A TWO-STEP FED SEQUENCING BATCH REACTOR COMBINED WITH PRE-NITRITATION FOR TREATING SWINE WASTEWATER

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ABSTRACT

Scientists have identified agricultural fertilizers as a primary culprit behind the eutrophication phenomenon booming in lakes and gulfs, among which the wastewater flushed from confined swine production and applied to cropland as fertilizers is identified to be one of the major responsible agricultural sources. When the scale of swine production keeps rising, manure that cannot be land applied according to the plant and soil testing has to be treated before discharge.

Shortcut nitrification and denitrification (SND) is a novel nitrogen removal process that has drawn significant attention from researchers lately. In this study, the application of SND in swine wastewater treatment was investigated using two sequencing batch reactors (SBRs) connected in series in order to reduce the needs for energy and carbon uses. The first SBR produced a nitrite rich effluent that was subsequently fed to the second SBR where both nitrogen and phosphorus removal were taking place.

Shortcut nitrification (also termed nitritation) is the first step of shortcut nitrogen removal from swine wastewater. As such, stably obtaining an effluent with a significant amount of nitrite becomes the premise to realize SND. The possibility of accumulating nitrite from swine wastewater was firstly investigated by adopting a continuous feeding strategy in an activated sludge SBR. The results showed that free ammonia and free nitrous acid in the system could reduce the activities of nitrite oxidizing bacteria, generating an effluent with 13-23% of NH_4-N, 15-21% of NO_3-N, and 56-72% of NO_2-N. Two cyclic modes with HRTs of 3 days (the ratio of aerobic feeding to total aerobic reaction time was 0.33) and 1.5 days (the ratio was 0.77) were employed. The cycle comparison between the two modes with different HRTs shows that there is no

big difference with regard to the whole nitritation process, which is characterized by continuous conversion of loaded ammonium to nitrite and nitrate in the aerobic feeding period and no further conversion after the loading was terminated, resulting relatively stable levels for all the three nitrogen components in the entire cycle. Compared to 3-day HRT mode, 1.5-day HRT cyclic mode has doubled daily output in volume.

In order to better understand the process of nitrite accumulation, more bench experiments were performed including an effluent nitrogen composition stability test and a reducing load test. The nitrite production stability was tested using four different ammonium loading rates, 0.075, 0.062, 0.053, and 0.039 $gNH_4/gMLSS \cdot d$ in a 2-month running period. The TIN composition in the effluent was not affected when the ammonium load was between 0.053 and 0.075 g NH_4/g $MLSS \cdot d$ (64% NO_2-N, 16% NO_3-N, and 20% NH_4-N). Under 0.039 g NH_4/g $MLSS \cdot d$, a little more NO_2-N was transformed to NO_3-N with an effluent of 60% NO_2-N, 20% NO_3-N, and 20% NH_4-N. The reducing load test has revealed the relationship between a declining FNA concentration and the decreasing nitrite production. The NH_4^+ load was gradually decreased from 0.081 to 0.011 $g/gMLSS \cdot d$. When the NH_4^+ load was between 0.081 and 0.035 $g/gMLSS \cdot d$, the ratio of $NO_2^-/(NO_2^- + NO_3^-)$ was kept stable around 0.75. When the NH_4^+ load dropped from 0.035 to 0.024 $g/gMLSS \cdot d$, the ratio dropped to 0.70, accompanied by an abrupt decline of FNA from 1.2 to 0.6. From that point forward, the nitrite dominance environment in the system was no longer existing. Combining the results from both reducing load and stability tests, it is concluded that an ammonium loading rate around 0.035 is the lower threshold for producing a nitrite dominance effluent from the activated sludge SBR.

In the denitrification step, three COD/NO_x-N ratios (3.6, 4.8 and 6) and two solid retention times (SRTs), 16 and 23 days, were selected to test the influence of carbon availability and SRT on the total inorganic nitrogen (TIN) reduction and phosphorus removal efficiencies for the step-fed SBR. The best operating combination of parameters would consist of a COD/NO_x-N ratio of 4.8 and an SRT of 23 days to achieve 97% TIN and 67% dissolved phosphorus (DP) removals.

Keywords: swine wastewater, shortcut nitrification and denitrification, nitrite accumulation, SRT, COD/NO_x-N ratio

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Chapter 1. Introduction

1.1 Background

Large-scale swine production is increasing in the United States at a very rapid rate, indicated by the number of hogs produced yearly from 2.6 million in 1990 (Hunt and Vanotti, 2001) to 61 million in 2007 (USDA, 2007). This fast growth has made pork industry one of the major agricultural sectors that have monumental waste-treatment challenges. The problems are related to flushing waste from high-density confinement facilities into anaerobic lagoons and then applying the wastewater to cropland. This practice can lead to nutrient losses from row-crop land and cause non-point source water quality problems and "impaired waters." Nitrogen (N) losses due to nitrate (NO_3) leaching may cause drinking water problems and possibly worsen the hypoxia (low oxygen) problem in streams and lakes. In a similar way, phosphorus (P) losses can cause euthrophication problems in surface waters (lakes, streams, and reservoirs) where algal blooms decrease oxygen, kill fish, and result in murky and bad tasting water. With the increased expansion and consolidation of the swine industry in the United States, continuing the traditional land disposal of excess manure has become the center of scrutiny because of its potential to harm the environment by nutrients runoff and leaching. To sustain the swine industry and maintain its continued growth, effective methods of controlling nutrient output from the manure need to be developed so a protected environment and a growing swine industry can coexist.

Animal waste is made up of two distinct components, urine and faeces. The total quantity of each component and the chemical composition of each are influenced by a number of factors, including species of animal, the composition of feed ration, age, sex, environmental condition, and management methods (Robertson, 1977). Swine waste is handled differently in different parts of the country, depending on the goals and needs of individual producers. In the Mid-West, swine waste is valued for its nitrogen and phosphorus content. The goal of producers in this region is to store the manure in concentrated form to preserve nutrients until it can be applied to cropland, usually to corn. Waste collection systems at these facilities typically employ slurry systems that use no added water. In the southeast, swine farms are often on smaller tracts of land that cannot utilize the available nutrients for corn production. These areas typically utilize water wash systems and anaerobic lagoon treatment to improve the air quality in the production houses and reduce odor generated during storage. These systems produce a dilute wastewater compared to the slurry systems. The solids level for wastewater from these systems may range from 0.5 and 2 percent.

There are different treatment methods once swine wastewater is generated. Physical methods are usually used to reduce solid content of swine wastewater by separating solids from liquid as preliminary treatments before further storage or treatment. Rotary vacuum filter, pressure filter, and centrifuge are three commonly used techniques in liquid-solid separation. Chemical precipitation is another way to separate the solid from liquid swine wastewater, where organic polymers, such as polyacrylamide (PAM), are used to increase separation efficiency of suspended solids and carbon compounds from manure liquid (Vanotti and Hunt, 1999; Zhang and Lei, 1998). Although efficient, the cost of dosing polymers and the subsequent disposal of separated solids with high organic nutrient content could pose other problems.

Some of the other commonly practiced systems, such as a series of lagoons (Oleszkiewicz, 1986), extended aeration (Moriyama et al., 1990), chemical coagulation (Sievers et al., 1994), and the systems featuring land application (Nicholson et al., 2005), are found not cost-effective. In contrast, the systems combining high-rate anaerobic and aerobic unit processes could perform the same job at half or even a third of the total annual cost based on analysis of full economic effectiveness (Oleszkiewicz, 1985). This has prompted extensive studies in recent years on biological reactors characterized by anaerobic and/or aerobic settings to treat swine wastewater. Osada et al. (1991) and Bicudo and Svoboda (1995) reported on good results in reducing biochemical oxygen demand, chemical oxygen demand, nitrogen, phosphorus, and metal ions by aerobic treatment. In order to make the aerobic biological technology more applicable, efforts have thus been made to enhance the efficiency, including prolonging the pre-anaerobic process (Gerrish et al., 1975), intermittent aeration (Osada et al., 1991; Bicudo and Svoboda, 1995), and use of proper aerators (Fallowfield et abl., 1994). From experience of Bortone et al. (1992), sequencing batch reactors (SBRs) offer a good possibility of treating swine wastewater quite easily and cheaply compared to other complex flow schemes on farm scale. Sequencing batch reactor (SBR) can treat either the liquid fraction of swine wastewater or the effluent of anaerobic digesters in just one stage (Tilche et al., 1999). Because it can combine both aerobic and anaerobic phases, the aeration time can be reduced. Through the operational control of the batch sequence, it can be applied to swine wastewater treatment and possibly meets well the standard wastewater criteria for removal of organic carbon, nitrogen and phosphate.

Regulations related to swine waste

The Congress passed the Federal Water Pollution Control Act, also known as the Clean Water Act (CWA) in 1972. The CWA (33 U.S.C. Sec. 1251(a)) prohibits discharge of pollutants from a point source to waters in U.S. except those authorized by the National Pollutant Discharge Elimination System (NPDES) permits established and regulated according to the CWA.

