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Original Article

Evaluation of upflow hybrid bioreactor system for treating low-strength nitrogenous wastewater under low-shear environment

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Abstract

Lab-scale upflow bioreactor system without biomass-liquid separation unit was built to treat low-strength nitrogenous wastewater based on intermittent aeration under low-shear environment. Biomass zone formed in the absence of gas bubbles provided simultaneous biomass retention, biodegradation of nitrogenous and carbonaceous compounds, and biomass-liquid separation. Biomass zone was stable as indicated by insignificant biomass washout rates (14–29 mg VSS/day) and relatively constant biomass zone height (26–30 cm) up to the shear gradient of 1.8 s^{-1}. Nitrogen treatment efficiency of wastewater containing 15 mg NH_4^+-N/L was 15.3±1.96% under continuous oxygen influx of 95 mg O_2/L/day and autotrophic environment, whereas it increased significantly, 88.2±7.05%, after intermittent aeration (3 hrs air-on and 3 hrs air-off) and organic carbon source were supplied to the bioreactor system. Carbon removal efficiencies for both continuous and intermittent aeration were comparable reported at 85±1.76% and 91±2.1%, respectively.

Keywords: nitrification, denitrification, nitrogen, wastewater, bioreactor

1. Introduction

Low-strength wastewater such as those from aquaculture farms or domestic wastewater from small community contains concentrations of ammonium nitrogen (NH_4^+-N) and chemical oxygen demand (COD) in the range from 10 to 30 mg N/L and 80 to 250 mg/L, respectively (Reyes *et al.*, 1999; Tchobanoglous and Burton, 2003; Chen *et al.*, 2011; Zhang *et al.*, 2011). Unfortunately, activated sludge process, which has been conventionally employed for biological wastewater treatment, does not perform efficiently in the treatment of low-strength wastewater due to the complexity of the system, high operational expense, and frequent occurrence of filamentous bulking (Chen *et al.*, 2011). Nitrogen and carbon removal from wastewater could be accomplished via nitrification and denitrification. These biological pathways conventionally require two separated bioreactors for aerobic nitrification and anaerobic denitrification. However, simultaneous nitrification and denitrification is possible in a single bioreactor given that suitable environments to support relevant bacterial communities could be met (Munch *et al.*, 1996). One of the strategies for achieving simultaneous nitrification and denitrification in a single bioreactor is an intermittent aeration in which aerated and non-aerated periods are alternated to accommodate nitrifying bacteria to oxidize ammonium to nitrate and denitrifying bacteria to reduce nitrate to nitrogen gas (Munch *et al.*, 1996; Cheng and Liu, 2001; Fan *et al.*, 2009; Bernat *et al.*, 2011; Chen *et al.*, 2011). A major advantage of intermittent aeration over the conventional nitrification and denitrification is the reduction of operational cost

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due to lower oxygenation and reduction of organic carbon source (Lim *et al.*, 2012). Most studies on intermittent aeration have focused on wastewater containing 50 to 600 mg N/L (Cheng and Liu, 2001; Chen *et al.*, 2008; Lim *et al.*, 2012) and yet the data associated with low-strength wastewater such as those from small rural communities, aquaculture facilities or polluted eutrophic lakes remained limited.

The upflow bioreactor system without biomass-liquid separation unit as initially developed by Sales and Shieh (2006) was selected for this study (Figure 1). The replacement of *in-situ* submerged bubble-oxygenation eliminates intensive gas effervescences to create a quiescent hydrodynamic environment inside the bioreactor that enables effective gravitational separation of biomass from the axial liquid flow. At the same time, vertical flow also prevents the formation of compression zone at the bottom of the bioreactor. Advantages of the proposed system over the completely-mixed activated sludge process, namely simultaneous biotreatment and biomass retention, effective oxygenation control, and the possible elimination of secondary clarifier, are related to the formation of biomass zone without any use of growth-support media (Sales and Shieh, 2006). It was postulated by Sales and Shieh (2006) that the formation of filamentous structures under long means cell residence time (MCRT) condition allowing suspended cells to attach to filamentous structures and separate from liquid was responsible to biomass zone stability. A previous study by Nootong and Shieh (2008) indicated that the formation of a biomass zone from mixed nitrifying sludge was possible in the absence of gas bubbles. In that study, the upflow bioreactor system was able to treat medium-strength nitrogenous wastewater containing 60 mg NH_4^+-N/L, obtaining the volumetric removal rates of 79.6 mg N/L/day as well as insignificant biomass loss via the effluent stream. Nonetheless, application of the upflow bioreactor system on the treatment of low-strength nitrogenous wastewater has not been tested by the original authors or subsequent works.

