvious reason of acclimatization of microorganisms in the startup phase. BOD removal efficiency was also lowest in the startup phase as compared with other phases ($\chi^2 = 30.9$ and $P = < 0.001$). During phase I and phase II, the pilot plant was operated at HRT of 32 ± 0.15 hours and the reactor temperature was 20 °C and 15 °C, respectively. Organic loading rate in these phases was 1.69 ± 0.7 kg

Table 1 | Pilot plant operating conditions

Phase	Startup	I	II	III	IV	V	VI
Days	13	15	13	10	10	13	12
Temperature (°C)	25 ± 0.378	20 ± 0.671	15 ± 2.529	15 ± 2.529	6 ± 0.556	6 ± 0.556	6 ± 0.556
Flow (L/day)	22.5 ± 1.06	22.5 ± 1.06	22.5 ± 1.06	42.9 ± 2.04	42.9 ± 2.09	59.4 ± 3.87	81.3 ± 2.81
HRT (h)	32 ± 0.15	32 ± 0.15	32 ± 0.15	18 ± 0.2	18 ± 0.2	12 ± 0.34	9 ± 0.51

COD $\text{m}^{-3} \text{d}^{-1}$ and $1.86 \pm 0.6 \text{ kg COD m}^{-3} \text{d}^{-1}$, respectively. The concentration of COD increased in effluent of pilot plant during initial days of phase II, which might be due to the sudden decrease in temperature, but on an average there was an enhanced removal of organic loading ($1.61 \pm 0.4 \text{ kg COD m}^{-3} \text{d}^{-1}$) in phase II as compared with phase I. In phase III, the HRT of the pilot plant was further decreased to 18 h by increasing the hydraulic loading rate to $42.9 \pm 2.04 \text{ L/day}$. The removed organic loading rate in this phase further increased to $2.58 \pm 0.5 \text{ kg COD m}^{-3} \text{d}^{-1}$ and average BOD removal efficiency was $92 \pm 5.5\%$. After that, the remaining three phases (IV, V and VI) were operated at 6°C and HRT was maintained as $18 \pm 0.2 \text{ h}$, $12 \pm 0.34 \text{ h}$ and $9 \pm 0.5 \text{ h}$, respectively. The incoming organic loading rate was maximum as $7.82 \pm 1.1 \text{ kg COD m}^{-3} \text{d}^{-1}$ in phase VI. Irrespective of increased organic loading, there was a proportional increase in removal efficiency of COD during these last three phases. This revealed that PVA gel based MBBR may remove organic loadings larger than what was removed in phase VI, i.e. $6.82 \pm 0.8 \text{ kg COD m}^{-3} \text{d}^{-1}$. In addition to this, the decrease in removal efficiency of total COD (TCOD) was only 3% when the

temperature was decreased to $6 \pm 0.56^\circ\text{C}$ in phase IV. This slight decrease showed the performance stability and adaptation characteristics of PVA gel based MBBR against temperature change. This feature in the PVA gel beads may be due to the entrapped microbial biomass system that acts as a shield and prevents the impacts of temperature on the activity of microorganisms. It is also worthwhile to highlight here that the COD and BOD levels in effluent were somewhat lower than the discharge limits prescribed by Indian standards, which are 150 mg/L and 30 mg/L, respectively (NRCD 2005). In spite of the variability in incoming BOD characterized by a maximum of 187 mg/L on the 87th day, the effluent concentration remained almost constant, with an average concentration ranging from 9 to 13 mg/L during the study.