The EPA's regulation of wastewater and manure from concentrated animal feeding operations (CAFOs) dates back to the 1970s. The existing national effluent limitations guideline and standards for feedlots were issued on February 14, 1974 (39 FR 5704). The existing NPDES CAFO regulations were issued on March 18, 1976 (41 FR 11458). In 1990s, the regulation and permitting of CAFOs was revised due to changes in the livestock industry, specifically the consolidation of the industry into fewer, but larger operations. In 1997, dialogues were initiated between EPA and the poultry and pork livestock sectors. On December 12, 1997, the Pork Dialogue participants, including representatives from the National Pork Producers Council (NPPC) and officials from EPA, U.S. Department of Agriculture (USDA), and several States, issued a Comprehensive Environmental Framework for Pork Production Operations, leading to development of a Compliance Audit Program Agreement (CAP Agreement) issued by

EPA on November 24, 1998 that is available to any pork producer who participates in the NPPC's environmental assessment program. Under the agreement, pork producers that voluntarily have their facilities inspected are eligible for reduced penalties for any CWA violations discovered and corrected.

President Clinton and Vice President Gore announced the Clean Water Action Plan (CWAP) on February 19, 1998. The CWAP describes the key water quality problems the nation faces today and suggests both a broad plan and specific actions for addressing these problems. The CWAP indicated that polluted runoff is the greatest source of water quality problems in the United States today and that stronger polluted runoff controls are needed. The CWAP goes on to state that one important aspect of such controls is the expansion of CWA permit controls, including those applicable to large facilities such as CAFOs.

The requirements that can apply to CAFOs can be found under the existing NPDES CAFO regulations and feedlot effluent limitations guidelines. The definitions of an animal feeding operation (AFO) and a CAFO can be found in existing 40 Code of Federal Regulations (CFR) 122.23. Once defined or designated as a CAFO, the operation is subject to NPDES permitting. A permit contains the specific technology-based effluent limitations (whether based on the effluent guidelines or best professional judgment (BPJ)); water quality-based limits if applicable; non-numeric effluent limitations in the form of specific best management practices; monitoring and reporting requirements; and other standard NPDES conditions.

Sequencing Batch Reactors

A sequencing batch reactor (SBR) is a fill-and-draw activated sludge system for biological wastewater treatment. It has received considerable attention since Irvine and Davis (1971) described its operation. With a flexible operation style, SBR has been successfully used to treat various biological wastewaters.

The unit processes of SBR are similar to conventional activated sludge systems that use a mixed microbial community to biodegrade wastewater constituents. It operates by a cycle of phases usually consisting of four basic sequences, i.e., FILLING, REACTION, SETTLEMENT and DISCHARGE (Figure 1.1). All of them fulfill in turns within a single bioreactor following a given regime. Compared to other wastewater treatment processes, the advantages of SBR include (Irvine and Ketchum, 1989):

- No need for flow equalization
- More rapid rates of microbial growth and substrate utilization
- The ability to achieve both biological nitrification and denitrification
- No need for an external solids separation device (e.g., gravitational clarifier)
- High flexible operational control

Among these advantages, the avoidance of both flow equalization and gravitational clarifiers are advantageous to treat swine wastewater wastewaters. The operational simplicity of SBR is particularly pertinent and desirable. On the other hand, biological wastewater treatment is a technology currently unfamiliar to most farmers, so research in this area can greatly promote the educational effort on this technology.

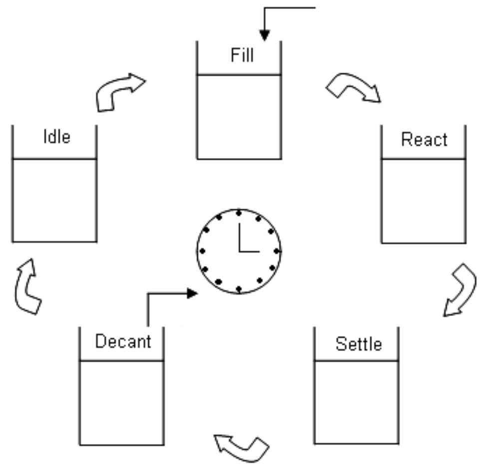


Fig. 1.1 Schematic of an SBR cycle.

Step-fed SBRs

Organic carbon source is a crucial parameter for nutrient removal in the biological removal system, since it generally serves as an electron donor for denitrification and is essential for phosphorus release by phosphorus accumulating organisms (PAOs). However, incomplete denitrification due to a deficient carbon supply was observed in most cases of SBR treating swine wastewater (Guo et al., 2007).

To solve the carbon deficiency issue, step-fed sequencing batch reactor (SFSBR), a new type of SBR with a modified feeding pattern, was recently demonstrated favorable for nutrients (N, P) removal of wastewater by providing an organic carbon source supplement. (Bortone, et al., 1992; Tilche et al, 1994; 2001; Andreottola et al., 2001; Puig , et al., 2004; Zhang et al., 2006; Han, et al., 2007). During the SFSBR process, the REACTION stage was divided into several consecutive non-aeration/aeration sub-cycles, and feeding was conducted at the beginning of each non-aeration phase. As a result, the organic matter contained in the influent was mainly anoxically or anaerobically consumed during the non-aeration phase as carbon source for denitrifiers and PAOs, and thus the process of biological nutrients removal could be enhanced significantly. A feeding ratio greater than 1.0 was adopted in some of the past studies (Bortone, et al., 1992; Zhang et al, 2006; Han, et al., 2007).

The previous research in our group (Zhang et al., 2005) achieved satisfactory removal of the total nitrogen (97.5%), total P (95%), COD (96%), and BOD_5 (100%) by utilization of the activities of denitrifying phosphorus accumulating organisms (DNPAOs) in a non-detectable DO environment under low-intensity aeration (1.0 L/(m^3·s)) in a bench-scale SBR system operated in a two-step fed mode. However, Han et al. (2007) could not be able to gain the same high efficiencies of dissolved phosphorus (DP) and total inorganic nitrogen (TIN) removal under the same non-detectable DO condition at aeration intensity of 2.1 L/m^3·s. Only 68% and 60% removal rates were obtained for DP and TIN, respectively. The results from my preliminary experiments also showed that good nitrification could be established but good denitrification could not be obtained with high concentrations of nitrate (25-115 mg/L) always present in the effluent. The reason for the difference between ours and previous studies might be attributed to the unstable performance of the bacteria and low fraction of DNPAO in the normal PAO. Additionally, another disadvantage of DNPAO is that it has a lower substrate utilisation efficiency in anoxic conditions compared with nornal PAO in aerobic conditions (Spagni et al., 2001).

Therefore, it was concluded that the overall removal efficiencies for nitrogen and phosphorus could be improved but might not be guaranteed by using step-fed mode of a SBR only for treating concentrated wastes like swine wastewater.

Shortcut Nitrification and Denitrification

Novel processes for removing nitrogen from wastewaters, such as shortcut nitrification and denitrification (SND), anaerobic ammonium oxidation (Anammox), completely autotrophic nitrogen removal over nitrite (Canon) process, have been under study for many years (Verstraete and Philips, 1998). Partial nitrification to nitrite was reported to be technically feasible and economically favorable, especially when wastewater with high ammonium concentrations or low C/N ratios was the center of concern (Hellinga et al., 1998).

In case of using complete nitrification-denitrification, a high amount of oxygen and supplementation of extra carbon sources are required. However, such requirements can be softened if ammonium is only oxidized to nitrite (rather than nitrate) that is subsequently denitrified in the biological nitrogen removal treatment. Therefore, the SND process is based on the fact that nitrite is an intermediate in both nitrification and denitrification steps, i.e., a partial nitrification up to nitrite is performed followed by nitrite denitrification, hence omitting the step of nitrite oxidation to nitrate as shown in Fig. 1.2 (Ferhan, 1996; Fdz-Polanco et al., 1996).

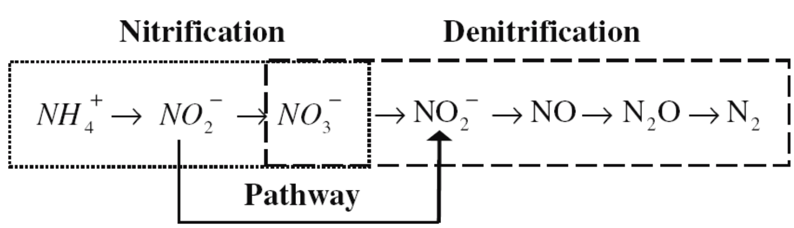


Fig. 1.2 Biological nitrification–denitrification via nitrite pathway.

Compared to the traditional nitrification and denitrification, the main advantages of shortcut nitrification and denitrification can be summarized as follows (Beccari et al., 1983; Turk and Mavinic, 1987; van Kempen et al., 2001):

(1) 25% lower oxygen consumption in the aerobic stage, implying 60% energy savings;

(2) lower electron donor requirement (up to 40%) in the anoxic stage;

(3) 1.5 to 2 times higher denitrification rates with nitrite than with nitrate;

(4) reduction of CO_2 emission by 20%;

(5) 33-35% lower sludge production in the nitrification process and 55% in the denitrification process.

With all these advantages, biological nitrogen removal via the nitrite pathway can be defined as an environmentally cleaner process, reducing aeration and chemical costs.

The critical and difficult part of applying this technology is to maintain partial nitrification of ammonium to nitrite. This could be accomplished by selectively inhibiting nitrite oxidizing bacteria (NOB) through appropriate regulation of the system's dissolved oxygen (DO) concentration, microbial retention time, pH, temperature, substrate concentration and load, operational and aeration pattern, and regulation of inhibitory factors (Peng and Zhu, 2006).