Therefore, the main objective of this experiment was to assess the performance of the proposed upflow bioreactor system by treating low-strength nitrogenous wastewater based on intermittent aeration method under low-shear reaction environment. Data on the stability of biomass zone and oxygen utilization will also be presented.

2. Materials and Methods

2.1 Bioreactor system

A glass column (inner diameter 4.6 cm, length 41 cm) with conical bottom (height 3 cm, inner diameter 4.6 cm) and enlarged top section (inner diameter 7 cm, length 10.7 cm) was used as the upflow bioreactor system. The bottom cone was filled with spherical perforated glass beads (diameter 0.6 cm) to evenly distribute the influent stream. Discharge and recycle ports were located 2 and 4 cm below the top of the bioreactor, respectively, thereby resulting in the bioreactor working volume of 950 mL. A glass flask with a side arm having the working volume of 300 mL was used as the external oxygenator where it was also used to accommodate air supply line and recycle stream. Wastewater feed was introduced to the oxygenated stream originated from the external oxygenator before the combined streams entered into the bioreactor column at the conical section. The bioreactor system was located in a laboratory where the ambient temperature was 26.1±1.52°C.

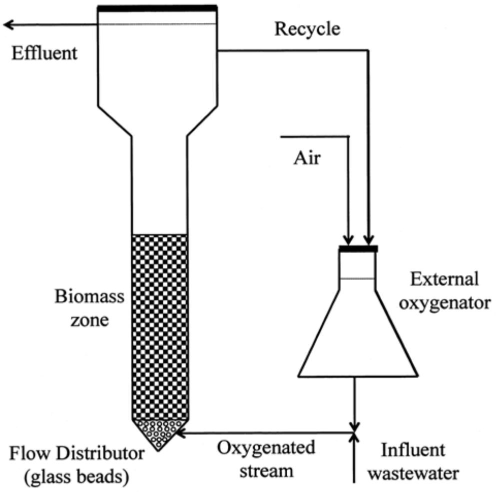

Figure 1. Schematic drawing (not to scale) of the proposed upflow bioreactor system based on Sales and Shieh (2006).

2.2 Bioreactor startup

The biomass employed in the present study was harvested from the aerated nitrifying biofilters for treating the effluent from intensive tilapia cultivation at the Marine Technology Research Center, Burapha University (Chanthaburi Campus). Harvested biomass was transferred into the upflow bioreactor system as described in the previous section to attain the initial biomass approximately 3,500 mg VSS (volatile suspended solids). Biomass was able to settle and separate from water column approximately 5 to 10 minutes after it was introduced into the bioreactor and finally formed the biomass zone, which appeared a loosely-space and mat-like structure. The initial height of the biomass zone was 26 cm. The upflow bioreactor system was fed with synthetic wastewater containing 15 mg NH_4^+-N/L as nitrogen source, $NaHCO_3$ as alkalinity source, KH_2PO_4 as buffer source and other essential minerals as followed: 5 g/L $MgSO_4$, 5 g/L $CaCl_2$, 5 g/L $FeCl_3$, 2.5 g/L $CoCl_2 \cdot 6H_2O$, 2.5 g/L $CuSO_4$, and 1.5 g/L yeast extract. The mass ratio of NH_4^+-N:$NaHCO_3$:KH_2PO_4 was maintained at 1:10:2.5 to ensure healthy bacterial growth. Synthetic wastewater (i.e., 15 mg NH_4^+-N/L) was refrigerated at the temperature below 10 °C to preserve its quality and fed into the bioreactor to attain the nitrogen loading rate of 34.6 mg N/L/day. Recirculated stream flow rates into the bioreactor were maintained at 0.65 L/hr. The described nitrogen loading rates and oxygenated stream volumetric flow rates were maintained at the specified values for two months to establish the complete nitrification as well as to acclimatize the biomass to upflow hydrodynamic environment. Accumulation of nitrite followed by nitrate was observed during the bioreactor startup. After one month, effluent concentrations of ammonium and nitrite were below 0.2 mg N/L while nitrate concentrations remained relatively constant at 14.4±0.53 mg N/L.

