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Analysis of an upflow bioreactor system for nitrogen removal via autotrophic nitrification and denitrification

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Abstract

The upflow bioreactor system without biomass-liquid separation unit was evaluated for its efficacy in sustaining autotrophic nitrification and denitrification (AND). The bioreactor system was capable of sustaining AND by means of carefully controlled oxygenation to achieve the maximum NH_4^+–N removal rate of 0.054 g N g VSS^{-1} day^{-1} (38% removal efficiency) at the oxygen influx and nitrogen loading rate of 3.68 mg O_2 h^{-1} L-$bioreactor^{-1}$ and 182 mg N day^{-1} L-$bioreactor^{-1}$, respectively. Additional nitrogen removal was achieved in a two-stage bioreactor configuration due to endogenous denitrification under long mean cell residence time. Quiescent conditions maintained in the bioreactor provided stable hydrodynamic environments for the chemoautotrophic biomass matrix, which revealed porous, loosely-structured, and mat-like architecture. More than 95% of the total biomass holdup (1.3–1.5 g VSS) was retained, thereby producing low biomass washout rate (~40 mg VSS day^{-1}) with VSS < 11 mg VSS L^{-1} in the effluent.

Keywords: Bioreactor; Nitrification; Autotrophic denitrification; Oxygenation; Biomass matrix

1. Introduction

Biological wastewater treatment is one of the most cost-effective means to reduce organic and nutrient contents from wastewaters prior to their final discharges (Tchobanoglous and Burton, 2003). Apart from economic consideration, biological treatment facilities must possess the following two key features including the ability to retain active biomass at high level in order to achieve the required treatment efficiency, and the ability to effectively separate and recycle biomass from the bioreactor effluent after completion of biological degradation. Suspended growth bioreactors in which biomass is thoroughly mixed with wastewater often fail to achieve sufficient biomass holdup largely due to low-substrate reaction environments (Nicolella et al., 2000; Tchobanoglous and Burton, 2003). This condition also encourages filamentous bulking, which is known to cause settling difficulty and poor compactability in the secondary clarifier (Davis and Cornwell, 1991). Alternatively, attached-growth bioreactors offer distinct advantages of achieving high biomass accumulation that is independent from the performance of the secondary clarifier (Shieh and Keenan, 1987). In spite of their ability to retain biomass, attached-growth bioreactors often experience mass transferred limitation that reduces the maximum biodegradation rate achievable (Nicolella et al., 2000). Other disadvantages include clogging in biofilters due to excessive biofilm growth, and intensive energy utilizations in BFB bioreactors to meet fluidization requirements (Shieh and Keenan, 1987).

A bioreactor system without a biomass-liquid separation unit was developed in the previous work by Sales and Shieh, 2006. Their design concept focused on the

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formation and stability of a *biomass matrix*, which was cultivated without assistance of growth-support media in an upflow bioreactor to offer integrated functions of biodegradation, biomass retention and biomass-liquid separation. In addition, an external oxygenator, in which the feed stream and the bioreactor effluent stream were mixed and oxygenated via diffusive aeration, produced a fully oxygenated liquid stream that was directly fed to the bioreactor to supply substrates, nutrients, and oxygen to the biomass matrix. The bioreactor was operated under gas effervescence-free conditions to enable the gravitational separation of biomass from the axial fluid flow and at the same time, the upflow conditions would prevent the formation of a compressed biomass zone near the bottom of the bioreactor. As a result, a biomass matrix with a porous and mat-like architecture was formed. The proposed bioreactor system was able to accommodate the heterotrophic biomass matrix, which provided up to 90% COD removal efficiency for a period more than 5 months despite subjecting to low F/M condition (Sales and Shieh, 2006). However, the experiment overlooked to investigate the bioreactor ability to capture biomass under autotrophic reaction environments, where the growth of microorganisms is limited. Therefore, this paper describes and discusses a continued laboratory study in which the bioreactor system developed similar to Sales and Shieh (2006) was tested for its efficacy in sustaining slow-rated autotrophic nitrogen removal mediated by mixed AOB/NOB species (AOB: ammonia oxidizing bacteria, NOB: nitrite oxidizing bacteria). In addition, the significance of controlled oxygenation on nitrogen removal and the stability of the biomass matrix were also discussed.

