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Removal of nitrogen from anaerobically digested swine wastewater using an anoxic/oxic (A/O) process complemented with a sulfur-packed biofilter

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A modified lab-scale anoxic/oxic process was designed incorporating an upflow sulfur-packed biofilter for the treatment of anaerobically digested swine wastewater. In this study, chemical oxygen demand (COD), NH$_4^+$-N and NO$_x$-N removal efficiencies were investigated. The experimental results showed that by increasing the internal recycle ratio from 1 to 3, the overall performance of the system improved. Organics removal efficiency was found to be fairly high and stable and the average total chemical oxygen demand (TCOD) removal efficiency ranged from 79 to 90%. This process removed up to 98% of the total NH$_4^+$-N from the nitrification reactor with proper pH control using excess alkalinity and a recycle ratio of 3. The average removal efficiency of NO$_x$-N in the anoxic reactor was above 80% with the poor effluent quality (25 mg/l). This high concentration of NO$_x$-N in the effluent of the anoxic reactor was removed by the sulfur-packed biofilter with the stable effluent concentrations between 0.4 and 4 mg/l. This result indicates that the sulfur-packed biofilter would be used as an efficient option for denitrification by autotrophic denitrifiers during swine wastewater treatment.

Key words: Biological nitrogen removal, nitrification, denitrification, chemical oxygen demand (COD), intermittent aeration, sulfur-packed bed reactor, swine wastewater, anoxic-oxic process, internal recycle.

INTRODUCTION

Swine wastewater contains high amounts of organic matter and nutrients, and hence it is widely applied as fertilizer for increasing crop productivity (Deng et al., 2006; Zhang et al., 2006). Generally, swine wastewater is treated anaerobically in order to reduce the level of pollutants and to recover methane gas (An et al., 2007; Deng et al., 2006; Lee et al., 2008). However, the inappropriate discharge of swine wastewater containing excess inorganic nitrogen (NH$_4^+$-N, NO$_x$-N and NO$_3$-N) into natural waters causes the overgrowth of algae that ultimately promotes eutrophication of lakes and streams (Deng et al., 2009; El-Hoz and Apperley, 1996; Lim et al., 2009; Sumino et al., 2006). Therefore, post-treatment is necessary in order to remove nitrogen as well as organic matter from swine wastewater (Bernet and Beline, 2009; Bortone, 2009; Obaja et al., 2003; Waki et al., 2008).

Biological nitrification-denitrification is the most studied and applied biological nitrogen removal (BNR) method used to remove ammonium from swine wastewater (Cooper et al., 1994; Sliekers et al., 2002). Nitrification process consists of two steps that convert ammonium into nitrate by autotrophic bacteria under aerobic conditions (Lim et al., 2009). In the first step, ammonium is oxidized into nitrite by ammonia oxidizing bacteria (AOB) and converted to nitrate in the second step by nitrite oxidizing bacteria (NOB) (Aslan and Dahab, 2008). Denitrification subsequently converts nitrate into nitrogen.
gas under anoxic conditions using organic matter as a source of carbon and also the electron donor (Shin et al., 2001; Sumino et al., 2006). The biological nitrification-denitrification method is widely used in the sequencing batch reactor (SBR) (Obaja et al., 2003), membrane bioreactor (MBR) (Kim et al., 2008) and the anoxic/oxic (A/O) process (Fu et al., 2009) to remove nitrogen from wastewater. The A/O process is considered an effective BNR method (Fu et al., 2009) with high ammonia removal efficiency, in which the recirculation of mixed liquid containing NO\(_x\) -N (NO\(_3\)-N and NO\(_2\)-N) from the anoxic reactor is necessary as the denitrifying microbes utilize the nitrite or nitrate in the anoxic reactor as the electron acceptor to oxidize the organic matter (Cheng et al., 1996; Fu et al., 2009).

The A/O process helps to reduce the competition between nitrifiers and heterotrophs in theoxic zone as most of the organic material is consumed in the anoxic zone (Fu et al., 2009). However, the A/O process is quite expensive as the circulation of liquid requires additional energy and external carbon source must be added to the anoxic reactor if the swine wastewater does not contain enough organic matter to fully denitrify (Cervantes et al., 2001; Zhu et al., 2009). Therefore, further study of the A/O process is needed. Recently, autotrophic denitrification using elemental sulfur or reduced-sulfur compounds (S, S\(^2\), S\(_2\)O\(_3\)\(^2\), S\(_2\)O\(_5\)\(^2\) and SO\(_3\)\(^2\)) as the electron donor has shown promise as an alternative to heterotrophic denitrification (Batchelor and Lawrence, 1978; Jang et al., 2005). Sulfur based autotrophic denitrification can reduce the operational cost significantly because sulfur is cheaper than other organic carbon sources (Lee et al., 2001).