2.3 Experimental procedure

The present study can be divided into two main experiments. The first experiment assessed the stability and oxygen utilization of the biomass zone under low-shear environment. The upflow bioreactor system was operated under room temperature (25–29°C) while the pH was controlled between 7 and 8 by the automatic addition of 2 M NaOH. Synthetic wastewater containing 15 mg NH_4^+-N/L was fed into the upflow bioreactor system at the volumetric flow rates of 0.12 L/hr while the flow rates of the oxygenated stream, which varied from 0.12 to 2.10 L/hr, were assigned as the main experimental variable. At the given oxygenated stream flow rates, effluent samples from the bioreactor were obtained and analyzed for the concentrations of ammonium, nitrite, nitrate, and biomass washout measured in terms of volatile suspended solids (VSS). Several samples of the biomass zone approximately 10 to 15 mL were obtained for each oxygenation rate condition and analyzed for the biomass concentrations. Moreover, DO concentrations at various depths of the biomass zone were measured by using two-channel (YSI) DO meter.

The second experiment focused on the steady state operation of the upflow bioreactor system when subjecting to continuous and intermittent aeration as depicted in Table 1. Synthetic wastewater containing 15 mg NH_4^+-N/L and other trace minerals as described in the previous section was fed at constant flow rate using a peristaltic pump at 0.12 L/hr to obtain the nitrogen loading rate of 34.6 mg N/L/day. The flow rate of oxygenated stream from the external oxygenator was maintained at 0.52 L/hr for the entire experiment. The upflow bioreactor system was at room temperature and maintained in a pH range between 7 and 8 by automatic addition of 2 M NaOH. Effluent samples were obtained weekly and measured for the concentrations of ammonium, nitrite, nitrate, chemical oxygen demand (COD), and biomass in terms of VSS.

2.4 Analytical methods

Water samples were obtained from the effluent outlet and analyzed for the following parameters according to APHA (1998); ammonium using method 4500-NH_3-D, for nitrite using method 4500-NO_2-B, for nitrate using APHA method 4500-NO_3-B, for volatile suspended solids using method 2540-Solids-D, and for COD using method 5220-COD-D.

3. Results and Discussion

3.1 Biomass zone under low-shear environment

The upflow bioreactor system was operated under low shear environment, which can be justified by using the parameter called root-mean-square shear gradient (G_m). This parameter can be interpreted as the rate of kinetic energy dissipation from upflow liquid to the biomass zone. The expression for G_m can be written as (Davis and Cornwell, 1991):

$$G_m = \sqrt{\frac{m_L(u_{in}^2 - u_{out}^2)}{2V_M \mu_L}} \tag{1}$$

where u_{in} is the velocity of the oxygenated stream at the point of entry to the bioreactor (i.e., tip of glass elbow connector) (cm/s); u_{out} is the superficial velocity at the top of biomass zone (cm/s); m_L is the mass flow rate of the oxygenated stream (g/s); V_M is the volume of biomass zone (mL); and μ_L is the dynamic viscosity of the liquid at the specified temperature (dynes-s/cm^2).

In the present study, the hydrodynamic condition in the biomass zone was quiescent as suggested by insignificant G_m values of less than 1.8 s^{-1} as opposed to conventional low-shear unit in wastewater treatment plant, such as flocculating tank, which was operated in a G_m range of 20 to 300 s^{-1} (Tchobanoglous and Burton, 2003). Despite large variations in shear gradients (i.e., 0.05 to 1.81 s^{-1}), the extent of kinetic energy dissipation was low to cause significant changes in biomass zone stability over a long term period as can be seen

Table 1. Operating condition of the upflow bioreactor system during the steady state operation.