1.1. Autotrophic nitrification and denitrification

A recent discovery depicts an interesting reaction pathway for biological nitrogen removal that is referred to as the autotrophic nitrification and denitrification (AND), which is the biological conversion of NH_4^+ and NO_2^- to N_2 under low-oxygen concentration conditions (Bock et al., 1995; Grommen and Verstraete, 2002; Li et al., 2006; Strous et al., 1997, 1999; van Dongen et al., 2001; Jetten et al., 1999; Han et al., 2001; Schmidt et al., 2002; Shrestha et al. 2002; Sliekers et al., 2002, 2003; Pynaert et al., 2004; Wyffels et al., 2004). Eqs. (1) and (2) describe AND reaction scheme.

$$NH_4^+ + 1.5\,O_2 \rightarrow 2H^+ + H_2O + NO_2^- \quad (1)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + H_2O \quad (2)$$

Some aerobic chemoautotrophs (e.g., *Nitrosomonas europaea*) are able to utilize NH_4^+ as the electron donor at low-oxygen concentrations via internal electron transfers between NH_4^+ and NO_2^- (Bock et al., 1995; Schmidt et al., 2002; Shrestha et al., 2002). Therefore, the absence of significant heterotrophic bacterial activities in the same reaction environment would be advantageous for the slow-growing chemoautotrophs to carry out the reactions (Littleton et al., 2003). If the bioreactor is populated by mixed AOB/NOB species, further oxidation of NO_2^- to NO_3^- supported by NOB activities may also occur to attain nitrification as demonstrated by Eq. (3).

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \quad (3)$$

The ability to supply oxygen in accordance with the stoichiometric demand of (1) without promoting further oxidation of NO_2^- to NO_3^- according to (3) will be pivotal to achieve a significant nitrogen removal in an AOB/NOB-populated bioreactor (US Environmental Protection Agency, 1993; Kuai and Verstraete,1998). A low-oxygen reaction environment that is coupled with controlled oxygenation will also likely inhibit the oxidation of NO_2^- to NO_3^-. In addition, the attainment of good biomass retention in the bioreactor will be crucial because of low chemoautotrophic yields.

2. Experimental approach

2.1. Bioreactor system

Two identical bioreactor systems were fabricated similar to Sales and Shieh (2006). A glass column (I.D.: 4.6 cm, length: 41 cm, volume: 681 cm^3) with bottom cone (altitude: 3 cm, volume: 17 cm^3) and enlarged top section (I.D.: 7 cm, volume: 336 cm^3) was used as the bioreactor. The discharged port was located 2 cm below the top of the bioreactor, yielding a working volume of 950 cm^3. The oxygenated stream was introduced downward into the bottom cone section via glass elbow connector that was fused to the bioreactor wall directly above the bottom cone. A glass flask with a sidearm (volume: 250 cm^3) was used as the external oxygenator. Aeration was provided using an aquarium aerator and diffusion stone. The top of the flask was covered with aluminum weighting dish that was tightly wrapped with paraffin film. Four holes were punched through the aluminum dish to accommodate air line, feed line, oxygenated stream line, and bioreactor effluent line. All tubes were neoprene and wrapped with multiple layers of the Teflon® thread seal tape to prevent permeation of ambient air.

2.2. Bioreactor startup

Mixed nitrifying biomass, harvested from nitrification biofilter used for treating water from tilapia cultivation, were employed as seeding to inoculate bioreactors for AND experiment. Each bioreactor was supplied with approximately 2 g of biomass measured as dried volatile solid (VS), and fed with synthetic wastewater containing NH_4Cl (nitrogen source), $NaHCO_3$ (alkalinity source), KH_2PO_4 (buffer), and essential minerals (i.e., Ca, Co, Fe, Mo and Mg). The mass ratio of NH_4^+–N/$NaHCO_3$/KH_2PO_4 was maintained at 1/10/2.5 to ensure healthy

bacterial growth. Bioreactor I (RI) was fed with 60 mg NH_4^+–N L^{-1} synthetic wastewater at 0.12 L h^{-1} (182 mg NH_4^+–N day^{-1} L-bioreactor^{-1}), and bioreactor II (RII) was fed with 40 mg NH_4^+–N L^{-1} synthetic wastewater at 0.09 L h^{-1} (91 mg NH_4^+–N day^{-1} L-bioreactor^{-1}). A biomass matrix with a mat-like architecture was promptly formed in the lower portion of each bioreactor when the oxygenated stream was maintained at 0.72 L h^{-1}. The resulting initial biomass matrix heights for each bioreactor measured from the top of the bottom cone, and initial biomass matrix volumes were 21 cm and 316 mL, respectively. Both bioreactors were operated under the prescribed conditions for 2 months to ensure that the chemoautotrophic cells, presumably AOB/NOB, in the biomass matrix were fully adapted to continuous flow conditions.