The maximum organic loading to the reactor was $7.82 \pm 1.1 \text{ kg COD m}^{-3} \text{d}^{-1}$ in this study when the temperature was 6°C . The maximum removal rate of $6.82 \pm 0.8 \text{ kg COD m}^{-3} \text{d}^{-1}$ was also observed in the same phase. Before this, the performance of PVA gel beads containing MBBR was studied at organic loading rate of $4.22 \text{ kg COD m}^{-3} \text{d}^{-1}$ and $2.14 \text{ kg COD m}^{-3} \text{d}^{-1}$ at 21°C and

Table 2 | Summary of the performance parameters of the pilot plant in various phases (\pm is standard deviation)

Phase	Startup	I	II	III	IV	V	VI
COD loading ($\text{kg COD m}^{-3} \text{d}^{-1}$)	1.48 ± 0.6	1.69 ± 0.7	1.86 ± 0.5	3.08 ± 0.7	2.18 ± 0.5	4.39 ± 0.6	7.82 ± 1.1
COD removal rate ($\text{kg COD m}^{-3} \text{d}^{-1}$)	1.22 ± 0.5	1.47 ± 0.6	1.61 ± 0.4	2.58 ± 0.5	1.77 ± 0.4	3.64 ± 0.5	6.82 ± 0.8
Ammonia loading ($\text{kg NH}_4^+\text{-N m}^{-3} \text{d}^{-1}$)	0.14 ± 0.1	0.15 ± 0.1	0.12 ± 0.02	0.27 ± 0.07	0.20 ± 0.1	0.62 ± 0.23	0.89 ± 0.2
$\text{NH}_4^+\text{-N}$ removal rate ($\text{kg NH}_4^+\text{-N m}^{-3} \text{d}^{-1}$)	0.11 ± 0.1	0.14 ± 0.09	0.13 ± 0.1	0.25 ± 0.07	0.18 ± 0.05	0.56 ± 0.2	0.75 ± 0.17
TN loading rate ($\text{kg N m}^{-3} \text{d}^{-1}$)	0.29 ± 0.01	0.25 ± 0.08	0.21 ± 0.03	0.51 ± 0.01	0.33 ± 0.05	0.92 ± 0.14	1.32 ± 0.12
TN removal rate ($\text{kg N m}^{-3} \text{d}^{-1}$)	0.26 ± 0.11	0.22 ± 0.08	0.18 ± 0.03	0.47 ± 0.07	0.27 ± 0.05	0.87 ± 0.14	1.22 ± 0.12
SS loading ($\text{kg SS m}^{-3} \text{d}^{-1}$)	0.56 ± 0.04	0.65 ± 0.05	0.97 ± 0.32	2.00 ± 0.34	1.84 ± 0.31	3.7 ± 1.10	9.49 ± 2.04
SS removal rate ($\text{kg SS m}^{-3} \text{d}^{-1}$)	0.44 ± 0.09	0.58 ± 0.02	0.95 ± 0.13	1.96 ± 0.33	1.79 ± 0.31	3.65 ± 0.61	9.35 ± 3.12
Influent $\text{NO}_3^- \text{-N}$ (mg/L)	2.2 ± 0.36	2.3 ± 0.26	2.4 ± 0.46	1.8 ± 0.48	2.0 ± 0.58	2.5 ± 0.48	1.8 ± 0.61
Effluent $\text{NO}_3^- \text{-N}$ (mg/L)	1.6 ± 0.24	1.7 ± 0.17	1.6 ± 0.19	1.3 ± 0.19	1.4 ± 0.33	2.2 ± 0.42	1.3 ± 0.46
TKN removal (%)	91.2 ± 16.5	93.3 ± 4.5	90.4 ± 7.0	91.6 ± 9.6	88.6 ± 6.4	90.3 ± 4.6	84 ± 7.7
TP removal (%)	67.1 ± 23.4	86 ± 6.1	92.5 ± 8.8	94 ± 7.5	92.4 ± 12.4	85 ± 11.7	89.8 ± 6.4

Loadings are based on total volume of PVA gel.