Phase	Day	Substrate Loadings	Mode of Aeration
1	1–21	NH_4^+-N at 34.6 mg N/L/day	Continuous
2	22–63	NH_4^+-N at 34.6 mg N/L/day	Intermittent: 3 hrs air-on and 3 hrs air-off
3	64–112	NH_4^+-N at 34.6 mg N/L/day Glucose at COD:N = 6:1	Intermittent: 3 hrs air-on and 3 hrs air-off
4	113–147	NH_4^+-N at 34.6 mg N/L/day Glucose at COD:N = 6:1	Continuous

by the gradual increase in biomass zone height (H_B) from 26 to 30 cm over the entire experiment (119 days) as well as small biomass washout rate (M_L) from 14 to 29 mg VSS/day (Figure 2). Successful biomass-liquid separation was also achieved as can be confirmed by extremely clear effluent quality containing insignificant biomass concentrations from 5 to 13 mg VSS/L as compared to the average biomass concentrations in the biomass zone measured at 4,714±213 mg VSS/L. In this experiment, operating the upflow bioreactor system under extremely long means cells residence time (MCRT) from 50 to 70 days favored the formation of filamentous biomass structures (Martin *et al.*, 2004), which in turn acted as the network of backbone to which cells could attach and form the biomass zone that was clearly separated from the liquid. Extensive entanglement of filamentous microorganisms provided the stability for the biomass zone was also observed elsewhere (Martin *et al.*, 2004; Sales and Shieh, 2006). Moreover, the plot of biomass specific volume (i.e. V_B/M_x where V_B is the biomass zone volume and M_x is the total biomass in the biomass zone) is demonstrated in Figure 2. This parameter was used because it was analogous to the sludge volume index (SVI), which is an indicator for filamentous bulking. High values of biomass specific volume ranging from 195 to 222 mL/g VSS were reported in the present study and suggested the presence of filamentous bulking condition in the biomass zone, and thus concurred with an earlier explanation regarding the existence of backbone filamentous structure. Clearly, the upflow bioreactor system was able to produce low-VSS effluent without any assistance from biomass-liquid separation unit to meet the discharged requirement. Successful operation of the upflow bioreactor system was attributed to several factors including the absence of gas bubbles in the biomass zone that enhanced gravitational sedimentation and subsided the extent of biomass washout via effluent stream. Another factor was the bioreactor operation under extended MCRT leading to filamentous backbone formation and low biomass yield. Results of this section, specifically the ability to retain biomass and solid-liquid separation despite subjecting to filamentous bulking condition, were important for the improvement of low-strength nitrogenous wastewater treatment since the conventional completely-mixed activated sludge process is often troubled by inefficient biomass retention under low substrate concentrations as well as poor biomass settling in the secondary clarifier (Tchobanoglous and Burton, 2003; Martin *et al.*, 2004; Chen *et al.*, 2011). Finally, the effect of kinetic energy dissipation emerged after the shear gradients exceeded 1.81 s^{-1}, which corresponded to the level of maintaining the oxygenated stream flow rates at 4 L/h and higher. Under this operating condition, the effluent biomass remained lower than 15 mg VSS/L but the biomass zone started to separate at the lower section into two pieces and floated upward along the bioreactor column.

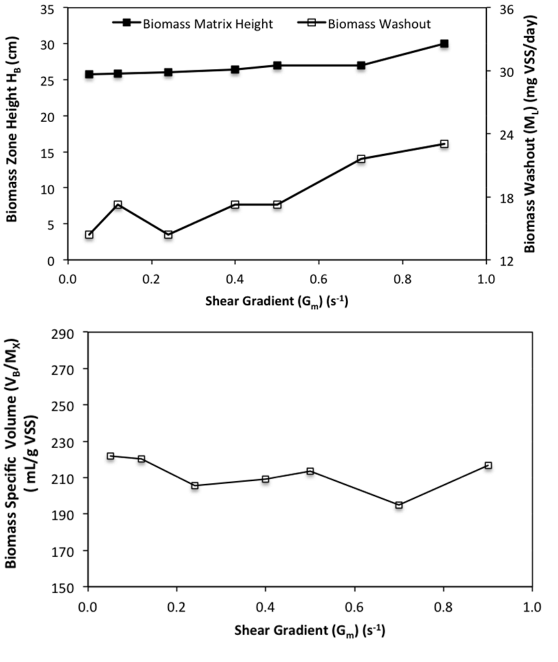

Figure 2. Effect of shear gradient (G_m) on biomass zone height (H_B), daily biomass washout rate (M_L), and biomass specific volume (V_B/M_X).