2.3. Experimental design

Two identical bioreactor systems (RI and RII) were employed to obtain the experimental data. The oxygen influx, which was varied by adjusting the oxygenated stream volumetric flow rates (Q_o) from 0.18 to 2.10 L h^{-1}, was chosen as the sole experimental variable. The oxygenator was able to achieve >95% oxygen dissolution at these flow rates. The resulting superficial upflow velocities in the bioreactors were from 0.181 to 2.094 cm min^{-1}. Upon the completion of the single-stage experiment, RII was connected to RI as the second-stage bioreactor. The nitrogen loading applied to RI remained at 182 mg NH_4^+–N day^{-1} L-bioreactor^{-1} and the effluent stream from RI was fed to RII at 0.12 L h^{-1}. The recycle stream flow rate of RII was maintained at 1.8 L h^{-1} to ensure completely mixed conditions in RII. At a given oxygen influx for both single and two-stage configurations, daily samples taken from the feed stream and bioreactor effluent streams were first filtered using Whatman GF/A glass fibre filters (pore size 1.5 μm) and then analyzed for NH_4^+–N, NO_2^-–N, NO_3^-–N, and SS/VSS (suspended solids/volatile suspended solids) to obtain steady-state performance data. Bioreactor pH and temperature were maintained at 7.1 ± 0.2 and 21 ± 1 °C, respectively. A two-channel YSI biological oxygen monitor was used to measure the dissolved oxygen (DO) concentrations in the oxygenator and at the various depths in the biomass matrix. At the given oxygen influx, two biomass matrix samples (sample size: ~10 mL) were taken from each bioreactor and used for the measurement of biomass concentration. A Hach DR-4000/U spectrophotometer was employed for the measurements of NO_2^-–N (diazotization method at 507 nm) and NO_3^-–N (chromotrophic acid method at 410 nm). The procedures described in *Standard Methods* (APHA-AWWA-WEF, 1998) were employed for the measurements of alkalinity (2320B), NH_4^+–N (4500-NH_3B and 4500-NH_3C), biomass holdup (2540D and 2540E), and SS/VSS (2540D and 2540E).

3. Results and discussions

3.1. Oxygenation and oxygen utilization

Oxygen utilization in the mixed chemoautotrophic biomass matrix was complete for each NH_4^+–N loading even at the oxygen influx as high as 19 mg O_2 h^{-1} L-bioreactor^{-1}. This observation was supported by the oxygen concentration profile in the biomass matrix measured at different depths that revealed a sharp decline of DO concentrations from approximately 8–9 mg O_2 L^{-1} to <0.5 mg O_2 L^{-1} at the lower portion of the biomass matrix and continued to fluctuate minimally within the anoxic range (DO < 0.5 mg O_2 L^{-1}) in the remaining portion of the biomass matrix. It is clear that the majority of oxygen inventory was almost completely consumed at the lower ends of the biomass matrix to establish a low-DO reaction environment that is necessary to initiate and sustain AND reactions. Moreover, rapid utilization of oxygen, in addition to microbial activities, could also be the consequence of Q_o/Q_f ratios (Q_o is the oxygenated stream volumetric flow rate; Q_f is the volumetric feed rate) maintained, which were sufficiently large to render the bulk liquids in both bioreactors approaching completely mixed conditions.

3.2. Single-stage nitrogen removal

It was assumed that reactions (1)–(3) were carried out only in the biomass matrix, which retained most of the biomass in the bioreactor (based on the biomass and bioreactor effluent VSS data). By considering the entire bioreactor system as the control volume, the specific nitrogen removal rate in the biomass matrix can be calculated and plotted as illustrated in Fig. 1. The nitrogen removal efficiencies achieved in RI, which was fed with 182 mg N day^{-1} L-bioreactor^{-1}, ranged from 7% to 38%. R_N peaked at 0.054 g N g VSS^{-1} day^{-1} at the oxygen influx of 3.7 mg O_2 h^{-1} L-bioreactor^{-1}, and

