# Investigation of Nitrification and Nitrogen Removal from Centrate in a Submerged Attached-Growth Bioreactor

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**ABSTRACT:** The purpose of this study was to investigate the nitrification and nitrogen removal from centrate produced in the dewatering process of anaerobically digested sludge, using a single-unit, single-zone submerged attached-growth bioreactor. The nitrogen loading varied from 0.54 to 1.51 kg-N/m<sup>3</sup>·d. Stable ammonia oxidation (nitritification) to nitrite was demonstrated. A nitritification efficiency of 98% was achieved, while the denitrification efficiency varied from 84 to 99% (with methanol). The average total nitrogen removal was 85%. Inhibition of nitrite oxidation by a limited penetration of dissolved oxygen into the biofilm and free ammonia resulted in the accumulation of nitrite, while inhibition of ammonia oxidation by free nitrous acid did not occur. The quantity of biomass, in terms of volatile solids, ranged from 10123 to 16034 mg-VS/L of media. *Water Environ. Res.*, **80** (2008).

**KEYWORDS:** nitrification, nitrogen removal, recycle stream, side stream treatment, submerged attached-growth bioreactor, downflow packed-bed reactor, upflow packed-bed reactor.

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## Introduction

A fixed-film technology that has been applied in wastewater treatment since the 1980s is the biological aerated filter, which is a specific type of submerged attached-growth bioreactor (SAGB). These reactors have commonly achieved complete nitrogen removal, by combining the aerobic oxidation of organics and nitrification into one operational unit and denitrification into a separate unit operation (Andersen et al., 1995; Holbrook et al., 1998).

The primary advantage of an SAGB is the high biomass concentration, which results in short hydraulic retention times and thus a small volume, when a media with a high specific surface area is used. Therefore, these highly compact systems have an application for sidestream treatment, which is often required as part of an upgrade to an existing plant where space is limited. In addition, these systems are relatively easy to operate compared with other technologies currently being investigated for the treatment of centrate.

Despite these potential advantages, the application of this technology for the sidestream treatment of centrate produced from anaerobically digested sludge has not been investigated. However,

two studies indicate that submerged filters can be used to treat highstrength ammonia wastes. High ammonia oxidation rates (0.4 to 0.6 kg-NH<sub>4</sub>-N/m<sup>3</sup> · d) were achieved on fertilizer waste (Çeçen, 1996). Chui et al. (2001) evaluated two submerged filter systems for the treatment of wastewater with high concentrations of ammonia. The systems demonstrated up to 90% reduction in nitrogen and 98% reduction in chemical oxygen demand (COD) for loading rates up to 5 kg-COD/m<sup>3</sup> · d and 0.5 kg-N/m<sup>3</sup> · d.

Treatment and management of recycle streams is a necessary part of the solution to meet more stringent nutrient limits. One important recycle stream is the anaerobic digester sludge centrate/filtrate, which typically contributes 0.3 to 1.5% of the influent flow and up to 20 to 30% of the influent total nitrogen load to the plant. In addition, this recycle stream typically does not contain the requisite carbon-to-nitrogen ratio for traditional biological nitrogen removal processes. Therefore, there are a number of emerging technologies for removing nitrogen from nitrogen-rich recycle streams, including the following:

- Enhanced nitrification/denitrification with bioaugmentation, such as In-Nitri and BABE processes;
- Partial nitrification (nitritification) and denitrification from nitrite, such as the Sharon process; and
- De-ammonification processes, such as Strass and Anammox.

Given the importance of sidestream treatment and the need for simplicity of operation, the purpose of this research was to investigate nitrification and nitrogen removal from a high ammonia concentration centrate in an SAGB. The research was divided into two phases. The purpose of phase 1 and the subject of this paper was as follows:

- (1) To examine the potential of nitrogen removal by ammonia oxidation to nitrite and then the subsequent denitrification from nitrite with a supplemental carbon source,
- (2) To determine if any inhibition to the ammonia-oxidizing bacteria by nitrous acid accumulation occurred, and
- (3) To determine the quantity of biomass within the reactor.