3.2 Oxygen utilization

Oxygen dissolution in the external oxygenator was nearly complete (> 95%) during the first experiment, which was confirmed by DO concentrations between 7.7 to 8.5 mg O_2/L. As a result, the extent of oxygenation rate introduced to the biomass matrix was adjusted by changing the flow rate (0.12–2.1 L/hr) of the oxygenated stream only, resulting in an oxygenation rate of 23 to 408 mg O_2/L/day. Figure 3 demonstrates the profiles of DO concentrations in the biomass zone at different heights from the bioreactor bottom given that nitrogen loading rate was maintained at 34.6 mg N/L/day. It is seen that DO concentrations decreased along the direction of upward flow with the rapid decrease occurred between the vicinity of oxygenated stream inlet and the lower section of the bioreactor. For the oxygenation rate greater than 126 mg O_2/L/day, the entire biomass zone was under aerobic condition (DO > 2 mg O_2/L), which was suitable for nitrifying bacteria to oxidize ammonium to nitrate and yet hinder the occurrence of denitrification. A sharp decrease of DO concentrations was noticed when the oxygenation rate of 58 mg O_2/L/day and lower was regulated. Under the described condition, DO concentrations quickly decreased from near saturated values (DO = 7.5-8.5 mg O_2/L) at the oxygenated stream inlet into anaerobic range (DO < 0.5 mg O_2/L) at the distance approximately 7 cm above the oxygenated stream inlet. DO concentrations continued to fluctuate in the anaerobic regime for the remaining section of the biomass zone. Oxygen limitation in the biomass zone under low oxygenation rate (<58 mg O_2/L/day) might present the difficulty in sustaining nitrification to produce adequate amounts of nitrate as the precursor for denitrification. Clearly, the main finding of this section was the recognition that suitable oxygen-regime environments for simultaneous nitrification and denitrification depended on the careful manipulation of oxygen influx into the biomass zone to support both nitrifying and denitrifying bacteria. Based on the results illustrated in Figure 3, it appeared that continuous oxygenation rates between 58 and 126 mg O_2/L/day should be maintained to sustain nitrogen removal via simultaneous nitrification and denitrification.

3.3 Nitrification

Nitrification was the primary biological pathway that occurred in the biomass zone during the first phase (Day 1–21) when the upflow bioreactor system was subjected to continuous nitrogen loading and oxygenation rates of 34.6 mg N/L/day and 95 mg O_2/L/day, respectively. Under the described operating conditions, the steady state concentrations of ammonium and nitrite in the effluent were both insignificant measured at 0.09±0.04 and 0.12±0.03 mg N/L, respectively, while nitrate concentrations increased from negligible level to the range between 12 and 13 mg N/L (Figure 4). The almost complete conversion of ammonium to nitrate from incoming wastewater as well as the insignificant accumulation of ammonium and nitrite in the effluent were indicators that confirmed the establishment of nitrification in the biomass zone. Based on the effluent data, nitrogen removal efficiency for the upflow bioreactor system was determined at 15.3±1.96%. Low nitrogen removal efficiency during the initial period was due to the existence of mainly aerobic biomass zone that is suitable for nitrification rather than denitrification, which requires oxygen-limited or anaerobic environment to proceed. Moreover, the presence of glucose as organic carbon source in wastewater was observed to exert the negative effect on nitrification during the final phase (Day 113–147) despite maintaining the same oxygen influx as in the first period. This was confirmed by the increase of ammonium (0.75±0.04 mg N/L) and nitrite (0.78±0.15 mg N/L) concentrations along with the decreased nitrate concentrations (3.48±0.74 mg N/L) in the effluent relative to those of the initial period (Figure 4). The adverse effect of organic carbon addition on nitrification could be explained by heterotrophic bacteria, which required organic carbon for their growth, and possessed a growth rate as high as 10 times greater than nitrifying bacteria (Tchobanoglous and Burton, 2003; Hargreaves, 2006; Schneider *et al.*, 2006). As a result, nitrifying bacteria lose their competitiveness in acquiring oxygen to complete nitrification.