Nitrite accumulation can be obtained by maintaining operational conditions favoring the growth of ammonium oxidizing bacteria (AOB) while inhibiting NOB such as:

(1) higher temperature. The specific growth rate of AOB is greater compared with NOB at temperatures more than 25°C. (Hellinga et al., 1998);

(2) lower DO. Since the oxygen saturation coefficients (Ks) of Monod kinetics for ammonia oxidation and nitrite oxidation are known to be 0.3 and 1.1mg/L, respectively. The critical values of DO for realizing nitrite accumulation recorded in the literature were in the range of 1.0–1.5 mg/l (Wiesmann, 1994; Ruiz et al., 2003; Ciudad et al., 2005).

(3) within the levels of free ammonia (FA, NH_3, 0.1-1.0 mg/L) and free nitrous acid (FNA, HNO_2, 0.2-2.8 mg/L) (Anthonisen et al., 1976).

It has been reported that denitrification rates with nitrite are 1.5-2 times greater than with nitrate (Abeling and Seyfried, 1992). However, the same researchers also proposed that an HNO_2 concentration of 0.13 mg/L was the toxicity threshold to denitrifying bacteria. An inhibition mechanism proposed for HNO_2 toxicity is that it acts as an uncoupler by donating a proton inside the cell, which directly interferes with the trans-membrane pH gradient required for ATP synthesis (Glass et al., 1997). Besides, nitrite also has a known influence upon microorganisms and their metabolic processes. And it is a known bacteriostatic molecule due to its affinity for the metal ions in the center of enzymes (Wild et al., 1995). Therefore, when using the shortcut nitrification and denitrification process, cautions are needed to avoid the adverse effect of nitrite or nitrous acid on the activities of microorganisms.

1.2 Objectives

The overall goal of this study is to evaluate the possibilities of applying SND to swine wastewater treatment using two SBRs connected in series in order to reduce energy and carbon supply needs. Specific objectives of the research are to: (1) test the potential of accumulating nitrite from swine wastewater in the first SBR with a continuous feeding strategy; (2) compare the nitrite production of two continuous feeding patterns with different feeding lengths and test the stability of the treatment by examining the effluent inorganic nitrogen composition; and (3) determine the optimum COD/NOx-N ratio and solid retention time (SRT) for the best performance of the second SBR, focusing on significant reduction of total inorganic carbon (TIN).

1.3 Dissertation Outline

The three main chapters of this dissertation are written in research paper format. Chapter 2 describes investigation of the possibility of accumulating nitrite from swine wastewater in a conventional activated sludge SBR (named nitritation SBR) run on a continuous feeding strategy and the comparison of nitrite production capabilities of two cyclic modes with different feeding lengths. Chapter 3 presents the results from a stability test and a reducing load test for the nitrite accumulation. Chapter 4 focuses on the effects of COD/NO_x-N and SRT on the removal efficiencies of TIN and DP. Finally, the conclusions and future research needs are provided in Chapter 5.

Chapter 2.

Possibility Test of Accumulating Nitrite from Swine Wastewater in a Sequencing Batch Reactor

2.1 Abstract

Shortcut biological nitrogen removal is a process based on nitrification and denitrification via nitrite, rather than nitrate, so both the aeration needed in nitrification and the organic carbon required by denitrifiers can be reduced. This research investigated the possibility of accumulating nitrite in swine wastewater treated in an activated sludge sequencing batch reactor (SBR). The SBR was operated under HRTs of 3 days and 1.5 days for one month. Ammonium loadings were increased from 0.04 kg NH_4-N/m^3/d to 0.7 kg NH_4-N/m^3/d. The nitrite/nitrate ratio (NO_2/NO_3) in the effluent lay mainly within the range of 3~4, generating an effluent with 13-23% of NH_4-N, 15-21% of NO_3-N, and 56-72% of NO_2-N. The results showed that free ammonia and free nitrous acid could reduce the activities of nitrite oxidizing bacteria but not completely inhibit them. Nitrite accumulation from swine wastewater could be achieved by using a continuous feeding strategy for the SBR.

2.2 Introduction

The cleanup and disposal of wastewater from swine-production is one of the nation's greatest environmental challenges. In practice, land application of pre-stored manure in lagoons (Barker, 1996), which usually occurs twice a year, is long considered to be a least expensive disposal method to restore the soil productivity. However, runoff from over-saturated soil carrying nitrogen and phosphorus would finally end up in surface waters, causing eutrophication which threatens aquatic lives and deteriorates water quality (Turner et al., 1997; Karlen et al., 2004). Thus, novel and cost effective methods for treating excess swine wastewater need to be explored in order to sustain the long-term development of swine industry.

Contrary to anaerobic treatment, aerobic treatment is more efficient but requires high energy input, especially for treating ammonium laden swine wastewater because a substantial amount of oxygen is needed for oxidizing ammonium all the way to nitrate in the nitrification process. To avoid the full-length aeration, a process called "shortcut nitrification" was developed in which ammonium is only oxidized to nitrite instead of nitrate, which could eliminate the oxygen needed in oxidation of nitrite to nitrate, thus potentially saving the oxygen demand by 25% (Ruiz et al., 2003).

To remove nitrogen from wastewater, successful denitrification of nitrate or nitrite in the treated wastewater to nitrogen gas is essential, which is closely related to the availability of easily biodegradable carbon present in the wastewater. Being the electron donor of denitrifiers, the biodegradable carbon is a critical factor determining the denitrification efficiency (Han et al., 2008). The ratio of chemical oxygen demand (COD) to total Kjeldahl nitrogen (TKN) lies between 3 and 6 according to the analysis of swine wastewater samples in our lab (data not shown here). As a rule of thumb, 4 g BOD_L/g NO_3^--N is required in denitrification (BOD_L is the ultimate biochemical oxygen demand

and is less than COD) (Rittmann and McCarty, 2001). The inert COD fraction, which includes inert soluble and particulate COD fractions, could account for 42−84% of the total COD in piggery wastewaters reported by Boursier et al. (2005). Thus it was suggested that the design of biological process for piggery wastewater should be done using biodegradable organic matter instead of total organic matter. Given the above two facts, complete denitrification of swine wastewater would hardly be achievable in practice due to the deficit of biodegradable organic carbon. Since shortcut denitrification via nitrite is a process that reduces the COD needed for the conversion of nitrate to nitrite, saving 40% of total carbon demand (Fux et al., 2006) could be achieved, providing a potential solution to the carbon shortage problem for swine wastewater treatment.

Different types of reactors have been successfully used to accumulate nitrite from wastewaters, such as completely and partially submerged rotating biological contactors (RBC) (Antileo et al., 2007), aerobic granular sludge sequencing batch reactors (SBR) (Wang et al., 2007), fixed airlift biofilm reactors (Kim et al., 2006), and suspended activated sludge reactors (Oyanedel-Craver et al., 2005). All of them used the competitive advantages of ammonia oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB) under conditions when either the dissolved oxygen (DO) was limited or free ammonia (FA) and free nitrous acid (FNA) were present at certain levels. The disadvantage of using DO as the controlling parameter is that the overall nitrification rate would decline when DO concentration is maintained too low (Wang et al., 2004; Joo et al.,2000) because it is the substrate for both AOB and NOB. In the meantime, inhibition by FA and FNA on NOB was confirmed by a number of researchers (Hellinga et al., 1999; Anthonisen et al., 1976; Yun et al., 2003). Anthonisen et al. (1976) investigated the range of inhibiting concentrations of ammonium for nitrite accumulation and found that the inhibition on NOB occurred at 0.1-1.0 mg/L of FA and 0.2-2.8 mg/L of FNA. By adjusting pH, the wastewater feeding strategy, and the reactor type, the FA and FNA concentrations could be controlled at desired levels, which are important in nitrite accumulation (Yun et al., 2003).

Sustained nitritation has also been reported in (sequencing) batch-type reactor operation (Yoo et al., 1999; Fux et al., 2003; Lai et al., 2004; Peng et al., 2004). The termination of aeration prior to or at the completion of ammonium oxidation in a process with nitrite accumulation has been suggested as a key factor leading to sustained nitritation (Yoo et al., 1999; Fux et al., 2003; Peng et al., 2004).

Swine wastewater contains a high level of ammonia, which can be used to produce either FA or FNA at inhibitory levels on NOB, leading to termination of the nitrification process at nitrite. This feature can be combined with a (sequencing) batch-type reactor operation which has been proved effective to achieve sustained nitritation by several researchers (Wang et al., 2007; Fux et al., 2003; Ganigue et al., 2007). Based on the above aspects, the objective of this study was to investigate the possibility and potential of accumulating nitrite by partial nitrification of swine wastewater containing high ammonium using an activated sludge SBR.

2.3 Materials and Methods

Manure Source

Raw swine wastewater was collected once a week from a reception sump of a finishing barn at the University of Minnesota Southern Research and Outreach Center, where fresh manure in a shallow pit inside the barn was flushed out biweekly. The characteristics of the raw wastewater analyzed included pH, suspended solids (SS), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), biochemical oxygen demand (BOD_5), NH_4-N, total Kjeldahl nitrogen (TKN), NO_2-N, and NO_3-N. If not used immediately, the collected manure was stored at 4°C or below.