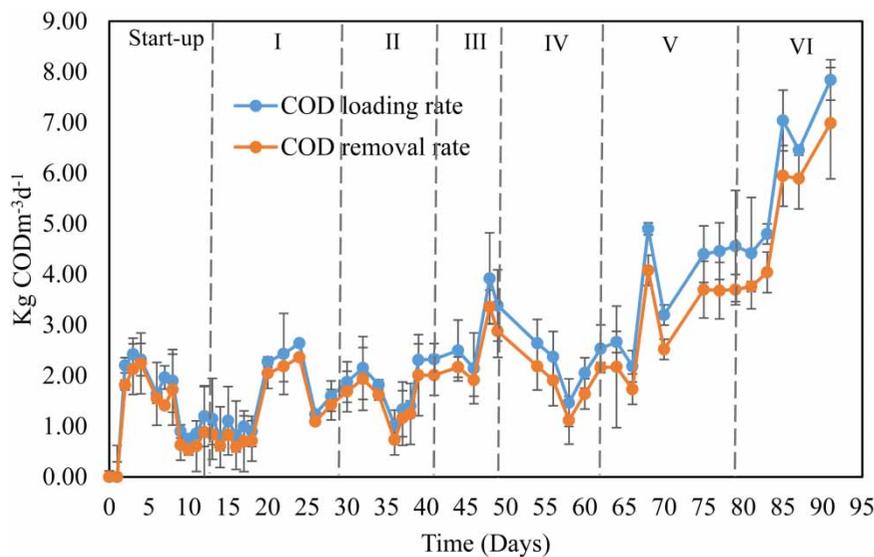


Figure 2 | Applied and removed COD loading rates during the study.

25 °C, respectively (Chen *et al.* 1998). The COD removal efficiency reported in these studies was >80%, which is in conformity with the obtained efficiency of 85% when the temperature was 20 ± 0.7 °C in our study.

Biomass in the beads was quantified by the difference in weight of fresh beads and used beads, and the average value was 304 mg volatile suspended solids (VSS)/g beads. The value was more than the 0.019 g VSS/g beads reported by Chen *et al.* (1998), which might be due to more organic loading in our study or to the difference in biomass measurement method. The COD loading rate in the reported study was 0.855–4.223 kg COD m⁻³ d⁻¹, and the measurement method was protein assay method which estimates the biomass content more precisely (Smith *et al.* 1985).

Nitrogen removal

The nitrogen removal efficiency of the pilot-scale MBBR in different phases is summarized in Table 2 and the variation of nitrogen loading is shown in Figure 3. Overall nitrification efficiency obtained in all phases was more than $80 \pm 3.4\%$. Phases I and II were statistically equivalent to each other ($\chi^2 = 0.34$ and $P = 0.6$) m which may be due to the relatively lower ammonia loading and quite favorable temperature of 20 ± 0.7 and 15 ± 2.5 °C.

Although the maximum ammonia removal efficiency should have been highest in phase I rather in phase VI because of the higher HRT and temperature in phase I, the results were contradictory. This may be because phase I was just after the startup period, due to which the ongoing

acclimatization might have affected the performance in this phase, while phase VI started after a long enough time period of 46 days from the startup phase, during which the growth of nitrifiers would have also increased. Moreover, the incoming loading during the initial phases (I–IV) was low (0.12 – 0.20 kg NH₄⁺-N m⁻³ d⁻¹), and the removal was in accordance with that.

For denitrification, anoxic conditions were developed in the second tank from the first day of operation of the pilot plant by feeding it with nitrified effluent from the first tank. The nitrate produced in the first tank along with nitrate present in the influent was denitrified in the second tank. For most of the period, the NO₃⁻-N concentration in effluent was lower than in influent (Table 2), which showed that the nitrate produced in the treatment stream and a portion (50%) of incoming nitrate was denitrified effectively. The concentration of NO₃⁻-N in effluent was higher than in influent on the 70th and 85th days of operation. These events occurred during phases V and VI, when the organic loading was large as compared with other phases for better denitrification. During the remaining phases, the effluent NO₃⁻-N concentration was always lower than influent NO₃⁻-N. The temperature in these operational phases was 6 °C; therefore, it might be the poor diffusion of substrate at low temperatures to the denitrifiers, which grew prolifically into the inner anoxic zones of the PVA gel beads (Chen *et al.* 1998), that caused the effluent concentration to be higher. In addition, the denitrification in tank 1 in the core of beads was very low as compared with tank 2 because of ongoing aeration. The contribution to denitrification in tank 1 was