3.4 Denitrification

Based on the results of previous sections, maintaining oxygen influx into the biomass zone exceeding 95 mg O_2/L/day would lead to the occurrence of complete nitrification in the biomass zone. Therefore, the extent of oxygen supply into the biomass zone must be lowered to create a suitable environment for both nitrification and denitrification. Reduction of oxygen influx was achieved by means of intermittent aeration, which was carried out in alternated cycles of 3 hrs aeration and 3 hrs non-aeration. This practice resulted in lower oxygenation rate estimated at 48 mg O_2/L/day. Under intermittent aeration, the steady state concentrations of ammonium, nitrite and nitrate were measured at 1.92±0.34, 2.13±0.48, and 5.57±0.53 mg N/L, respectively, during the second phase (Day 22–63) given that the same nitrogen loading rate of 34.6 mg N/L/day was used (Figure 4). Based on the steady state data, nitrogen removal efficiency (i.e. nitrogen loss) in the second period was determined at 35.9±5.92%. An increase of nitrogen removal efficiency as compared to the first period was the result of providing less oxygen influx to create suitable environment in the biomass zone for denitrification. Due to maintenance of an autotrophic reaction environment (i.e., no organic carbon addition), autotrophic denitrification was the most likely biological pathway responsible for the nitrogen loss observed in this phase. Mixed nitrifying bacteria were reported to be capable of denitrifying ammonium directly to nitrogen gas under

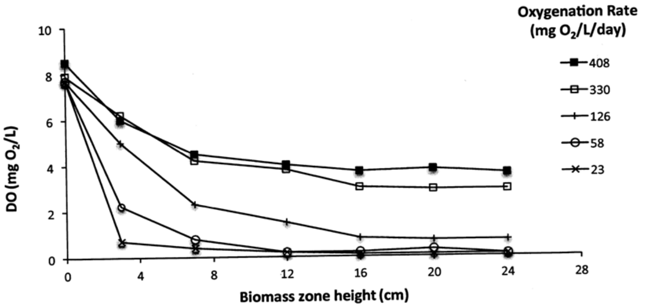

Figure 3. Profiles of dissolved oxygen (DO) concentrations in the biomass zone under different oxygenation rates.

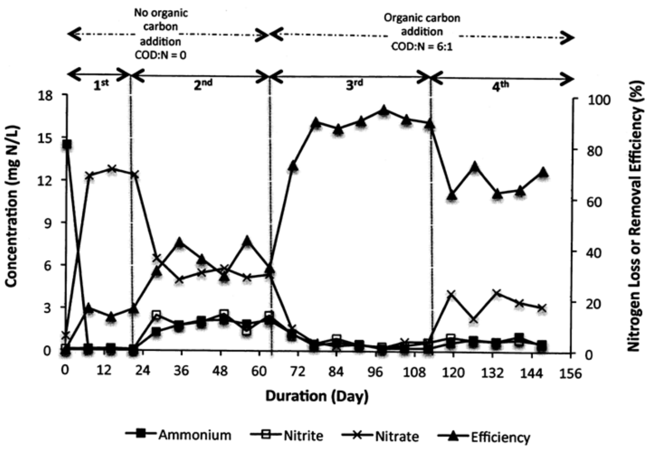

Figure 4. Variation in concentrations of ammonium, nitrite, nitrate, and nitrogen removal efficiency (i.e. nitrogen loss) during different phases of the steady state operation (1st phase: continuous aeration, 2nd phase: intermittent aeration, 3rd phase: intermittent aeration and wastewater COD:N = 6:1, and 4th phase: continuous aeration and wastewater COD:N = 6:1).

oxygen-limited or anaerobic environment (Bock *et al.*, 1995; Schmidt and Bock, 1997). By using the entire bioreactor system as control volume, the nitrogen removal rate for the second phase was determined at 12.4±2.05 mg N/L/day (i.e., 7.18±1.19 mg N/g VSS/day). However, the magnitude of nitrogen removal rate obtained in the second phase was substantially less than the results from previous works, which reported autotrophic nitrogen removal rates from 20 to 1,500 mg N/L/day in the single-stage bioreactor configuration (Bock *et al.*, 1995, Han *et al.*, 2001; Slieker *et al.*, 2003, Wyffels *et al.*, 2004; Nootong and Shieh, 2008). Low nitrogen removal rate as compared to past experiments might be related to low ammonium concentrations in wastewater as well as maintaining extremely long MCRT that yielded low biodegradation rates.