Nitritation Sequencing Batch Reactor (NSBR) Set-Up

A 12 L lab-scale SBR with a working volume of 10 L was used in this study, which was equipped with influent feeding/effluent discharging and air supply subsystems (Fig. 2.1 and Fig. 2.2). The SBR was operated at room temperature (20±3°C) without temperature control. The air was provided by a vacuum pressure pump (Barnant 60010-2392, USA) with the aeration intensity kept at 10 liters per minute (LPM) to keep the biomass completely mixed without using a mechanical stirrer. The running time of the air pump was controlled by a timer (BH-94460-45, Cole-Parmer Instruments Co., USA). Two peristaltic pumps (MasterFlex 7550-30, USA) were used for feeding and discharging, respectively. A mixer (Servodyne 50003-20, Cole-Parmer Instruments Co., USA) was installed in the influent tank for complete mixing at a constant rate of 80 rpm during the feeding period of SBR. The pumps and the mixer were automatically controlled by a computer program WIN LIN V1.2 (Cole-Parmer Instrument Co., USA).

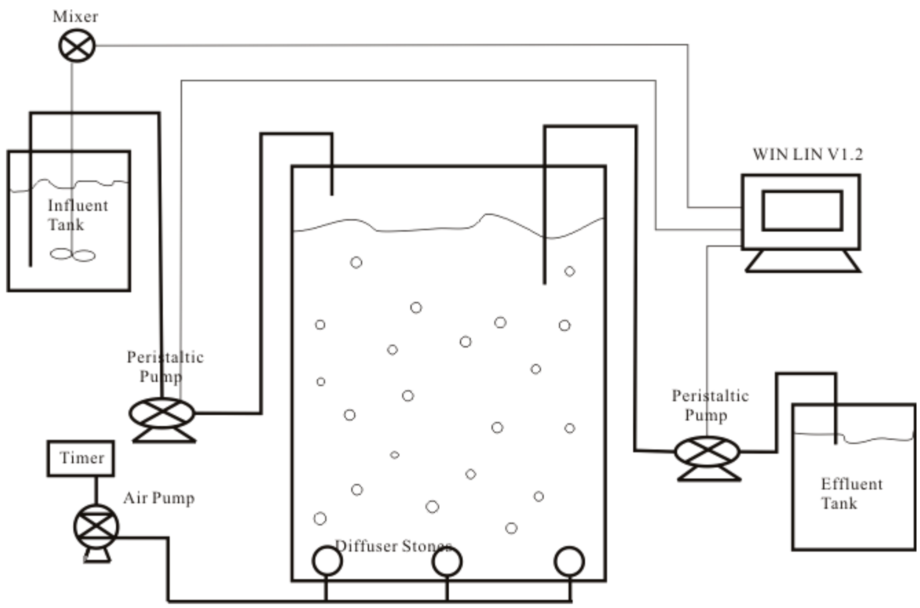


Fig. 2.1 The diagram of the nitritation SBR.

Fig. 2.2 The real system of the nitritation SBR (Omitting the influent and effluent tanks).

NSBR Operation

The SBR was first seeded with sludge from a steady-state SBR in our lab treating swine wastewater. After seeding, the reactor was fed with the collected swine wastewater at different dilutions to examine its flexibility of handling wastewater at different ammonium levels, and also to allow for an adaptation period for the microorganisms inside the reactor to acclimate to the increasing ammonium loading rates. The dilution rate was calculated by dividing the sum of volumes of the swine wastewater and added tap water by the volume of the swine wastewater. The dilution rate applied, which was gradually decreased from around 10 to 1.2, was determined by desired influent ammonium concentrations (from around 100 mg/L to 1300 mg/L) and the ammonium concentration in the raw swine wastewater on every single day during the experimental period.

During the 1-month test period of the NSBR, two 8-h cyclic operation modes were examined (Fig. 2.3), one running for 20 days (HRT = 3 days) followed by the other for 9 days (HRT = 1.5 days). The first mode of 20 days consisted of 2h 19 min aerobic feeding, 4h 41 min aerobic reaction, 30 min settling, 12 min withdrawal and 18 min idle (so the ratio of aerobic feeding to total aerobic reaction time is calculated as 2h 19 min/(2h 19 min+4h 41 min) which equals 0.33. Influent of 1.11 L was added to the reactor for each cycle so the hydraulic retention time (HRT) was kept at around 3 days, which means 3.33 L influent was added into the reactor on a daily basis (the reactor working volume: 10 L). In order to increase the daily treatment capacity, the second mode of 9 days doubled the aerobic feeding time to 4h 38 min, with 2.22 L influent added for each cycle and 6.66 L added daily. The corresponding HRT was thus reduced to 1.5 days. To maintain the 8-hour cycle time, the aerobic reaction was shortened to 1h 22min, with 30 min settling, 24 min withdrawal, and 1h 6min idle. As such, the ratio of aerobic feeding to total aerobic reaction time is calculated as 4h 38 min/(4h 38 min+1h 22 min) which equals 0.77. Sludge withdrawal was minimally performed in order to maintain the proliferated biomass in the system during the increasing loading period, obtaining a sufficient solid retention time (SRT) for autotrophic nitrifiers which require a SRT of 20 days in an activated sludge SBR to achieve complete nitrification with a synthetic wastewater containing approximately 1,000 mg ammonia-nitrogen (Zimmerman et al., 2004). Within the first cyclic mode test period (20 days), the influent ammonium concentration was increased from 127 mg/L to 1301 mg/L (127, 137, 206, 466, 555, 502, 545, 1023, 1005, 931, 1138, 1272, 1135 and 1301 mg/L), with one effluent sample for each ammonium level collected and analyzed (14 total). The mixed liquid suspended solid (MLSS) increased from 2150 to 7449 mg/L in this period. Within the second cyclic mode test period (9 days), the influent ammonium concentration was varied between 708 mg/L and 1018 mg/L (791, 708, 666, 890, 1018 and 848 mg/L), from which 6 effluent samples were collected and analyzed. The MLSS varied between 5867 and 9567 mg/L in this period.

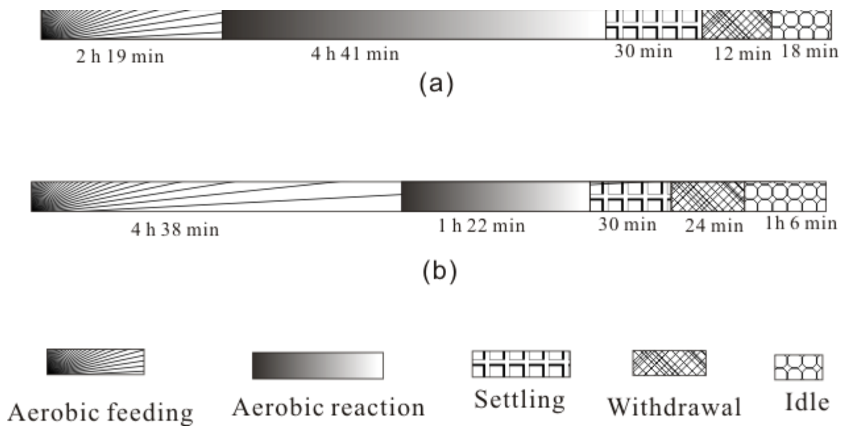


Fig. 2.3 Two applied operational modes. (a) Cycle mode 1; (b) Cycle mode 2.

The experiment was designed to study the possibility of obtaining a nitrite rich effluent. The control (independent) variables included HRT and the influent ammonium concentration, while the dependent variables were the respective concentrations of nitrite, nitrate and ammonium in the effluent. Two HRTs were chosen for the study, i.e., 3 and 1.5 days. Under the 3-day HRT, a total of 14 different influent ammonium concentrations were recorded, while under the 1.5-day HRT, the number was reduced to 6. The influent of different ammonium concentrations was obtained by diluting the raw swine wastewater with tap water. No replications within each sampling day were performed, which was common for this kind of experiments with data evoluting along the timeline, and was previously used by others in similar research (Yun et al., 2003; Ruiz et al., 2003). The comparison between the two HRTs, 3 days and 1.5 days, was done by conducting pH, nitrite, nitrate and ammonium analysis for one of their typical cycles respectively.

Analytic Methods

Influent samples were drawn from the homogenized liquid manure at approximately the mid-depth of the influent tank during agitation with a motorized paddle-stirrer. Effluent samples of 100 mL each were taken from the effluent tank when one cycle was completed. Parameters of DO, pH, MLSS, and sludge volume index (SVI) were measured daily for samples collected 20 minutes before the end of aeration in a cycle according to the standard methods (APHA, 2005). DO in the reactor was measured by a dissolved oxygen meter (YSI 5000, USA). pH was measured by a portable pH meter (Orion 210A, Thermo Scientific, USA). For on-line chemical measurement within a cycle, 20 mL of mixed liquid was drawn at an interval of 0.5 h by a 25 mL syringe then injected into a 15 mL centrifuge tube. After centrifuging at 5000 rpm for 10 min, the supernatant was obtained for analyses of ammonium (NH_4^+–N), nitrite (NO_2^-–N) and nitrate (NO_3^-–N). Measurements of NH_4^+, NO_2^-, NO_3^-, and COD were performed for both influent and effluent liquid samples following the Hach DR2800 Spectrophotometer Manual (Hach, 2005). Chemical oxygen demand (COD) was measured by the reactor digestion method using COD digestion TNT 822 vials (Hach, USA), nitrite (NO_2^--N) by the diazotization method using TNT 839 vials (Hach, USA), and nitrate (NO_3^--N) by the cadmium reduction method using TNT 835 vials (Hach, USA). Total Kjeldahl nitrogen (TKN) in raw swine wastewater was measured by the digestion-titration method using a nitrogen analyzer (Kjeltec™ 2300 Analyzer Unit, Foss, Denmark).