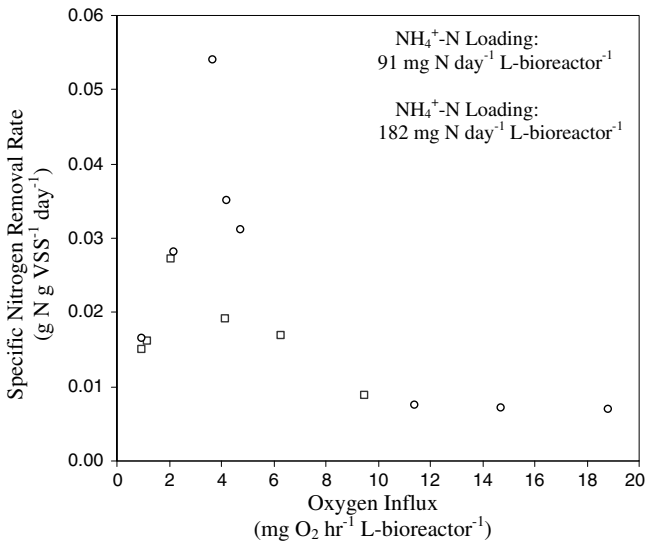

Fig. 1. The specific nitrogen removal rate as a function of oxygen influx.

then decreased sharply until it leveled off at roughly 0.007 g N g VSS^{-1} day^{-1} as the oxygen influx exceeded 10 mg O_2 h^{-1} L-bioreactor^{-1}. The maximum R_N observed in RII (nitrogen loading: 91 mg N day^{-1} L-bioreactor^{-1}) was 0.027 g N g VSS^{-1} day^{-1} (20% nitrogen removal) which was achieved at the oxygen influx of 2.1 mg O_2 h^{-1} L-bioreactor^{-1}. As in the case of *R*1, R_N peaked and then rapidly decreased with the oxygen influx until it leveled off at roughly 0.007 g N g VSS^{-1} day^{-1} at the oxygen influx greater than 9.5 mg O_2 h^{-1} L-bioreactor^{-1}. Similar trends of nitrogen removal observed in both bioreactors indicated an importance of controlling the oxygenation extents that enable AND reactions to proceed without NOB interferences. The results also suggested narrow ranges of oxygen influx optimum, which confirmed the importance of coupling the low-DO reaction environment with controlled oxygenation in an AOB/NOB-inoculated bioreactor designed to carry out AND reactions. At low-oxygen influxes the mass of oxygen delivered to the biomass matrix was limited and utilized primarily for the formation of NO_2^- according to (1). At the same time, the low-DO reaction environment in the biomass matrix was conducive to forming N_2 according to (2). Under these circumstances, R_N would increase almost linearly with increasing oxygen influx until it maximized at a critical oxygen influx. Beyond that, oxygen would become available to NOB in addition to that utilized by AOB to oxidize NH_4^+. Therefore, the concurrent of oxidation and reduction of NO_2^- would occur. However, it appeared that AND reactions would not be absent completely because oxygen was largely absent in the bulk liquid.

The maximum AND rates observed in single-stage bioreactors were somewhat low as compared to the rates reported elsewhere, which ranged from 0.002 to 0.4 g N g VSS^{-1} day^{-1} (Bock et al., 1995; Han et al., 2001; Sliekers et al., 2002, 2003; van Dongen et al., 2001; Wyffels et al., 2004). Since both bioreactors were operated at long MCRT (mean cell residence time) that ranged from 17 to 22 days, the AOB/NOB species embedded in the biomass matrixes would have low degradation activities. Moreover, concurrent oxidation and reduction of NO_2^- in the bioreactors would also reduce AND performance, as clearly illustrated in Fig. 1. It is noteworthy that a number of high nitrogen removal rates reported elsewhere occurred in the bioreactors seeded with the Anammox sludge that contained highly active bacterial species (e.g., *Brocadia Anammoxidans*) (Bock et al., 1995; Han et al., 2001; Sliekers et al., 2002, 2003; van Dongen et al., 2001; Wyffels et al., 2004), or could be the results of simultaneous nitrification and denitrification of wastewater containing high organic contents (Obaja et al., 2005; Andrade de Conto et al., 2008).

3.3. Two-stage nitrogen removal

An important outcome of the single-stage experiments was the recognition that the promotion of AND reactions did entail manipulation of oxygen influx. It was also clear that nitrogen removal rates via AND were low (i.e., <38%) and susceptible to variations in oxygen influx. Since the bulk liquid would remain free of DO, further removal of NH_4^+ and NO_2^- could be carried out by the biomass matrix maintained in a separated reaction environment without oxygenation (i.e., an anoxic reaction environment). Therefore, upon the conclusion of the single-stage experiments, RII was connected to RI and served as a second-stage bioreactor. The nitrogen loading to RI remained at 182 mg N day^{-1} L-bioreactor^{-1} and the feed rate to RII was 0.12 L/h. The nitrogen removal performance in both RI and RII was evaluated at a number of oxygen influxes, and the results are illustrated in Fig. 2.