## **Pilot Plant and Experimental Procedure**

An SAGB pilot plant at the Massachusetts Water Resources Authority, Deer Island Wastewater Treatment Plant (Winthrop, Massachusetts), shown in Figure 1, was operated to treat the centrate generated from dewatering the sludge from the anaerobic digestion process at the main wastewater treatment plant. The system consists of one 6.2-m<sup>3</sup> (1600-gal) centrate storage tank, one

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Figure 1—SAGB pilot plant.

1.667-m<sup>3</sup> (440-gal) SAGB, one 8.33-m<sup>3</sup> (2200-gal) equalization tank, and one 6.2-m<sup>3</sup> (1650-gal) clear well (Figure 1). The wastewater from the centrate storage tank was pumped into the anoxic/ equalization tank and flowed by gravity into the SAGB. The hydrostatic pressure created by the differential liquid levels between the tanks was the driving force of the forward flow. Flow through the SAGB alternated between downflow (forward flow) and upflow (reverse flow) modes. During the forward flow, the SAGB operated like a downflow packed-bed reactor. Because the forward flow was a result of the liquid level over the filter, the flowrate was a function of the level and resulted in the Reynolds number varying between 1.55 and 4.66. The reverse flow was achieved by pumping from the clear well up through the filter; therefore, the SAGB operated like an upflow packed-bed reactor. Although there was a small change in the reverse flowrate, as the liquid level in the clear well (i.e., over the pump) changed, it was negligible, and the Reynolds number was 15.9. Although the flow through the filter was both down and up, the daily influent went through the equalization tank, where any solids were separated before it went to the SAGB and flowed down through, thereby eliminating any potential for heavy solids to plug the underdrain.

To achieve the desired aerobic and anoxic conditions within the biofilm, process air was supplied intermittently via an underdrain at the bottom of the reactor. The process blowers operated for an average of 620 min/d (43% of the time) and supplied air at a rate of 124 L/min (4.4 cfm), or 272 L/min/m<sup>2</sup> (0.9 cfm/ft<sup>2</sup>) of filter area.

Sludge was wasted from the filter once per day by a backwash consisting of a high volumetric air flowrate and water. The backwash water was returned to the equalization tank, where the sludge settled out. The volume of backwash water that was returned to the equalization tank was  $0.84 \text{ m}^3$  (222 gal), or roughly 4.5 times the daily flow of 0.19 m<sup>3</sup> (50 gpd). The average quantity of sludge removed from the filter was 0.21 kg-VSS/d (0.46 lb-VSS/d).

The SAGB is 0.762 m (2.5 ft) in diameter and has a media depth of 1.22 m (4 ft.). The sand media, with a 3.2-mm nominal diameter, results in a media porosity of approximately 40%. The media specific surface area was 820 m<sup>2</sup>/m<sup>3</sup>. Four sampling ports are installed at discrete depths of 0.15 m (0.5 ft), 0.46 m (1.5 ft), 0.76 m (2.5 ft), and 1.07 m (3.5 ft) from the top of the filter bed (see Figure 2). At each depth, a pipe with slits extends across the diameter of the reactor and is connected to a sampling port. A ball valve at each port may be opened to collect a liquid sample withdrawn from within the media at its respective depth. Samples from these four depths were collected and analyzed to determine concentration profiles within the filter bed.

In this phase of the research (phase 1), the objectives were as follows:

- (1) To remove 80 to 90% of the total nitrogen from the centrate at a flowrate of 0.19 m<sup>3</sup>/d (50 gpd),
- (2) To determine both the free ammonia and free nitrous acid levels and evaluate if nitrous acid accumulated to a concentration that was inhibitory to the ammonia-oxidizing bacteria (AOB), and
- (3) To estimate the quantity of biomass within the SAGB.

With the exception of two events, in which excess centrate was processed through the system, a relatively constant flowrate was maintained. Before the start of denitrification, sodium bicarbonate was supplied for supplemental alkalinity, and adjustments to the aeration cycle were made. After several weeks of operation and no evidence of inhibition by nitrous acid buildup, 20% methanol was added to the bioreactor at a rate of 3.8 L/d (1 gal/d), or approximately 3.0 g-methanol/g-NO<sub>x</sub>. At this time, the addition of supplemental alkalinity was terminated, as a result of the subsequent alkalinity recovery from the increased denitrification, as shown in Figure 3. The system was then maintained at steady-state for 6 months.