2.4 Results and Discussion

Analysis of Raw Swine Wastewater

The characteristics of raw swine wastewater collected from the finishing barn at Waseca were pH 8.21-8.33, 6000-15,000 mg L^{-1} SS, 8,000-11,000 mg L^{-1} TCOD, 6,400-8,500 mg L^{-1} SCOD, 3000-5000 mg L^{-1} BOD_5, 1400-1800 mg L^{-1} NH_4-N, and 2000-2400 mg L^{-1} TKN. NO_2-N and NO_3-N were negligible.

Start-Up of Nitrite Accumulation

The start-up of the production of a nitrite dominant effluent was trial tested by applying different ammonium concentrations to the system. When the MLSS in the SBR was initially around 1800 mg/L with an influent ammonium concentration around 220 mg/L, nitrite and nitrate were rarely produced, indicating that the nitrifying bacteria could not adapt to this ammonium load and all nitrifying activities were largely reduced, if not inhibited. The nitrite started to accumulate when the influent ammonium was initilally reduced to 65 mg/L for one day (the corresponding nitrite concentration was 11.2 mg/L) and kept at around 100 mg/L for two days with a resulting nitrite concentration of 104 mg/L on the fourth day. Then on the fifth day when the influent ammonium was 127 mg/L, the effluent nitrite, nitrate and ammonium concentrations were 104 mg/L, 32.2 mg/L and 9.63 mg/L respectively, implying that nitrite became the dominant product in the effluent. The reason why the sum of the effluent nitrite, nitrate and ammonium was greater than the influent ammonium is possibly due to the conversion of the residual ammonium in the system from previous cycles.

COD Removal Efficiencies

Fig. 2.4 shows the profiles of influent and effluent COD over the experimental period. The effluent COD concentrations were corrected for NO_2-N interference (minus 1.1 mg/L COD per 1 mg/L NO_2-N). In the period of the first HRT (3 days), the COD removal efficiencies in the first two days were around 66%, indicating that the sludge was still adapting itself to the organic loading. After that, the average removal efficiency was 85% in the remaining days under the HRT of 3 days. After day 20, the COD removal efficiency dropped to the range of 62-77% due to the operating scheme change from 3-day HRT to 1.5-day HRT that increased the hydraulic loads.

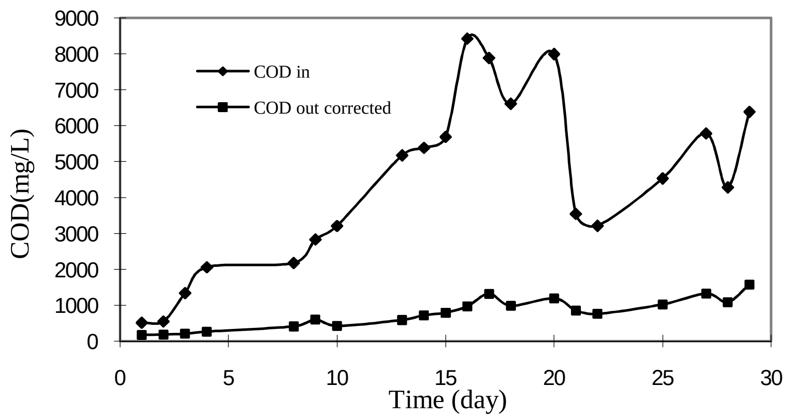


Fig. 2.4 Trends of COD in influent and effluent after corrected for NO_2^- interference

The COD removal efficiency appears to be not entirely associated with the operating HRT of the SBR. Take day 20 for example, the dilution rate was set around 1.2, i.e., the reactor almost was fed with raw swine wastewater. Given the adopted aeration intensity (10 LPM), biomass concentration (7450 mg MLSS/L), and HRT (3 days), the COD in the effluent after correction was 1188 mg/L as opposed to 7992 mg/L in the influent, indicating that a portion of COD in the treated swine wastewater was not easily biodegradable. Bortone et al. (1992) achieved COD removal of 93% by adopting a HRT of 10 days, SRT of 34 days, and MLSS of 13500 mg/L, but still observed a certain amount of of non-biodegradable COD present in the effluent of an SBR. In wastewater effluents, the non-biodegradable fraction constitutes compounds present in the raw wastewater plus those non-biodegradable substances produced by the microorganisms. Pribyl et al. (1997) showed that the amount of soluble microbial products (SMP) in the effluent from an SBR depended on the sludge age. The minimum SMP concentrations could be achieved at sludge ages ranging between 5 and 15 days. At sludge ages outside that range, SMP concentration would increase. Under the possibility test period studied here, the withdrawal of excessive sludge was rarely performed, surely resulting in a sludge age greater than 15 days, which could partially contribute to the considerable amount of non-biodegradable COD remaining in the effluent.

Activated Sludge Properties

The mixed liquid suspended solids (MLSS) and sludge volumetric index (SVI) over the study period were shown in Fig. 2.5. The MLSS rose continuously from 2000 mg/L at the beginning to about 7500 mg/L on day 20. Meanwhile, the SVI decreased gradually from initially 240 mL/g to around 60 mL/g on day 20, indicating that good settleability was achieved eventually, which was often accompanied by increasing sludge age (Kargi et al., 2006). Before the second operating mode started, some sludge was washed out from the system, causing MLSS to decline from 7500 mg/L to around 6000 mg/L. Nonetheless, the SVI was still kept under 80 mL/g afterwards.

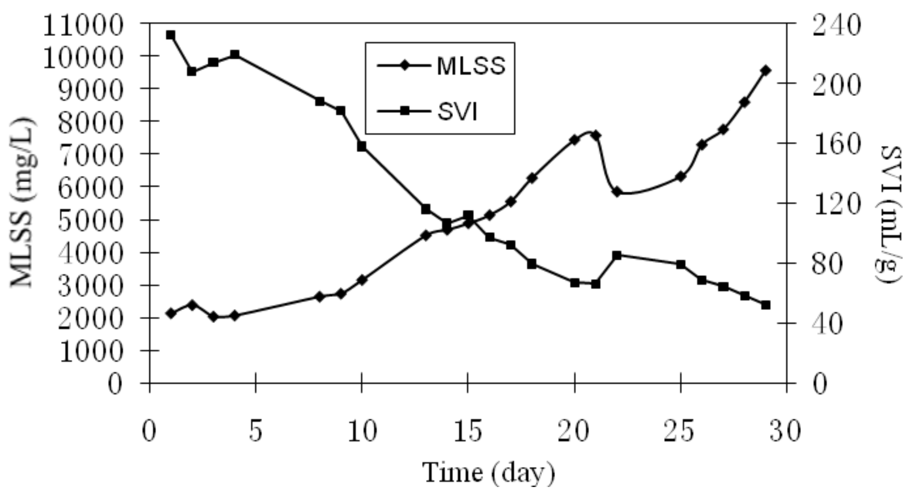


Fig. 2.5 Changes in MLSS and SVI over time

Profiles of NH_4^+, NO_2^-, NO_3^-

Fig. 2.6 presents the trend of nitrogenous compounds (NH_4^+ in the influent and effluent, NO_2^-, NO_3^- in the effluent) in the experimental period. The nitrite ratio (NO_2-N/(NO_2-N+NO_3-N)) in the effluent during the whole operation under two different HRTs was within the range of 73%-82%, which showed that the activity of NOB had been successfully reduced (Ciudad et al., 2005). The nitrite/nitrate ratio (NO_2-N/NO_3-N) in the effluent lay mainly within the range of 3~4 (Fig. 2.7), implying that a certain ratio of ammonia oxidizers to nitrite oxidizers was established in the system, with an effluent containing 13-23% of NH_4-N, 15-21% of NO_3-N, 56-72% of NO_2-N generated constantly. Lower HRT means larger daily feeding volume, which is always preferred if the same treatment efficiency can be obtained. Our results showed that HRT didn't obviously affect the composition of effluent nitrogenous compounds so a longer feeding time would lead to a greater nitrite production rate (Table 2.1). For example, the nitrite production rate was 0.34 kg NO_2-N/m^3/d under the HRT of 1.5 days compared to 0.17 kg NO_2-N/m^3/d under the HRT of 3 days for the same influent ammonium concentration of around 1000 mg/L.

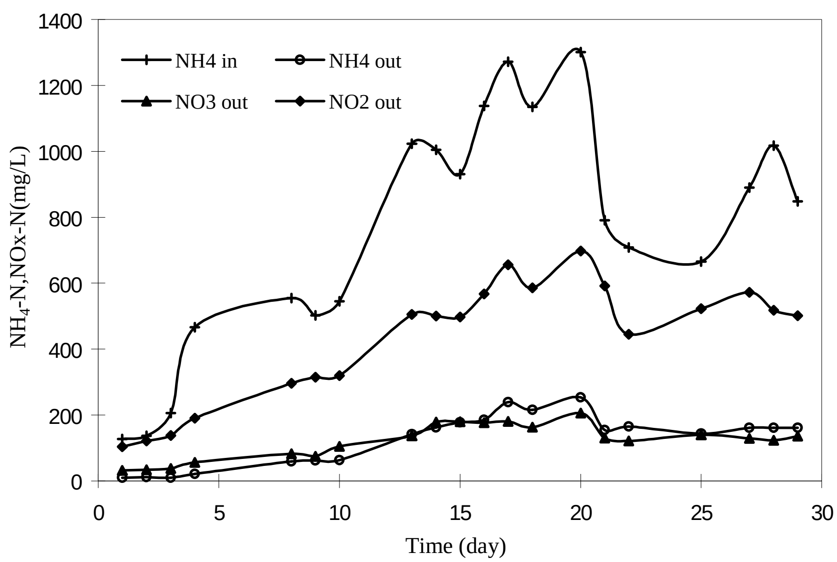


Fig. 2.6 The profiles of nitrogenous compounds (NH_4-N, NO_3-N, NO_2-N) in influent and effluent.