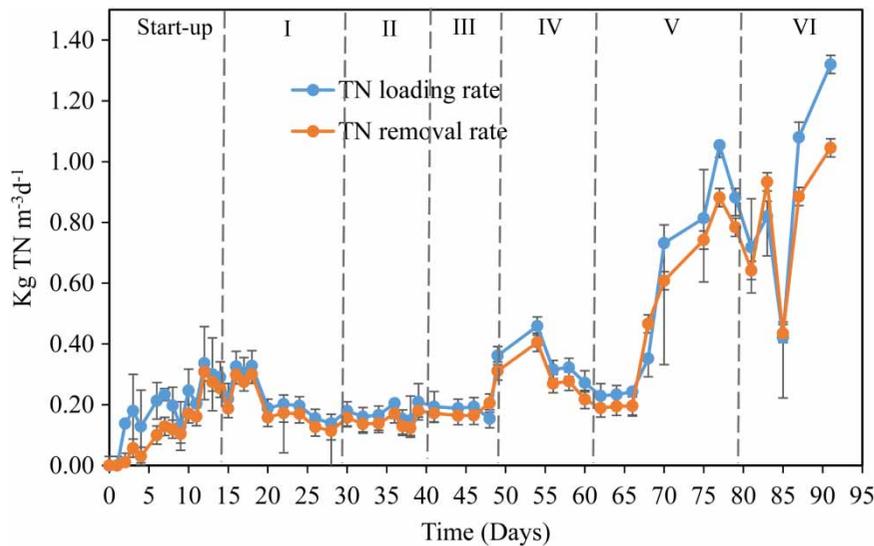


Figure 3 | Total nitrogen (TN) loading and removal rates during the study.

approximately 1–4%, and the maximum was observed in phase III, which may be a favorable temperature of 15 °C, which allows diffusion of substrate to the core of the beads. Although the increased organic loading in phase VI should also favor this denitrification because of more organic loading (making carbon source more available for nitrate), the low temperature in this phase might have decreased the diffusion of organic substrate in these beads, which lowers denitrification in tank I.

The performance stability is an important feature of immobilized biomass systems, especially in nitrification, because it is instantly affected by external stresses (Chang *et al.* 2005; Zhang *et al.* 2015). During all operational phases, the concentration of ammonia in influent changed over a wide range of 14.8 ± 3.8 to 33.5 ± 7.4 mg/L, but the removal efficiency was not affected too much. The variation in removal efficiency for all operational phases was only 84.2–93%. The filling fraction of biomass carriers in a biofilm process is one of the factors that decide cost and treatment performance of the treatment plant (Odegaard 2006). A lower fraction reduces the cost and allows large hydraulic loadings by leaving enough volume for wastewater in the reactor. The filling fraction of PVA gel used in this study was only 10% of reactor volume, and a nitrogen removal efficiency of 84–93% was observed during the whole study (Table 2). Mostly biomass carriers such as Kaldness (K1), Anox Kaldness (K3) and KMT[®] are reported at a filling fraction of 35–50% in literature (Rusten *et al.* 1995; Sabzali *et al.* 2012; Leyva-Diaz *et al.* 2013), with comparatively lower removal efficiency, ranging from 67 to 80%. This indicated that PVA gel may result as a cost reducing factor also in full-scale application.