Figure 2—Cross-sectional views of the SAGB.

The effluent was analyzed twice weekly for ammonia, nitrate, alkalinity, dissolved oxygen, and pH. The routine testing with kits was used for process control only, and the results were not included in the data analysis. The chemical data used in the analysis were obtained from samples of the influent, effluent, and from the four sampling depths within the biofilter. The samples were collected and analyzed to determine the concentrations of the following: total COD (TCOD), total 5-day biochemical oxygen demand (TBOD<sub>5</sub>), total Kjeldahl nitrogen (TKN), ammonia (NH<sub>3</sub>), nitrate (NO<sub>3</sub><sup>-</sup>), and nitrite (NO<sub>2</sub><sup>-</sup>). All sample analyses were conducted at the Massachusetts Water Resource Authorities Central Laboratory at the Deer Island Treatment Plant, according to U.S. Environmental Protection Agency (Washington, D.C.) test methods.

To determine the quantity of biomass within the SAGB, samples of media were extracted from approximately the middle of the media depth. Each sample contained approximately  $2.47 \times 10^{-3}$  m<sup>3</sup> (0.087 ft<sup>3</sup>) of media. Gravimetric tests were conducted to determine volatile attached solids (VAS) and volatile loosely attached solids (VLAS), and the sum of the two was considered as the total attached biomass and expressed as volatile solids (VS). The amount of suspended biomass in the interstitial volume of the media was determined by analyzing liquid samples collected at the four different depths in the filter for total suspended solids and volatile suspended solids (VSS).

## **Results and Discussion**

The average influent wastewater characteristics were as follows: TKN = 1260 mg/L, NH<sub>3</sub> = 851 mg/L, COD = 3283 mg/L, BOD<sub>5</sub> = 530 mg/L, and alkalinity as bicarbonate (HCO<sub>3</sub><sup>-</sup>) = 79.2 mmol/L. From the data (including the ammonium  $[NH_4^+]$  generated by ammonification), the molar ratio of alkalinity to ammonium ion  $(\text{HCO}_3^-:\text{NH}_4^+-\text{N})$  was 1.14:1 and is in agreement with the typical ratio of 1.1:1 for centrate reported in the literature (Hellinga et al., 1998). The influent carbon-to-nitrogen ratio, in terms of BOD<sub>5</sub> to TKN, was 0.42.

The total nitrogen removal rates presented in Figure 4 ranged from 0.49 kg-N/m<sup>3</sup> · d at a loading of 0.58 kg-N/m<sup>3</sup> · d to 1.19 kg-N/m<sup>3</sup> · d at a loading of 1.51 kg-N/m<sup>3</sup> · d. The total nitrogen removal efficiency varied from 78 to 96%; however, once the addition of methanol as an electron donor for denitrification was started and stable conditions were established, the average total nitrogen removal efficiency was 85%. The average effluent total nitrogen was 268 mg/L, while the ammonia was 117 mg/L. The specific nitrogen removal rate, assuming 100% of the media surface area was covered with biofilm, was 0.844 g-N/m<sup>2</sup> · d. The low removal



Figure 3—Effluent alkalinity concentration before and after methanol addition.



Figure 4—Total nitrogen loading and total nitrogen removal rate.

rates of 0.016 and 0.3 kg-N/m<sup>3</sup>  $\cdot$  d at a loading rate of approximately 0.6 kg-N/m<sup>3</sup> were the result of both insufficient alkalinity and insufficient carbon for denitrification.