Table 2.1 Nitrite production rates in the 1-month operation period

HRT (days)	3					1.5	
Time (Day)	1	3	9	14	20	22	28
Influent NH_4^+ concentration (mg/L)	127	206	502	1005	1301	708	1018
NO_2^- production ($kg/m^3/d$)	0.035	0.046	0.10	0.17	0.23	0.30	0.34

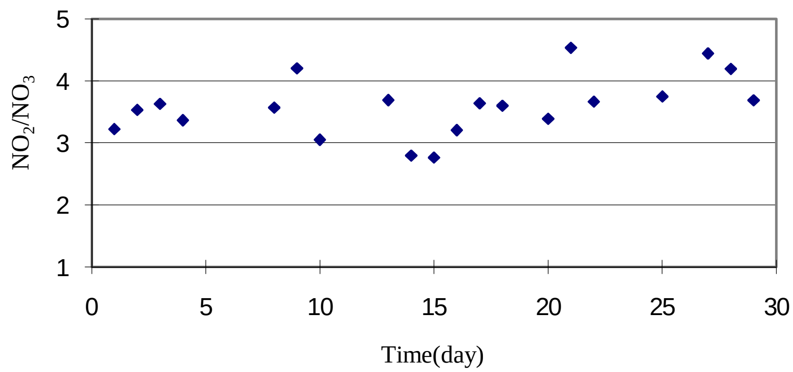


Fig. 2.7 Time course of nitrite ratio (NO_2-N/NO_3-N) during the experimental period.

Profiles of DO and pH

The profiles of DO and pH evolution are presented in Fig. 2.8. Since aeration was intensively supplied, the level of DO was always maintained between 5.9-9.0 mg/L in the bulk liquid without control, which is close to the DO saturation concentration in water, 9.07 mg/L, at 20°C. Compared with 0.7 mg/L used by other researchers (Kim et al., 2006; Oyanedel-Craver et al., 2005) as the controlling parameter for nitrite accumulation, the DO level in our study was obviously not the limiting factor contributing to nitrite production. A bell-shaped empirical model on the pH-dependent behavior of the maximum specific substrate utilization rates of ammonium and nitrite oxidizers (Park et al., 2007) suggested that a higher pH is good for nitrification and nitrite accumulation. As shown in Fig. 2.8, the value of pH was approaching 5.5 on day 17, suggesting that protons were accumulated in the reactor without pH control. The ammonia oxidizing rate could be greatly reduced when pH was too low. When the ammonia loading time doubled, pH was raised from 5.5 to above 6.5, accompanied by a doubled nitrite production rate even with reduced aeration (1 hour less).

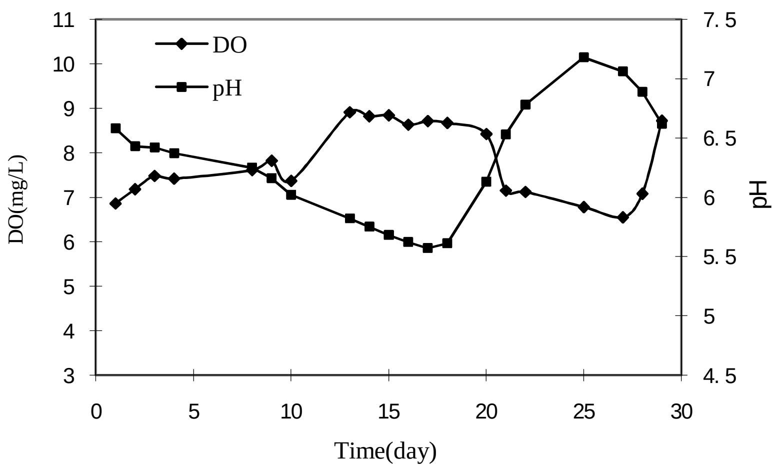


Fig. 2.8 Variation of pH and DO during the experimental period.

Free Ammonia and Free Nitrous Acid Production

The FA and FNA concentrations can be expressed as follows (Equ. (2-1) and Equ. (2-2)) (Anthonisen et al., 1976):

$$NH_3\text{-N (FA)} = NH_4\text{-N}*10^{pH} /[\exp(6344/(273+T))+10^{pH}] \qquad (2\text{-}1)$$

$$HNO_2\text{ (FNA)} = NO_2\text{-N} /[\exp(-2300/(273+T))\times 10^{pH}] \qquad (2\text{-}2)$$

The time courses of calculated FA and FNA levels are shown in Fig. 2.9 and 2.10. The FA concentrations in the mixed liquid increased from 0.02 mg/L at the beginning to 0.2 mg/L on the last day of the first operating mode period. Meanwhile the FNA concentration increased from 0.2 mg/L to around 15 mg/L on day 17 (the highest level in the study). In the second operating period, the FA had a significant rise within the range of 0.3-1.1 mg/L and the FNA concentration dropped into the range of 0.3-1.5 mg/L. According to the research done by Anthonisen et al. (1976), NOB were inhibited by FA at levels above 0.1 mg/L and all nitrifying bacteria were inhibited by FNA at levels above 0.2 mg/L. Apparently, the FNA was the inhibitor in the first operating period contributing to nitrite accumulation when the FA concentration was not reaching 0.1 mg/L until day 20. And in the second period, the FA was kept at above 0.3 mg/L which could become an inhibitor to nitrite oxidizers. Nitrous acid had always been kept at above 0.2 mg/L, although it dropped dramatically in the second operating period compared to the first one because of the elevated pH due to the increased ammonium loading. However, the activities of ammonia oxidizers seemed not completely inhibited by the persistent presence of FNA at the inhibitory level, evidenced by the continuous production of nitrite and nitrate during the whole experimental period. This phenomenon illuminates that nitrite oxidizers could adapt to a high FNA level through gradual acclimatization. Adaptation of nitrite oxidizers to FA and FNA was also observed by Turk and Mavinic (1986) at a FA concentration of 22 mg/L.

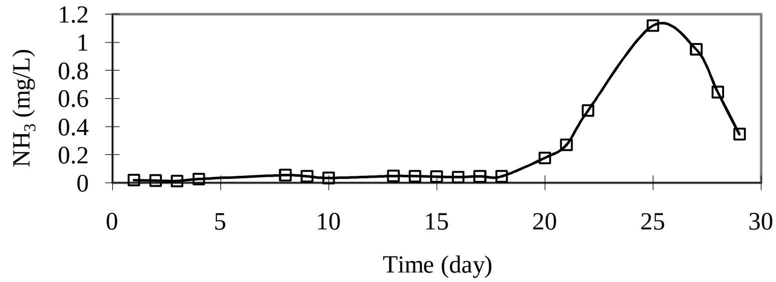


Fig. 2.9 Time course of free ammonia (FA, NH_3) in bulk liquid.

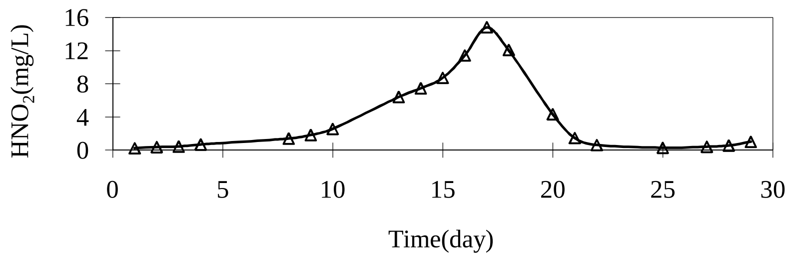
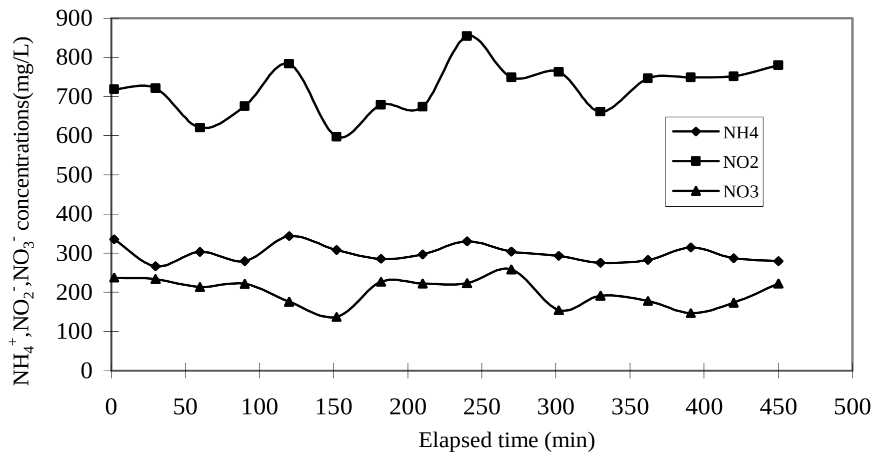