In this study, we designed a modified lab-scale A/O process that is complemented with a sulfur-packed bed reactor for denitrification and an integrated fixed film activated sludge (IFAS) system for nitrification. The objective was to study the performance of a laboratory scale A/O system under various operating conditions. The anoxic reactor is intermittently aerated and hence the possibility of simultaneous nitrification and denitrification (SND) was evaluated in the anoxic reactor. This study also evaluates the possibility of enhancing the biological performance, especially nitrogen removal by the sulfur-packed biofilter.

### MATERIALS AND METHODS

#### Characteristics of feedstock solution

Anaerobically digested swine wastewater was collected from a local pig farm in Anseong, Gyeonggi Province, Korea. The wastewater was diluted 1:10 with tap water and suspended solids were filtered using a 1 mm diameter sieve. Characteristics of these diluted wastewaters are given in Table 1.

#### Lab-scale A/O process set-up

The schematic diagram of the modified lab-scale A/O process used in this study is shown in Figure 1. The lab-scale A/O system consisted of an anoxic reactor for heterotrophic denitrification complemented with an upflow anoxic sulfur-packed biofilter for autotrophic denitrification and an aerobic rectangular tank for nitrification. The effective working volume of the anoxic reactor, sulfur-packed biofilter and the nitrification reactor were 57, 8 and 47 L, respectively. The anoxic reactor was intermittently aerated (MASTER; A/S: 02-764-5556) (1 h aerating and 1 h non-aerating) at a flow rate of 0.2 L air/min so that SND will occur in the single reactor. The upflow sulfur-based biofilter was 90% packed with sulfur particles (2 to 4 mm in diameter).

The nitrification reactor was divided into three equal-sized parts to create a plug-flow system. High levels of organic matter and solids in the swine wastewater led to the wash-out of the nitrifying bacteria due to their slow growth and low reaction rate in comparison to heterotrophs (Vanotti and Hunt, 2000). In order to achieve good BNR efficiency, nitrifying bacteria must be able to oxidize ammonium ions completely. Therefore, fixed-film media were integrated into each compartment to support fixed biofilm growth and enhance nitrification. Two biocube (a free floating sponge media) namely polyurethane (PU) and poly vinyl alcohol (PVA) were used for spontaneous attachment of nitrifying bacteria (AOB and NOB) in the medium. The nitrification tank was further supplemented with pH control to keep the pH within the range of 7 and 8.5 using 0.1 N H\(_2\)SO\(_4\) and 1 N NaHCO\(_3\).

#### Reactor operating conditions

The seed sludge for the nitrification reactor was taken from the Hongcheon wastewater treatment plant, Gangwon Province, South Korea. The hydraulic retention time (HRT) was fixed at 15 days throughout the entire study. Experiments were designed to evaluate BNR with variable nitrogen loading using different internal recycle flow rates. The variable operating parameter was the internal recycle ratio, R, defined as the ratio between the recirculation of nitrogen and liquid recycle flow rate (Q\(_R\)) and the influent wastewater flow rate (Q\(_I\)):

### Table 1. Characteristics of swine wastewater used in this study.

| Parameters       | Range of values | Average value |
|------------------|-----------------|---------------|
| TCOD (mg/l)      | 2152 - 3856     | 2878          |
| SCOD (mg/l)      | 1900 - 3680     | 2632          |
| NH\(_4\)-N (mg/l)| 400 - 700       | 522           |
| NO\(_3\)-N (mg/l)| 0.89 - 2.1      | 1.46          |
| PO\(_4\)-P (mg/l)| 2.23 - 7.24     | 4.6           |
| SO\(_4\)-S (mg/l)| 20.9 - 39.1     | 31.1          |
| pH               | 8.04 - 8.86     | 8.49          |
| Alkalinity (mg/l as CaCO\(_3\)) | 1456 - 2214 | 1912         |
Table 2. The operational parameters of the AO process.

| Parameters                          | Phase I | Phase II | Phase III |
|-------------------------------------|---------|----------|-----------|
| Operating time (days)               | 1 - 30  | 31 - 60  | 61 - 90   |
| Nitrate recycle ratio (nitrification reactor → anoxic reactor)\(^a\) | 1       | 2        | 3         |

\(^a\)Based on influent flow rate (Q\(_I\) = 3.7, 7.4 and 11.1 L/day).

With regard to the internal recycle flow (Q\(_R\)), three different rates: 1, 2 and 3 with respect to the influent flow rate (Q\(_I\)) were tested throughout the experiment (Table 2). The influent flow rate was maintained at 3.7 L/day.