An average ammonia oxidation (nitritification) efficiency of 91% was achieved throughout the experiment and followed zero-order kinetics, as indicated in Figure 5. This is in agreement with the theoretical statements of Harremoës (1978) and with results reported by Çeçen (1996). The implication of a zero-order rate is that ammonia has fully penetrated the biofilm (Çeçen 1996; Harremoës, 1978; Henze et al., 2002). With full penetration of ammonia into the biofilm and a relatively high rate of nitritification, the question arises as to the role of both alkalinity and oxygen and nitrite predominating in the effluent. For complete oxidation of NH<sub>4</sub>-N, a molar ratio of alkalinity to ammonium of 2 is required, accounting only for catabolic reactions (Gaul et al., 2005). In this experiment, the ratio was shown to be 1.14:1. Furthermore, it is accepted that a limited amount of oxygen suppresses the oxidation of nitrite, as a result of the nitrite-oxidizing bacteria's (NOB) lower affinity to oxygen than that of the ammonia oxidizers (Wiesmann, 1994). The following two important factors have previously been demonstrated:

- If alkalinity is limiting, then ammonium oxidizers will be limited and will no longer compete for oxygen, resulting in the formation of nitrate by nitrite oxidizers, which are catabolically independent of alkalinity; and
- (2) At high loading rates, alkalinity governed the formation of nitrite, because the  $HCO_3^-$  flux into the biofilm was greater



Figure 5—Zero-order kinetic model for ammonia oxidation rate.



Figure 6—Ammonia oxidation rate and total ammonia loading.

than the oxygen  $(O_2)$  flux (Gaul et al., 2005). In other words, if alkalinity is limited, then dissolved oxygen must also be limited for nitrite to be the predominate species (Gaul et al., 2005).

Although the influent molar ratio of alkalinity to ammonium was insufficient for complete ammonia oxidation, relatively high ammonia oxidation rates were demonstrated, as shown in Figure 6. These high rates were likely achieved as a result of the alkalinity recovered during denitrification. Once the methanol addition was started, excess alkalinity was measured in the effluent, as shown in Figure 3. The results indicate that both alkalinity and oxygen penetrated deep enough into the biofilm, but that oxygen—not alkalinity—was limiting. Therefore, the ammonia oxidizers, because of their higher affinity for oxygen than the nitrite oxidizers, were able to accomplish the high rate of ammonia oxidation demonstrated. In addition, the straight-line fit through the data in Figure 6 also indicates that an increase in the total ammonia loading may be possible.

The dissolved oxygen concentration in the bulk liquid varied from 0.5 to 1.0 mg/L during the periods without aeration and from 1.6 to 3.4 mg/L during aeration. The dissolved oxygen at each depth within the filter varied during both the aeration periods and the nonaeration periods. However, the large variation of 1 to 1.5 mg/L during aeration is likely a result of the fact that the samples were collected randomly. Therefore, some may have been collected at the beginning of the aeration cycle and some at the end of the aeration cycle.

As mentioned previously, one of the objectives of this research was to oxidize to nitrite rather than completely to nitrate. The concentrations of the nitrogen species within the bioreactor, both with and without methanol addition, are shown in Figure 7; in both cases, there is no evidence of nitrate in the reactor. During the initial tests with sodium bicarbonate addition, but without methanol, the ammonia concentration within the filter averaged 365 mg/L, and further ammonia oxidation appears to have been inhibited. With the addition of methanol and the subsequent recovery of alkalinity, the ammonia concentrations and the nitrite concentrations were reduced. It should be noted that the level of ammonification within the biofilter ranged from 73 to 96% and averaged 89%, and the resulting ammonia was included in the ammonia loading calculations.

Nitrite accumulation is common in the treatment of highammonia-concentration waste streams and has been reported to occur as a result of high levels of free hydroxylamine (Yang and Alleman, 1992), free ammonia, pH, and temperature (Anthonisen



Figure 7—Nitrogen species concentrations within the bioreactor.

et al., 1976; Kim et al., 2003; Randall and Buth, 1984; Turk and Mavinic, 1989). The nitrite concentrations ranged from 0.01 mg/L with methanol addition to 351 mg/L without methanol. Throughout the testing, the nitrate concentration never exceeded 6 mg/L.

To determine the type and level of inhibition, the concentrations of un-ionized or free ammonia and free nitrous acid were calculated with the following equations (Anthonisen et al., 1976):

$$C_{FA} = \frac{C_{NH_4}(10^{pH})}{((k_b/k_w) + 10^{pH})}$$
(1)

Where

$$C_{\rm FA}$$
 = concentration of free ammonia (mg NH<sub>3</sub><sup>-</sup>-N/L),  
 $C_{\rm NH4-N}$  = concentration of total ammonia mg (NH<sub>3</sub><sup>-</sup>-N/L).