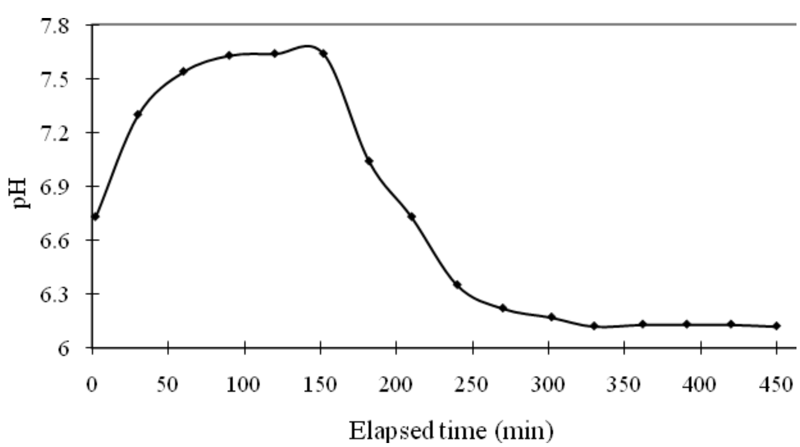
Fig. 2.10 Time course of free nitrous acid (FNA, HNO_2) in bulk liquid.

Cycle Data of the Two Different Operational Modes Under 3 and 1.5 Days of HRT

Fig. 2.11(a) shows the profiles of NH_4^+, NO_2^-, NO_3^- during one typical cycle under the HRT of 3 days with an influent NH_4^+ concentration of 1301.2 mg/L, in which NH_4/MLSS load can be calculated to be 0.058 g/g·d. During the whole cycle, NO_2^- concentration was between 600 and 850 mg/L; NO_3^- was between 140 and 260 mg/L and NH_4^+ was between 270 and 340 mg/L. So the effluent was a mixture of 61% NO_2^-, 17% NO_3^-, and 22% NH_4^+. The fluctuations of NO_2^- and NO_3^- concentrations within the cycle might relate to the simultaneous nitrification and denitrification (SND) happened in the reactor within the activated sludge flocs where nitrification was restricted to the outer oxic zone whereas denitrification occurred mainly in the inner anoxic zones (Satoh et al., 2003; Zeng et al., 2003). The three nitrogen components were kept relatively stable all along in the cycle without considering those local waves, even after the feeding was finished, indicating the inhibitory effect of a relatively acidic environment on the activities of nitrification microorganisms. There were no obvious further transformations from NH_4^+ to NO_2^- and NO_3^- in the aerobic reaction period as shown in Fig. 2.11(a).



(a)



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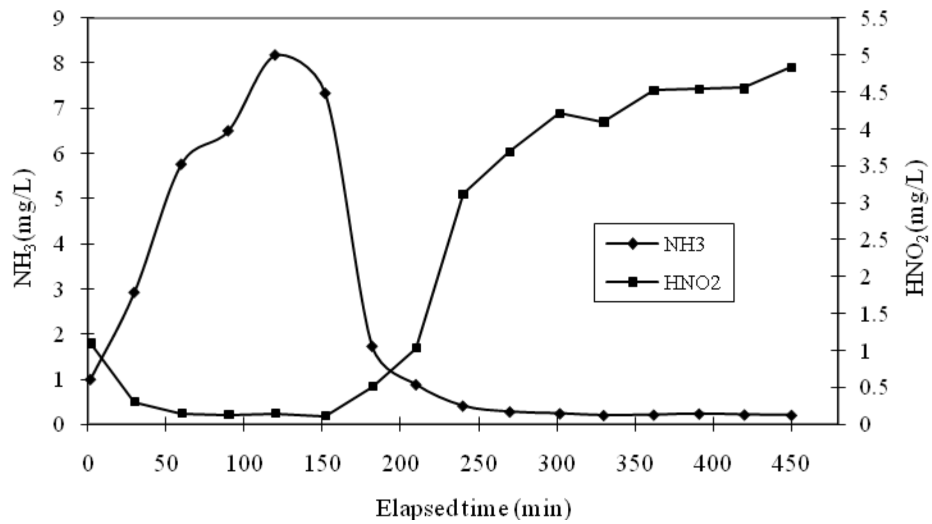


(c)

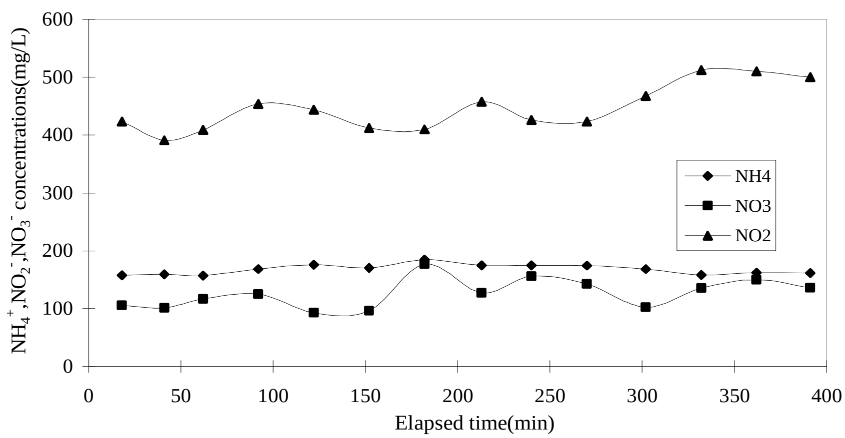
Fig. 2.11 (a) Nitrogen components' evolution; (b) Change in pH over time; (c) Profiles of free ammonia and free nitrous acid under HRT of 3 days and NH_4^+ of 1301.2 mg/L.

Fig. 2.11(b) shows the change of pH over the cycle with a pattern consistent with the time allotments of the 139-min aerobic feeding and 301-min aerobic reaction. This implies that pH was maintained at a higher level of 7.3 ~ 7.6 after mixing with the residual liquid from the previous finished cycle (first point at pH=6.7) during the feeding period. Afterwards the pH was gradually decreased to 6.1 without the buffering capacity added by the feed. Free ammonia and free nitrous acid were calculated using Equ. (2-1) and (2-2) and plotted in Fig. 2.11(c). Due to the higher pH and NH_4^+ replenished by the feeding, FA initially increased from 1 to 8 mg/L during the aerobic feeding period before heading down to 0.2 mg/L in the subsequent aerobic reaction period. Contrarily, FNA stayed virtually flat at around 0.1 mg/L during the feeding period. Then the trend was reversed after the feeding was terminated while aeration was still in operation. There was no new NH_4^+ supplied as a buffer so the free nitrous acid gradually rose to 4.8 mg/L along with the declining pH.

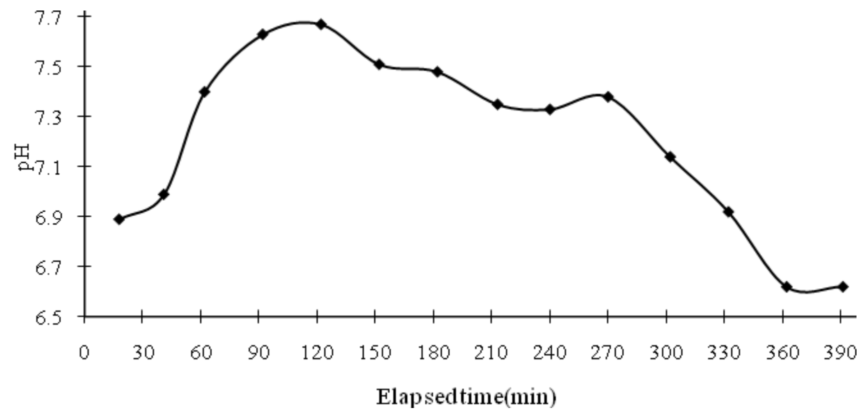
Based on the results from the 3-day HRT cycle, the fact was evidenced that extended aeration without loading in a cycle was not beneficial in producing more nitrogen in oxidized forms. In order to increase the daily output and improve energy efficiency, another cycle was studied using a doubled feeding time and decreased total aeration time. Fig. 2.12(a) shows the profiles of NH_4^+, NO_2^-, and NO_3^- in one typical cycle under the HRT of 1.5 days and an influent NH_4^+ concentration of 848.4 mg/L. The NH_4/MLSS load was also kept at 0.058 g/g·d. During this cycle, the NO_2^- , NO_3^-, and NH_4^+ produced were between 400 and 510 mg/L, 100 and 177 mg/L, and 160 and 185 mg/L, respectively, making the effluent a mixture of 63% NO_2^- , 17% NO_3^- and 20% NH_4^+. For pH (Fig. 2.12(b)), during the 278-min aerobic feeding, it was in a higher range of 7.3~7.7 after initial mixing, while in the 82-min aerobic reaction period, it was gradually decreased to 6.6 with a pattern similar to the first cycle (Fig. 2.11(b)).