Concentration profiles of inorganic nitrogen compounds during the second phase also revealed sizeable accumulation of nitrite (1.4–2.6 mg N/L) and nitrate (5.2–6.5 mg N/L) in the effluent that pointed to the possibility of insufficient organic carbon source in wastewater (Figure 4). Limitation of organic carbon source in wastewater usually led to incomplete heterotrophic denitrification that can be characterized by the accumulation of nitrite and nitrate as well as other intermediate products such as N_2O and NO_2 (Painter, 1977; van Rijn *et al.*, 2006). Based on this evidence, changing the influent composition to include organic carbon seemed to be reasonable way to improve the performance of upflow bioreactor system. Glucose as organic carbon source was added to the original wastewater to obtain the mass COD:N ratio at 6:1 during the third phase (Day 64–112). The chosen COD:N ratio in this study was within the optimal range, which was reported to vary from 3:1 to 6:1 depending on bacterial species and types of organic carbon (Narcis *et al.*, 1979; Skinde and Bhagat, 1982; van Rijn *et al.*, 2006). Under the operating condition used in the third phase (i.e., nitrogen loading rate = 34.6 mg N/L/day, intermittent aeration and influent COD:N = 6:1), heterotrophic denitrification was established in the biomass zone as indicated by negligible concentrations of ammonium (0.48±0.37 mg N/L), nitrite (0.63±0.31 mg N/L) and nitrate (0.66±0.44 mg N/L) that yielded a nitrogen removal efficiency (i.e. nitrogen loss) as high as 88.2±7.05% (Figure 4). The corresponding nitrogen removal rate was determined at 30.5±2.44 mg N/L/day (i.e., 17.6±1.86 mg N/g VSS/day). Based on the results obtained, it was possible to conclude that the treatment of low-strength nitrogenous wastewater was possible in the upflow bioreactor system when periodic oxygenation was given and that an organic carbon source was present in sufficient quantity and proper oxygen environment to support and sustain relevant bacterial communities.

The oxygen delivery method to the biomass zone was switched back to continuous mode during the final phase (Day 113–147). This led to an increase in the oxygen influx from 48 to 95 mg O_2/L/day. Under the operating condition of the final period (i.e., nitrogen loading rate = 34.6 mg N/L/day, continuous aeration and influent COD:N = 6:1), it was seen that the trends of ammonium and nitrite were similar to those of the third period with average concentrations measured at 0.75±0.23 mg N/L for ammonium and at 0.78±0.15 mg N/L for nitrite, while the increased nitrate concentrations in the effluent were determined at 3.48±0.74 mg N/L. Nitrogen removal efficiency and removal rate for the final phase were calculated at 66.6±5.25% and 23±1.81 mg N/L/day (i.e., 13.3±1.05 mg N/g VSS/day), respectively, which were lower than the results of the third period in which intermittent aeration was employed. Clearly, results of the final phase reconfirmed the importance of maintaining suitable oxygen environment during heterotrophic denitrification although an organic carbon source was present in the wastewater in adequate quantity. Previous works also agreed on the necessity of maintaining anaerobic conditions in a denitrifying system and it is generally known that DO concentrations in the bulk liquid should be lower than 0.5 mg O_2/L in order to sustain successful heterotrophic denitrification (Irvine *et al.*, 1971; Painter, 1977; van Rijn *et al.*, 2006). Moreover, according to Figure 3, which illustrates DO concentration profiles in the biomass zone under different oxygenation rates, it can be seen that more fractions of biomass zone with DO concentrations greater than 0.5 mg O_2/L were observed after the oxygen influx was increased from 48 to 95 mg O_2/L/day.

3.5 Carbon removal

Carbon loading rate into the upflow bioreactor system was maintained at 208 mg COD/L/day during the third and final phases (Day 64–147). The steady state COD removal efficiency was determined at 91±2.1% and 85±1.76% for the third and final phases, respectively. Carbon removal during the steady state operation was thought to be proceeding by two biological pathways, namely aerobic biodegradation and heterotrophic denitrification, depending on which oxygen-regime was imposed on the biomass zone. The magnitude of COD removal efficiency reported in this experiment were comparable to the results of previous works focusing on the treatment of low-strength nitrogenous wastewater with the COD loading rates from wastewater ranged from 600 to 1,062 mg COD/L/day (Fan *et al.*, 2009; Chen *et al.*, 2001; Moura *et al.*, 2012).