Nitrogen mass balance

To find out the fate of nitrogen in the MBBR, nitrogen mass balance was done. Figure 4 shows the percentage of total influent nitrogen removed with the effluent, waste sludge and in the denitrification process during phases I–VI of the operational period. The influent nitrogen concentration ranged from 14.8 ± 3.8 to 33.5 ± 7.4 mg/L and the major species was ammonia. The major portion of the influent nitrogen was removed in the denitrification process. The percentage of influent TN removed by the denitrification process in phase I–VI was 64–71% (average 68.3%), and the removal percentage was approximately the same in all phases. This showed the biological nitrogen removal efficacy of the reactor system at each HRT and temperature. Nitrogen in the wasted sludge was calculated using Equation (4). The percentage of influent TN removed in it during phases I–VI was 12–18% (average 15%). This portion of nitrogen in sludge is also advantageous for agricultural purposes after using the stabilized sludge for soil improvement. The remaining portion of influent TN exits the system with the effluent, calculated by quantifying various forms of nitrogen present in it. This percentage was 11–16% (average 13%) of the influent TN. The accuracy of TN mass balance achieved in each phase was calculated as percentage of removed amount of nitrogen from influent TN. The results for phases 1–6 were 95, 98, 94, 98, 92 and 93%, respectively, which demonstrated that the fate of nitrogen in PVA gel based MBBR can be accounted for by mass balances close to 100% at different HRTs and temperature. The discrepancy of 2–8% in mass balance can be accounted for the

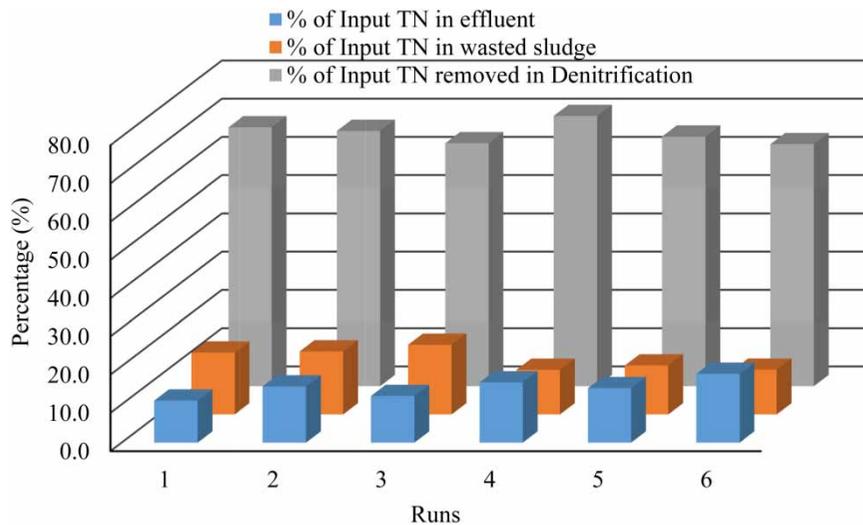


Figure 4 | Distribution of TN mass balance of pilot plant.

other nitrogen losses, such as incorporation of nitrogen into biomass, release of nitrous oxide and nitric oxide, and ammonia stripping (Rostron *et al.* 2001).

Phosphorus and solid removal

The phosphorus removal efficiency obtained in different phases is shown in Table 2. The chemical addition was started after one week of startup of operation of the pilot plant on observing the inefficient removal of phosphorus ($67 \pm 6.5\%$) during the startup phase. The results showed more than $85 \pm 5\%$ removal efficacy in the remaining phases. Since phosphorus was removed chemically by addition of PACl prior to the sedimentation tank, it was unaffected by change in HRT and temperature.

The suspended solid removal efficiency of the pilot plant remained almost constant in all phases (Table 2). Similar to phosphorus removal, the average removal efficiency of suspended solids was $98 \pm 6.7\%$, with the exception of $64 \pm 3.5\%$ during the startup phase, which may be due to the absence of PACl dosage during this phase.

SLUDGE SETTLEABILITY

Sludge settleability was determined by measuring the SVI. Average MLSS and MLVSS of the sludge wasted from the pilot plant were $4,703 \pm 11.3$ mg/L and $2,425 \pm 12.5$ mg/L, respectively. The SVI of the sludge was 185 ± 8.5 mL/g. A SVI value between 100 mL/g and 200 mL/g is considered