Additional removal of nitrogen species were achieved in RII that increased the overall efficiencies to as high as 80%. At the oxygen influx <4.7 mg O_2 h^{-1} L-bioreactor^{-1}, NH_4^+ and NO_2^- were removed in equimolar proportions according to (2) while the removal of NO_3^- was negligible. At the oxygen influxes >4.7 mg O_2 h^{-1} L-bioreactor^{-1}, however, the removal of NO_2^-–N proceeded at the rates that could not be accounted for solely on the basis of reaction (2), because the bulk-liquid NH_4^+–N concentrations were too low to support the extent of reactions observed. Moreover, the removal of NO_3^-–N also became evident. Since both RI and RII were operated under the long MCRT conditions, it was hypothesized that much of the decrease in NO_X^- $NO_2^- + NO_3^-$ observed in RII could be attributable to the heterotrophic degradation of decay cellular materials under anoxic conditions using NO_X^- as terminal electron acceptors (i.e., endogenous denitrification). To test this hypothesis, a number of COD measurements were performed on the filtered bioreactor effluent samples in the last three oxygenation rates applied of two-stage experiment. Table 1 summarizes the mean rates calculated on COD and NO_X^-–N. It was assumed that oxygenation in RI would suppress endogenous denitrification activities and the production of COD in both bioreactors occurs at similar rates in order to permit the assessment of fate of NO_X^-–N and COD in RII by respective mass balance calculation. The rate data on COD production confirmed the biological origins of COD in both RI and RII because the feed stream used was free of organic matters. In addition, both NO_X^-–N and COD were removed at the rates that suggested noticeable endogenous denitrification activities in RII. The oxygen equivalent of NO_X^- (i.e., $\frac{\Delta COD}{\Delta NO_X^- - N}$) calculated ranged from 1.86 to 1.96 mg mg^{-1}, which located between 1.71 mg mg^{-1} for $\frac{\Delta COD}{\Delta NO_2^- - N}$ and 2.86 mg mg^{-1} for $\frac{\Delta COD}{\Delta NO_3^- - N}$ (Tchobanoglous and Burton, 2003). The rate data validate the hypothesis that enhanced nitrogen removal achieved in RII could be attributable to concurrent AND and endogenous denitrification activities.

3.4. Hydrodynamic stability of biomass matrix

Since the oxygenated stream delivered oxygen, NH_4^+–N, and necessary nutrients to the biomass matrix, the hydro-