3.6 Comparison to other processes

Performance of the upflow bioreactor system measured in terms of nitrogen removal rate was satisfactory as compared to the results from past researches employing different biological systems to treat low strength nitrogenous wastewater. The maximum nitrogen removal rate (30.5±2.44 mg N/L/day) obtained in the current study was significantly higher than the removal rates from outdoor microalgal ponds typically used for the treatment of aquaculture effluent that reported the nitrogen removal rates from 0.45 to 1.5 mg N/L/day (Hargreaves, 1997; Burford *et al.*, 2003). Higher nitrogen removal rate from the upflow bioreactor system was perhaps the results of maintaining higher ammonium concentrations (15 mg NH_4^+-N/L) in wastewater as opposed to the aquaculture effluent, which reported ammonium concentrations varying from 0.5 to 2 mg N/L (Hargreaves *et al.*, 2006). Another advantage of the upflow bioreactor system over the outdoor microalgal ponds was the predictable and steady performance due to lesser susceptibility of the system to seasonal variation as well as avoidance of the bloom and crash cycle of phytoplankton (Hargreaves, 2006). By comparing with microalgal photobioreactors, which are closed-systems specially designed to treat wastewater containing ammonium in a range of 10 to 42 mg N/L, it was discovered that the nitrogen removal rates in the present study were approximately 3 to 40 folds higher than the work of Voltolina *et al.* (2005) and Cabanelas *et al.* (2013) who employed *Scenedesmus obliquus* and *Chlorella vulgaris* to treat tertiary effluent and domestic wastewater, respectively. However, the maximum nitrogen removal rate obtained in the present work was smaller than the results of past researches by Chen *et al.* (2011) and Moura *et al.* (2012). In their reports, nitrogen removal rates varied from 50 to 103 mg N/L/day for biological fixed-bed biofilters, which were subjected to intermittent aeration (2 hrs air-on and 1 hr air-off) and nitrogen loading rate ranged from 61 to 77 mg N/L/day. Despite this setback, the upflow bioreactor system still possessed advantages over other systems used in previous works due to operating the upflow bioreactor system under long MCRT that resulted in lower sludge production and lower sludge handing expense. In addition, the upflow bioreactor system was also compact and did not require large space for construction as in the case of natural or constructed wetland systems.

4. Conclusions

Biomass zone was formed under low-shear hydrodynamic environment (G_m<1.8 s^{-1}) and showed the capability in retaining biomass (>96%) and produced clear quality effluent (i.e., effluent VSS = 5–13 mg VSS/L) without an assistance a from biomass-liquid separation unit. Careful manipulation of oxygenation rate into the biomass zone was critical for the coexistence of both aerobic and anaerobic environments required for nitrification and denitrification, respectively. In the present study, the upflow bioreactor system was able to treat low-strength nitrogenous wastewater containing 15 mg NH_4^+-N/L by applying the oxygen influx at 48 mg O_2/L/day based on intermittent aeration strategy (i.e., 3 hrs air-on and 3 hrs air-off) along with maintaining wastewater COD:N ratio at 6:1. Under this operating condition, nitrogen and COD removal efficiencies were determined at 88.2% and 91%, respectively.

Nomenclature

G_m = Root-mean-square shear gradient (s^{-1})
u_{in} = Velocity of oxygenated stream at the point of entry to the bioreactor (cm/s)
u_{out} = Superficial velocity at the top of biomass zone (cm/s)
m_L = Mass flow rate of oxygenated stream (g/s)
H_B = Biomass zone height (cm)
M_L = Biomass washout rate (mg VSS/day)
M_x = Total biomass in the biomass zone (mg VSS)
V_B = Biomass zone volume (mL)
V_m = Volume of the biomass zone (mL)
μ = Dynamic viscosity of the liquid at specified temperature (dynes-s/cm^2)
COD = Chemical oxygen demand (mg COD/L)
DO = Dissolved oxygen (mg/L)
MCRT = Means cell residence time (day)
SVI = Sludge volume index (mL/L)
VSS = Volatile suspended solids

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