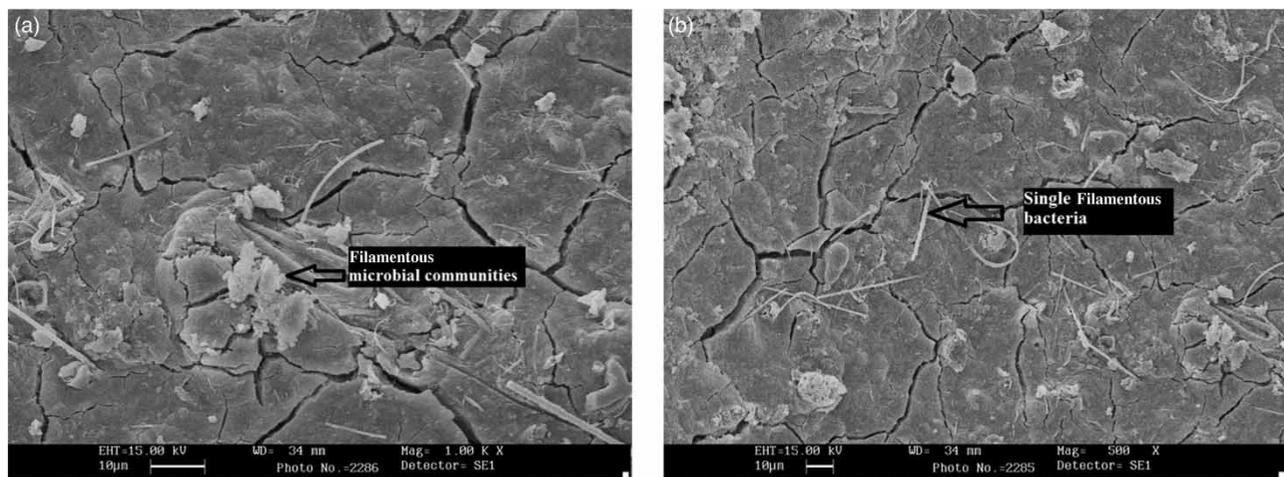


Figure 5 | (a) Filamentous microbial communities (arrows) were apparent along exterior surfaces of flocs. (b) Single filamentous bacteria.

as light weight sludge (Eckenfelder & Petr 1998). Janczukowicz *et al.* (2001) also reported a threshold SVI value of 150 mL/g for good settling features of the sludge. In this study, the SVI value of sludge indicates the light weight characteristics of the sludge. Therefore, the obtained result also supports the necessity of using PACl to enhance the settling properties of sludge.

SCANNING ELECTRON MICROSCOPY

Typical SEM micrographs of sludge samples are shown in Figure 5. The images were taken when the plant was operating at the temperature of 6 °C and 9 h HRT. The morphology of different dominant microorganisms in the biomass revealed the presence of isolated as well as joint microbial communities. The dominated cells observed were filamentous and rod shaped. Figure 5(a) shows joint microbial communities with filament shape like structure pointing outwards. We suspect that these are filamentous aerobic heterotrophic bacteria, which remain on the periphery and compete with aerobic nitrifiers and denitrifiers residing in the inner layer of gel beads. The diffusion gradient between substrate and oxygen also led to competition between heterotrophs, nitrifiers and denitrifiers. The diffusion gradient increases at low temperatures due to low solubility of oxygen and substrate, thereby resulting in low nitrification and denitrification, respectively. Calderon *et al.* (2012) also reported the presence of filamentous bacteria in the SEM micrographs on AQWISE ABC5, K1 and BIOCONS carriers during treatment of synthetic wastewater in a MBBR.

CONCLUSIONS

The study demonstrated the effectiveness of using PVA gel in a MBBR for the removal of organic carbon and nitrogen from real municipal wastewater at various temperatures and hydraulic loadings. The pilot plant was capable of performing efficiently at different HRTs and lower temperatures. The effluent COD and BOD values satisfied the Indian discharge regulations during all phases of the study. Minimum HRT and temperature at which the performance of the pilot plant was optimum were 9 h and 6 °C, respectively. Addition of PACl gave better removal efficiency of total phosphorus and suspended solids, which were reduced by a percentage larger than 90% at all operating conditions. Since the medium is new to the market of wastewater treatment, the lowest PVA gel filling

percentage of 10% used and the results obtained in this study will help in further developing the concept of PVA gel based wastewater treatment systems.

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