 $k_{\rm b}$  = ionization constant for ammonium, and

$$k_{\rm w} = 10012ation$$
 constant for water

$$C_{\text{FNA}} = \frac{C_{\text{NO}_2\text{N}}}{(k_a 10^{\text{pH}})} \tag{2}$$

Where

 $C_{\rm FNA}$  = concentration of free nitrous acid (mg HNO<sub>2</sub><sup>-</sup>-N/L), and

 $k_{\rm a}$  = ionization constant for nitrite.

The concentration of free ammonia within the filter ranged from 0.29 to 36 mg-NH<sub>3</sub><sup>-</sup>-N/L. Inhibition to nitrite oxidation has been reported at concentrations of 0.1 to 1 mg/L (Turk and Mavinci, 1986), 5 to 20 mg/L (Ford et al., 1980), and 9.5 to 73 mg/L (Mines, 1980). The concentration of free ammonia in the influent centrate, equalization tank, and within the biofilter is shown in Figure 8. At very high levels in the centrate, the concentration of free ammonia in the equalization tank was significantly lower because of dilution resulting from a recycle ratio of 4.5 caused by daily backwash of the filter.

It is well-established that high concentrations of nitrite are inhibitory to ammonium oxidation and that inhibition is caused by the presence of free nitrous acid (HNO<sub>2</sub>) (Beccari et al., 1979). In this experiment, the free nitrous acid concentration ranged from  $3.88 \times 10^{-8}$  to 0.23 mg–HNO<sub>2</sub><sup>-</sup>-N/L and had no inhibitory effect.

The concentrations of both free ammonia and free nitrous acid observed in this study are in agreement with values reported by Çeçen (1996). It has been demonstrated that the relative ratios of



Figure 8—Concentration of free ammonia within the system.

 $C_{\text{DO}}/C_{\text{NH4-N}}$  and  $C_{\text{DO}}/C_{\text{FA}}$  may be used to predict whether nitrite or nitrate will predominate in the effluent Çeçen (1996). The percent inhibition to nitrite oxidation I is defined as follows (Çeçen, 1996):

$$I = \frac{[NO_2 - N]}{[NO_x - N]}$$
(3)

The plot of the fraction NO<sub>2</sub> in NO<sub>x</sub> produced versus the ratio  $C_{\text{DO}}/C_{\text{NH4-N}}$  in the bulk liquid (Figure 9) indicates that, for this study, the ratio of 0.01 to 0.12 resulted in almost 100% (i.e., ratio I = 1) production of nitrite and very little nitrate. This is in agreement with the concentrations of nitrite and nitrate shown in Figure 7 and the results of Çeçen (1996), which indicate that, at a  $C_{\text{DO}}/C_{\text{NH4-N}}$  ratio less than 0.1, nitrite is the predominant form of NO<sub>x</sub>. The scatter is a result of the fact that denitrification lowered the effluent nitrite concentration, thereby lowering I; however, the plots still confirm the results obtained. It is evident, in Figure 10, that, in all but two tests, the  $C_{\text{DO}}/C_{\text{NH4-N}}$  ratio was less than 0.012, indicating that, with increased aeration, the  $C_{\text{DO}}/C_{\text{NH4-N}}$  ratio could be maintained below 0.1 and may allow an increase in ammonia loading to the filter, while still only oxidizing ammonia to nitrite.

Examination of the fraction NO<sub>2</sub> in NO<sub>x</sub> produced versus the ratio  $C_{\rm DO}/C_{\rm FA}$  (free ammonia) in the bulk liquid (Figure 10) indicates that significant inhibition occurred at ratios of dissolved oxygen to free ammonia approaching 10 and is in agreement with the results of Çeçen (1996). From Figure 10, it is evident that the majority of tests were conducted at  $C_{\rm DO}/C_{\rm FA}$  ratios between 0.1 and



Figure 9—Relationship of inhibition (ratio  $NO_2/NO_x$ ) to the ratio of dissolved oxygen (DO) to ammonium.