Analytical methods

Samples were taken from the influent to the lab-scale A/O plant, the anoxic reactor, the sulfur-packed biofilter and the mixed liquor in each compartment of the nitrification reactor. The samples were analyzed for different parameters such as temperature, pH, alkalinity (as the equivalent of CaCO\(_3\)), chemical oxygen demand (COD), concentration of NH\(_4\)^+-N, nitrate (NO\(_3\)^-) and nitrite (NO\(_2\)^-) N). The pH was measured using a portable pH meter (Orion model 410A, Boston, USA). Total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) and alkalinity were determined in accordance with standard methods (APHA, 1998). TCOD and SCOD were measured by a close reflux digestion and a titrimetric method. The liquid samples were filtered through low protein binding, non-pyrogenic membrane filters (Pall Corporation, USA) with 0.45 µm pore size prior to SCOD analysis. The concentration of NH\(_4\)^+-N was measured by an ammonia-gas sensing electrode (Orion model 9512) connected to a multimeter (Orion 5 Star Bechtop). The concentrations of NO\(_3\)^-N and NO\(_2\)^-N were measured by ion chromatography with suspended conductivity detention using an ion chromatograph (model DX-120, Dionex Corporation, Sunnyvale, Cal.) equipped with an IonPack® AG14 guard column (4 x 50 mm), and IonPack®AG14 analytical column (4 x 250 mm), a Dionex ASRS-II suppressor, a CDM-3 conductivity detector and an AS40 automated sampler. The eluent utilized was a mixture of 3.5 mM Na\(_2\)CO\(_3\) and 1.0 mM NaHCO\(_3\) and the flow rate was 1.00 ml/min. Liquid samples were filtered through sterile, non-pyrogenic hydrophilic membrane filters (Sartorius, Germany) with 0.20 µm pore size prior to chromatographic analysis. DO was measured by DO meter SG6 (SevenGo™Mettler Toledo AG, 8603 Schwerzenbach, Switzerland).

RESULTS AND DISCUSSION

Organic material removal

The high concentration of the COD in the livestock wastewater makes it difficult to carry out biological treatment as it contains a large amount of non-biodegradable matter which cannot be easily broken down (Kim et al., 2008). Figure 2 presents the performance of the TCOD removal throughout the experiment. In the case of pre-denitrification, most of the organic material is consumed in the anoxic zone for denitrification and the remaining organic material will eventually be degraded aerobically in the nitrification reactor (Fu et al., 2009). The average TCOD
removal efficiency was 85% when the average organic loading rate was 0.19 kg COD/m³/d. This value is similar to a previous study carried out by Rim and Han (2000) who observed an average TCOD removal rate of 80 to 95% when the organic loading rate was maintained in the range of 0.4 to 3.1 kg COD/m³/d.

Average TCOD removals were 79, 90 and 90% with effluent concentrations of 611 ± 156, 309 ± 39 and 260 ± 14 mg/l during phases I, II and III, respectively. It was found that with increasing R value, the COD removal efficiency increased by 10% in the later phases (II and III). It can be concluded that the A/O system was very consistent in maintaining a fairly high and stable COD removal throughout the experimental period.

**NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N removal**

The nitrification process was observed by measuring the concentrations of NH₄⁺-N, NO₂⁻-N and NO₃⁻-N in the effluent of the nitrification reactor. Figure 3 illustrates the performance of the A/O process in removing NH₄⁺-N from the digested swine wastewater under various R.

During the nitrification process, the oxidation of NH₄⁺-N releases hydrogen ions (H⁺) that decreases the pH in the nitrification reactor and could inhibit the nitrification efficiency (An et al., 2007; Vanotti and Hunt, 2000). Lack of alkalinity in the nitrification reactor could lead to poor NH₄⁺-N removal efficiency as alkalinity is consumed during nitrification (Yoo et al., 1999). In order to overcome this effect, sodium bicarbonate (NaHCO₃) as an external source of alkalinity was added throughout the study.

Similarly, the temperature was maintained in the range of 20 to 25°C using two heating coils as temperature is another critical environmental factor that could significantly impact the activity of nitrifying bacteria in the nitrification reactor (Zhang et al., 2009). Zhang et al. (2009) observed that the highest ammonium oxidation rate was obtained at temperature of 31°C (4.7 mg NH₄⁺-N/L-h) and the lowest value was observed at a temperature of 15°C (2.1 mg NH₄⁺-N/L-h).

During the phase I, the average NH₄⁺-N removal efficiency was 86% with the highest residual effluent concentration of 130 mg/l from the nitrification reactor. The optimum pH condition for nitrifying bacteria is 7.5 to 8.6 (Yoo et al., 1999). At phase I, the average pH value was 6.9 ± 0.55 (Figure 4). So, the poor effluent quality in the nitrification reactor could be explained by the low pH value which was lower than the optimum pH value for nitrification. Excess alkalinity was supplied to the nitrification reactor by maintaining the alkalinity/NH₄⁺-N ratio at 10:1. During phases II and III, the NH₄⁺-N removal efficiencies increased to 96 and 98% with the effluent NH₄⁺-N concentrations of 22 and 10 mg/l, respectively. In the later two phases (II and III), the pH values were within the optimum pH range, that is, 8.3 ± 0.3 and 7.75 ± 0.12 (Figure 4) in which nitrifying bacteria can perform optimally.