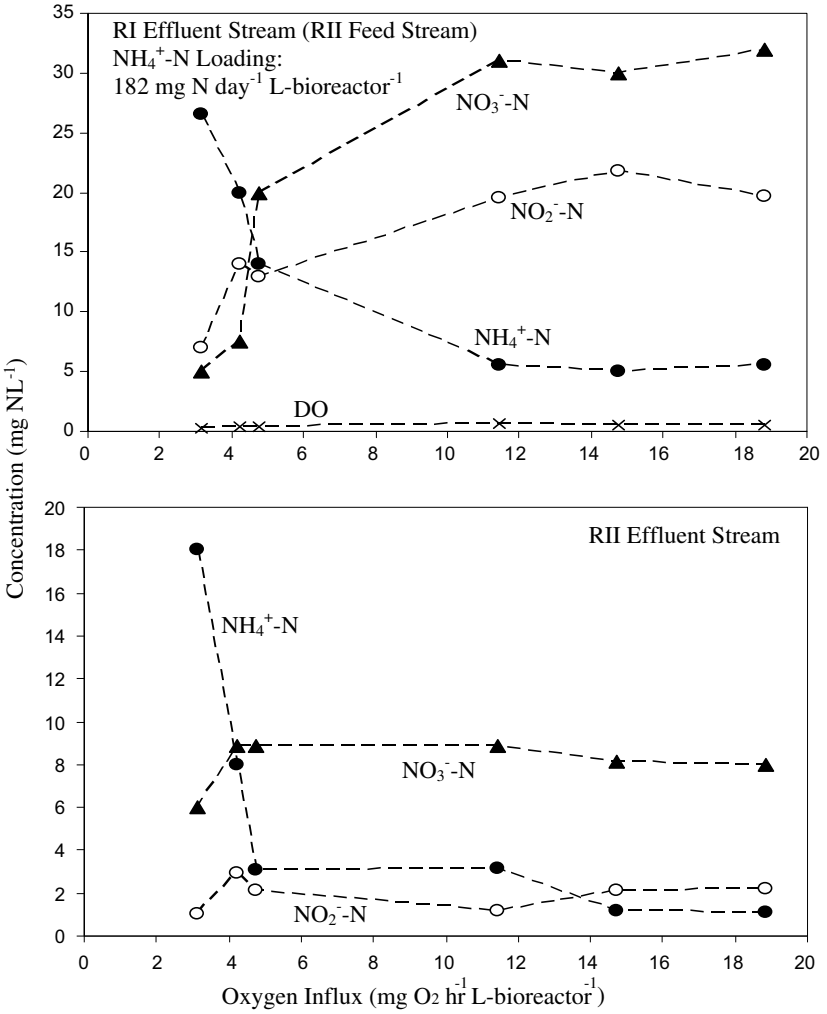

Fig. 2. The distribution of nitrogen species in two-stage bioreactor system effluent streams as a function of oxygen influx.

Table 1
Mean rate data on COD and NO_X^-–N in two-stage experiments

Oxygen Influx (mg O_2 h^{-1})	11	14	18
Production rate of COD in RI (mg h^{-1})	6.0	5.8	5.8
Production rate of COD in RII (mg h^{-1})	6.0	5.8	5.8
Removal rate of COD in RII (mg h^{-1})	9.6	9.7	9.4
Removal rate of NO_X^-–N in RII (mg h^{-1})	4.9	5.2	5.0
$\frac{\Delta COD}{\Delta NO_X^-N}$ (mg mg^{-1})	1.96	1.86	1.88

dynamics in the bioreactor could be assessed in terms of the RMS (root-mean-square) shear gradient (G, s^{-1}), which constitutes parameters related to the oxygenated stream flow and the biomass matrix. The RMS shear gradient characterizes the rate at which the fluid kinetic energy was dissipated in the biomass matrix, and its expression can be written as (Davis and Cornwell, 1991):

$$G = \sqrt{\frac{\dot{m}_l}{2V_M\mu_l}}\sqrt{u_{in}^2 - u_{out}^2} \quad (4)$$

where $\dot{m}_l$ is the mass rate of the oxygenated stream (g s^{-1}); V_M is the biomass matrix volume (mL); μ_l is the dynamic viscosity of water (dynes-s cm^{-2}); u_{in} is the velocity of the oxygenated stream at the point of entry in the bioreactor (i.e., the tip of the glass elbow connector) (cm s^{-1}); and u_{out}

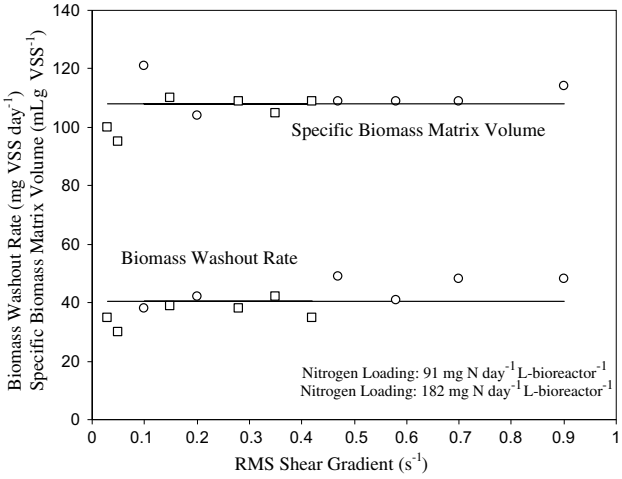

Fig. 3. Biomass matrix stability data as a function RMS shear gradient.

is the axial fluid upflow velocity at the top of the biomass matrix (cm s^{-1}). The hydrodynamic stability of the biomass matrix will be assessed using two biomass parameters: the biomass washout rate ($\dot{M}_X$) and the specific biomass matrix volume ($\hat{V}_M$), which is defined as V_M/M_X. Fig. 3 illustrates the biomass data plotted as a function of G. Despite the fact that the oxygenated stream flow varied over a wide range (i.e., 0.18 to 0.2089 L h^{-1}), the degree of kinetic energy dissipation within the bioreactor was too low (i.e., $G < 0.9\ s^{-1}$) to produce noticeable impact on the biomass matrix as indicated by the constant $\hat{V}_M$ and $\dot{M}_X$ at 110 mL g VSS^{-1} and 40 mg VSS day^{-1}, respectively. Constant $\hat{V}_M$ and $\dot{M}_X$ also suggested a stable biomass matrix with balanced biological growth and biomass detachment. To further establish that the biomass matrix was insusceptible to RMS shear gradient applied, a series of statistical techniques were performed according to Sales and Shieh, 2006. For instance, by obtaining G and $\dot{M}_X$ information from the chemoautotrophic biomass matrix, a simple linear regression was proposed to relate $\dot{M}_X$ to G as $\dot{M}_X = \beta_0 + \beta_1 G + \varepsilon$, where β_0 and β_1 are regression coefficients and ε is random error, then the values of β_0 and β_1 could be determined by least square technique (Montgomery et al., 2004). The following hypothesizes were tested with the level of significance $\alpha = 0.05$:

$H_0 : \beta_1 = 0$

$H_0 : \beta_1 \neq 0$

Student t-test (T_v) was used to test the hypothesizes, where $v = n - 2$ is the degree of freedom and n is the number of data point employed in the calculation. For this example, T_v was determined at 2.2101, which was smaller than the reference value of $t_{0.025,12} = 2.2179$ (Montgomery et al., 2004). Therefore, $\dot{M}_X$ appeared independent from G under the range investigated (up to 0.9 s^{-1}). Similar conclusion could be obtained for $\hat{V}_M$. In addition, more than 95% of total bioreactor biomass holdup was captured in the biomass matrix (i.e., 1.3–1.5 g VSS). The biomass matrix developed a continuous, loosely-structured, mat-like architecture with a mean matrix porosity of 0.65–0.70, which allowed an easy passage of axial upward flow without significant detachment of biomass from biomass matrix. The absence of gas effervescence in the bioreactor was primarily responsible for forming the biomass matrix with the desired properties. Since the washout of biomass was sufficiently low (~40 mg VSS day^{-1}), both RI and RII were able to produce low-VSS effluent streams (i.e., <11 mg L^{-1}) without requiring further biomass-liquid separation.

4. Conclusions

1. The AOB/NOB-inoculated biomass matrixes, which were formed under the gas effervescence-free and low-oxygen environments in upflow bioreactors, were able to performed AND reactions via carefully controlled oxygenation to achieve as much as 38% removal efficiency. However, the competition for oxygen between AOB and NOB species in biomass matrixes rendered the AND performance susceptible to variations in oxygenation. Oxidized nitrogen species would accumulate in bulk liquid once the oxygen influx was increased beyond its optimum range.
2. The two-stage bioreactor configuration offered enhanced nitrogen removal performance (i.e., as high as 80%) as compared to the single-stage configuration. Much of the additional nitrogen removal was achieved in the 2nd bioreactor that could be attributable to the heterotrophic degradation of decay cellular materials under the anoxic conditions using NO_X^- as the terminal electron acceptors.
3. The absence of gas effervescence in upflow bioreactors created hydrodynamic conditions that were conducive to forming the biomass matrixes with a porous, continuous, loosely-structured, mat-like architecture that was capable of retaining >95% of the bioreactor biomass holdup. The biomass losses from the bioreactors were sufficiently low (~40 mg VSS day^{-1}) and therefore, low-VSS effluent streams (i.e., <11 mg L^{-1}) were produced without further biomass-liquid separation.

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References

Andrade do Canto, C.S., Rodrigues, J.A.D., Ratusznei, S.M., Zaiat, M., Foresti, E., 2008. Feasibility of nitrification/denitrification in a sequencing batch biofilm reactor with liquid circulation applied to post-treatment. Bioresour. Technol. 99, 644–654.

APHA-AWWA-WEF, 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, DC.

Bock, E., Schimdt, I., Stuven, R., Zart, D., 1995. Nitrogen loss caused by denitrifying *Nitrosomonas* cells using ammonium or hydrogen as electron donors and nitrite as electron acceptor. Arch. Microbiol. 163, 16–20.

Davis, M.L., Cornwell, D.A., 1991. Introduction to Environmental Engineering, second ed. McGraw-Hill, New York, NY.

Grommen, R., Verstraete, W., 2002. Environmental biotechnology: the ongoing quest. J. Biotechnol. 98, 113–123.

Han, D.W., Yun, H.J., Kim, D.J., 2001. Autotrophic nitrification and denitrification characteristics of an upflow biological aerated filter. J. Chem. Technol. Biotechnol. 76, 1112–1116.