Figure 10—Relationship of inhibition (ratio  $NO_2/NO_x$ ) to the ratio of dissolved oxygen (DO) to free ammonia.

1.0; however, nitrate inhibition occurs at  $C_{\rm DO}/C_{\rm FA}$  ratios as high as 10. This implies that the quantity of air supplied to the reactor could be increased, which could lead to lower ammonia numbers than were achieved in this experiment, or the ammonia load to the filter could be increased, or a combination of the two. However, in all cases, nitrite would still be the predominant nitrogen species.

The total quantity of fixed biomass, which was measured in terms of VAS and VLAS, ranged from 10 123 to 16 034 mg-VS/L of media or 12.3 to 19.6 g-VS/m<sup>2</sup>. These are average values based on samples taken in the upper and lower halves of the filter. The percentage of loosely attached solids to the total biomass ranged from 1.8 to 4%; therefore, if subtracted from the overall total, the quantity of suspended biomass within the interstitial volume of the media ranged from 11.5 to 368 mg VSS/L, with an average value of 76.4 mg VSS/L, and was therefore not considered in the overall biomass estimation. The small amount of suspended solids within the interstitial spaces of the filter suggests that the SAGB functioned predominately as a fixed-film reactor.

#### Conclusions

High ammonia oxidation (nitritification) efficiencies, averaging 91%, were demonstrated in the SAGB. Zero-order kinetics, with respect to nitrification, were observed, indicating that ammonium had fully penetrated the biofilm. Alkalinity and dissolved oxygen penetrated sufficiently into the biofilm, so that ammonia oxidation to nitrite was possible; however, dissolved oxygen was the limiting substrate. This is evidenced by the fact that oxygen was present for the oxidation of ammonia to nitrite, but that the dissolved oxygen scarcity resulted in conditions conducive to the AOB, as a result of their affinity for oxygen. Under these conditions, the NOB, with a lower affinity for oxygen than the AOB, are either suppressed or non-existent. The nitrite accumulation within the biofilter demonstrated that denitrification from nitrite predominated. The low  $C_{\rm DO}/$  $C_{\rm NH4-N}$  ratio may allow for increased aeration and therefore an increased ammonia load to the filter, while still only oxidizing ammonia to nitrite, and will be the focus of the second phase of research. Nitrite oxidation was also likely inhibited by the free ammonia concentration within the filter, while inhibition of free nitrous acid was not observed. The  $C_{\rm DO}/C_{\rm FA}$  ratio was much lower than the upper limit required for maintenance of an inhibitory environment for nitrification (nitrate formation), which implies that increased aeration and therefore an increased ammonia load to the biofilter may be possible. With methanol addition, denitrification within the same biofilter was demonstrated, and an average total nitrogen removal of 85% was achieved.

The quantity of biomass within the biofilter was predominately fixed and ranged from 10 000 to 16 000 mg VS/L of media.

The results suggest that, with no inhibition to ammonia oxidation and the ability to control air and therefore nitrite accumulation, this SAGB can be operated such that the aerobic contact time only allows for the oxidation of ammonium to nitrite. It was further demonstrated that, through denitrification, a neutral pH was maintained, and sufficient alkalinity was recovered to sustain the ammonium-oxidizing reaction.

The process offers the potential for point-source reduction of the nitrogen loading created by the all the recycle streams, not just the high temperature centrate produced from anaerobically digested sludge, and therefore may affect the options available for future plant upgrades. The high biomass concentration allows for a relatively small footprint for such a high-strength wastewater. It is further reasoned that, in the application of the biofilter for sidestream treatment, the effluent from the bioreactor (low levels of ammonium, high levels of nitrite, and some level of nitrate) could be discharged to the influent piping of the treatment plant. The environmental conditions within the influent piping system and the headworks of a wastewater treatment plant are conducive to denitrification because of the anoxic and anaerobic conditions that exist and the quantity of organic carbonaceous matter within the wastewater. Therefore, the collection system and headworks could be used as a denitrification reactor by introducing the partially treated (high nitrite concentration) recycle stream to the collection system. This process of ammonia oxidation and pipe denitrification (AOx – PD) will be investigated in the next phase of research.

#### Credits

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