The anoxic reactor was intermittently aereated so that SND could take place. The average DO concentration during aeration and non-aeration was 2.5 and 0.95 mg O₂/l, respectively. Figure 5 shows the concentration of NO₂⁻-N in the anoxic and oxic reactors and the NO₃⁻-N removal efficiency throughout the operating period.
phase I, high concentration of NO$_2^-$-N accumulated in the effluent of the nitrification reactor along with NO$_3^-$-N. However, the NO$_2^-$-N concentration gradually decreased in later phases. NO$_2^-$-N accumulates when DO levels become low in the nitrification reactor. In some circumstances, the activity of Nitrobactor is inhibited even at the DO concentration of 7 mg/l (Munch et al., 1996). Average NO$_3^-$-N removal efficiencies of 73, 84, and 86% were obtained at phases I, II and III, respectively.

The highest NO$_3^-$-N removal efficiency of 86% was observed during phase III. The main reason for this result is that an increase in the R value increases the NO$_3^-$-N load supplied to the anoxic reactor which consequently increases the denitrifying activity in the denitrifying reactor. However, the effluent of the anoxic reactor contained relatively high concentration of NO$_3^-$-N (25 mg/l).
This could be due to the high DO levels in the anoxic reactor (0.95 mg/l non-aeration and 2.5 mg/l aeration). The denitrification efficiency increases initially with R even at a higher DO concentrations, but further increases in R shows an inhibitory effect on denitrification (Tan and Ng, 2008). An R value greater than 5 is not recommended in the A/O process (Baeza et al., 2004). The average NO\textsubscript{x}-N removal efficiency during the whole experiment was found to be 81% with relatively poor effluent quality with respect to NO\textsubscript{x}-N.

The high NO\textsubscript{x}-N concentration in the effluent of the anoxic reactor was removed through a sulfur-packed biofilter. Autotrophic denitrification is an alternative to heterotrophic denitrification using \textit{Thiobacillus} denitrifi-
Table 3. Mass balance of NH$_4^+$-N in the anoxic reactor.

|                | Phase I | Phase II | Phase III |
|----------------|---------|----------|-----------|
| Measured NH$_4^+$-N (g/l) | 0.252   | 0.232    | 0.112     |
| Calculated NH$_4^+$-N (g/l)  | 0.261   | 0.224    | 0.140     |

Autotrophic denitrification occurs at pH values between 7.5 and 8.0 and at the temperature of 30°C (Claus and Kutzner, 1985). During all operational phases, the pH was nearly close to the optimum pH range of 8.17 ± 0.2, 8.10 ± 0.08 and 8.20 ± 0.20, respectively. The high concentrations of NO$_3^-$-N from the effluent of the anoxic reactor was successively removed during phases I, II and III in the sulfur biofilter. The effluent of the sulfur biofilter contained the NO$_3^-$-N concentrations of 1.2 to 5.3, 0.8 to 4.2 and 1.5 to 4.1 mg/l, respectively (Figure 6). It can be concluded that the sulfur-packed biofilter was effective in removing NO$_3^-$-N.

A model mass balance revealed that over 90% of the influent NH$_4^+$-N was removed from the nitrification reactor. There was no oxidation of NH$_4^+$-N in the anoxic reactor (Table 3). Along with aerobic nitrification, other physical processes such as ammonia vitalization or ammonia stripping could have been involved in removing NH$_4^+$-N in the process. More than 80% NO$_3^-$-N was removed in the anoxic reactor, whereas, an average of 9% of the NO$_3^-$-N was removed from the sulfur biofilter.

Conclusions

The A/O system was operated at three different recycle ratios to treat the digested swine wastewater. According to the results of this experiment, the average TCOD removal efficiencies were 79, 90 and 90% during phases I, II and III, respectively. Heterotrophic denitrification is responsible for reducing TCOD since heterotrophs utilize organic substrates as a source of carbon. The average total NH$_4^+$-N removal efficiency of the nitrification reactor was found to be 93% with effluent concentrations between 9 and 130 mg NH$_4^+$-N/l. The average total NO$_3^-$-N removal efficiencies during phases I, II and III were found to be 73, 84 and 86%, respectively in the anoxic reactor. The effluent NO$_3^-$-N concentrations were lower than 5 mg/l in the sulfur biofilter throughout the operational phases.

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