Jetten, M.S.M., Strous, M., van de Pas-Schoonen, K.T., Schalk, J., van Dongen, U.G., van de Graaf, A.A., Logemann, S., Muyzer, G., van Loosdrecht, M.C., Kuenen, J.G., 1999. The anaerobic oxidation of ammonia. FEMS Microbiol. Rev. 22, 412–437.

Kuai, L., Verstraete, W., 1998. Ammonium removal by oxygen-limited autotrophic nitrification–denitrification system. Appl. Environ. Microbiol. 14, 4500–4506.

Li, Z.H., Kuba, T., Kusuda, T., 2006. Aerobic granular sludge: a promising technology for decentralized wastewater treatment. Wat. Sci. Technol. 53 (9), 79–85.

Littleton, H.X., Daigger, G.T., Strom, P.F., Cowan, R.A., 2003. Simultaneous biological nitrogen removal: evaluation of autotrophic denitrification, heterotrophic nitrification, and biological phosphorous removal in full-scale systems. Wat. Environ. Res. 75, 138–150.

Montgomery, D.C., Runger, G.C., Hubele, N.F., 2004. Engineering Statistics, third ed. John Wiley and Son, Inc., New York, NY.

Nicolella, C., van Loosdrecht, M.C.M., Heijnen, S.J., 2000. Particle-based Biofilm Reactor Technology. Trends Biotechnol. 18, 312–320.

Obaja, D., Mace, S., Mata-Alvarez, J., 2005. Biological nutrient removal by sequencing batch reactor (SBR) using an internal organic carbon source in digested piggery wastewater. Bioresour. Technol. 96, 7–14.

Pynaert, K., Smets, B.F., Beheydt, D., Verstraete, W., 2004. Start-up of autotrophic nitrogen removal reactors via sequential biocatalyst addition. Environ. Sci. Technol. 38, 1228–1235.

Sales, M.S., Shieh, W.K., 2006. Performance of an aerobic/anaerobic hybrid bioreactor under nitrogen deficient and low F/M condition. Wat. Res. 40, 1442–1448.

Schmidt, I., Sliekers, O., Schmid, M., Cirpus, I., Strous, M., Bock, E., Kuenen, J.G., Jetten, M.S.M., 2002. Aerobic and anaerobic ammonia oxidizing bacteria – competitors or natural partners? FEM Microbiol. Ecol. 39, 175–181.

Shieh, W.K., Keenan, J.D., 1987. Fluidized Bed Biofilm Reactor for Wastewater Treatment. In: Fiechter, A. (Ed.), Advances in Biochemical Engineering/Biotechnology, vol. 33. Springer, Berlin, pp. 131–169.

Shrestha, N.K., Hadano, S., Kamachi, T., Okura, I., 2002. Dinitrogen production from ammonia by *Nitrosomonas europaea*. Appl. Catal. A: General 237, 33–39.

Sliekers, A.O., Derwort, N., Gomez, J.L.C., Strous, M., Kuenen, J.G., Jetten, M.S.M., 2002. Completely autotrophic nitrogen removal over nitrite in one single reactor. Wat. Res. 36, 2475–2482.

Sliekers, A.O., Third, K.A., Abma, W., Kuenen, J.G., Jetten, M.S.M., 2003. Cannon and Anammox in a gas-lift reactor. FEM Microbiol. Lett. 218, 339–344.

Strous, M., Kuenen, J.G., Jetten, M.S.M., 1999. Key physiology of anaerobic ammonium oxidation. Appl. Environ. Microbiol. 65, 3248–3250.

Strous, M., van Gerven, E., Zheng, P., Kuenen, J.G., Jetten, M.S.M., 1997. Ammonium removal from concentrated waste streams with anaerobic ammonium oxidation (Anammox) process in different reactor configuration. Wat. Res. 31, 1955–1962.

Tchobanoglous, G., Burton, F.L., 2003. Wastewater Engineering: treatment, disposal and reuse, fourth ed. McGraw-Hill, New York, NY.

US Environmental Protection Agency, 1993. Nitrogen Removal EPA/625/R-93/010, Washington, DC.

van Dongen, U., Jetten, M.S.M., van Loosdrecht, M.C.M., 2001. The Sharon-Anammox process for treatment of ammonium rich wastewater. Wat. Sci. Technol. 44, 153–160.

Wyffels, S., Boeckx, P., Pynaert, K., Zhang, D., Van Cleemput, O., Chen, G., Verstraete, W., 2004. Nitrogen removal from sludge reject water by a two-stage oxygen-limited autotrophic nitrification and denitrification process. Wat. Sci. Technol. 49, 57–64.