Profiles of FA and FNA are shown in Fig. 2.12(c). The FA was in a level ranging from 2 to 4.5 mg/L in the aerobic feeding period. Compared with the first cycle, the influent NH_4^+ concentration applied was lower in this cycle, resulting in lower ammonium and FA in the bulk liquid. Initially, FNA was at around 0.1 mg/L but climbed to 1 mg/L in the aerobic reaction period. Further comparing the two cycles shows that under the same NH_4^+/MLSS load, the nitrite dominated in both cycles (Table 2.2). The 2nd cycle has a slightly higher nitrite percentage (63%) in the effluent and also a higher nitrite production rate (0.33 g/L·d), suggesting that longer feeding time benefits the nitrite production ability in the system.

Anthonisen (1976) reported that the NOB were inhibited by FA at levels above 0.1 mg/L and all nitrifying bacteria were inhibited by FNA at levels above 0.2 mg/L. Although complete inhibition was not verified in both cycles under much higher FA and FNA levels than proposed by Anthonisen (1976), no obvious further transformation was observed after the feeding was completed in both cycles. The comparison between the two cyclic modes with different HRTs shows that there is no big difference with regard to the whole nitritation process, which is characterized by continuous conversion of loaded ammonium to nitrite and nitrate in the aerobic feeding period and no further conversion after the loading was terminated, resulting in relatively stable levels for all the three nitrogen components in the entire cycle. The ratios of NO_2^-:NO_3^-:NH_4^+ in effluent were 61%:17%:22% and 63%:17%:20% for 3-day HRT and 1.5-day HRT, respectively (Table 2.2). Compared to 3-day HRT mode, 1.5-day HRT cyclic mode has doubled daily output in volume, so it is employed in all of our further experiments.



(a)



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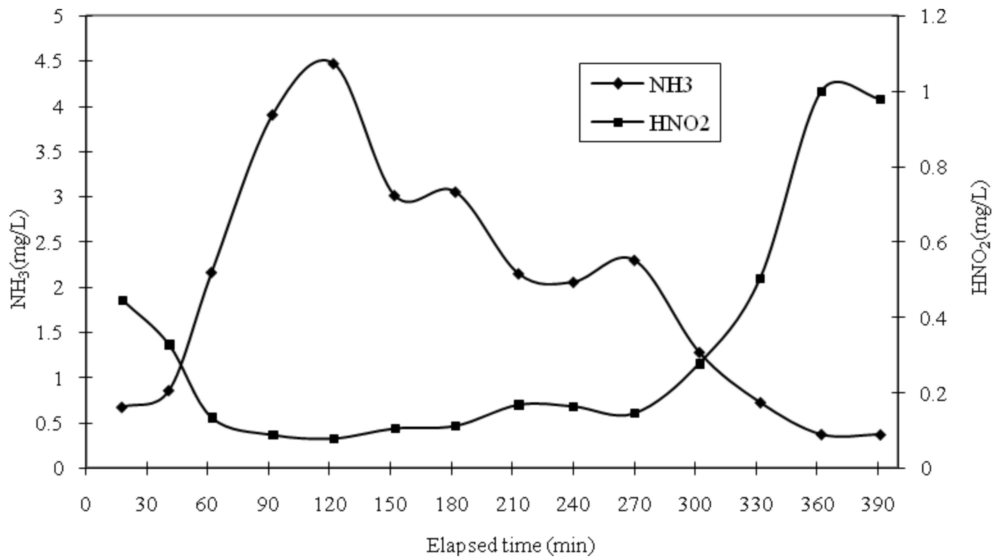


(c)

Fig. 2.12 (a) Nitrogen components' evolution; (b) Change in pH over time; (c) Profiles of free ammonia and free nitrous acid under HRT of 1.5 days and NH_4^+ of 848.4 mg/L.

Table 2.2 Comparison of two cyclic modes

Items	Cycle 1	Cycle 2
NH_4^+ /MLSS (g/g·d)	0.058	0.058
MLSS (mg/L)	7449	9750
NH_4^+ in (mg/L)	1301.2	848.4
Influent volume (L/d)	3.33	6.67
HRT(d)	3	1.5
NO_2^-:NO_3^-:NH_4^+ in effluent	61%:17%:22%	63%:17%:20%
NO_2^- production rate(g/L·d)	0.26	0.33
pH	6.42(6.12-7.64)	7.21(6.62-7.67)
NH_3 in average (highest) (mg/L)	0.6 (8.17)	1.96 (4.48)
HNO_2 in average (highest) (mg/L)	2.96 (4.83)	0.32 (1.00)

2.5 Conclusions

The outcomes from this study have provided evidence that it is possible and feasible to accumulate nitrite by partial nitrification of ammonium containing swine wastewater in an activated sludge SBR by producing FA and FNA at inhibitory levels for nitrite oxidizers. The SBR was operated under an HRT of 3 days for 20 days followed by a reduced HRT of 1.5 days for 9 days after initial start-up. Lower HRT means larger daily feeding volume, which is always preferred if the same treatment efficiency can be obtained. Our results showed that HRT didn't affect the composition of effluent nitrogenous compounds so a longer feeding time would lead to a greater nitrite production rate. For example, the nitrite production rate was 0.34 kg NO_2-N/m^3/d under the HRT of 1.5 days compared to 0.17 kg NO_2-N/m^3/d under the HRT of 3 days for the same influent ammonium concentration of around 1000 mg/L. However, although it appeared that HRT had little effect on the composition of nitrogenous species in the final effluent, the inhibitor accounting for nitrite accumulation might be different between FA and FNA under the two HRTs studied. The FA concentration in the mixed liquid was mostly between 0.02-0.05 mg/L in the first operating period and only reached 0.18 mg/L on day 20, while the FNA concentration increased from 0.2 mg/L to around 15 mg/L on day 17, declined to 4 mg/L on day 20, averaging 5.2 mg/L in the same period. In the second operating period, the FA had a significant rise to the range of 0.3-1.1 mg/L and the FNA concentration dropped into the range of 0.3-1.5 mg/L. According to this observation, it can be concluded that FNA was the inhibitor in the first HRT period contributing to nitrite accumulation when the FA concentration was not reaching its inhibitory level of 0.1 mg/L until day 20. In the second HRT period, FA stayed at above 0.3 mg/L so it joined FNA to become another inhibitor to nitrite oxidizers. Since either or both of FA and FNA was always present in the system, a nitrite dominant effluent could thus be produced from the SBR experimented in this study with swine wastewater, regardless of the HRT used.

Data also indicated that the SBR system could handle swine wastewater with varying ammonium concentrations without significantly impacting its capability of producing a nitrite rich effluent. Although ammonium loads were increased from 0.04 kg NH_4-N/m^3/d on the 1st day of experiment to 0.7 kg NH_4-N/m^3/d on the 28th, the nitrite/nitrate ratio (NO_2/NO_3) in the effluent fell mainly within the range of 3~4, stably generating an effluent with 13-23% of NH_4-N, 15-21% of NO_3-N, and 56-72% of NO_2-N under different influent ammonium concentrations and for both cyclic modes.

The cycle comparison between the two modes with different HRTs shows that there is no big difference with regard to the whole nitritation process, which is characterized by continuous conversion of loaded ammonium to nitrite and nitrate in the aerobic feeding period and no further conversion after the loading was terminated, resulting in relatively stable levels for all the three nitrogen components in the entire cycle. The ratios of NO_2^-:NO_3^-:NH_4^+ in effluent were 61%:17%:22% and 63%:17%:20% for 3-day HRT and 1.5-day HRT respectively. Compared to 3-day HRT mode, 1.5-day HRT cyclic mode has doubled daily output in volume, so it is employed in all of our further experiments.

Chapter 3.

More Investigation on the Nitrite Accumulation Characteristics: Reducing Load and Stability Tests

3.1 Abstract

Shortcut nitrification (SN) is the first step of shortcut nitrogen removal from swine wastewater. Stably obtaining an effluent with a significant amount of nitrite is the premise for the subsequent shortcut denitrification (SD). In this chapter, more features of nitrite accumulation were investigated using 1.5-day HRT and 8-h cycle in a 10-L (working volume) activated sludge sequencing batch reactor (SBR). The nitrite production stability was tested using four different ammonium loading rates, 0.075, 0.062, 0.053, and 0.039 $gNH_4/gMLSS \cdot d$ in a 2-month running period. The total inorganic nitrogen (TIN) composition in the effluent was not affected when the ammonium load was between 0.053 and 0.075 g NH_4/g MLSS·d (64% NO_2-N, 16% NO_3-N, and 20% NH_4-N). Under 0.039 g NH_4/g MLSS·d, more NO_2-N was transformed to NO_3-N with an effluent of 60% NO_2-N, 20% NO_3-N, and 20% NH_4-N. The reducing load test was able to show the relationship between a declining FNA concentration and the decreasing nitrite production, indicating that the inhibition of FNA on NOB depends on its levels and an ammonium loading rate around 0.035 $gNH_4/gMLSS \cdot d$ is the lower threshold for producing a nitrite dominance effluent in the activated sludge SBR.

3.2 Introduction

Agricultural fertilizers have been identified as a primary culprit behind the eutrophication phenomenon booming in lakes and gulfs (Howarth et al., 2000). Among the fertilizers used, the wastewater flushed from the confined swine production facilities and then applied to the cropland is one of the major responsible agricultural sources (VanDyne et al., 1978; Karlen, 2004). When the scale of swine production keeps rising (Hunt et al., 2001, USDA, 2007), manure that cannot be land applied according to the plant and soil testing has to be treated before discharge to avoid potential pollutions to water resources and the environment.

The energy input for aeration is one of the major costs associated with aerobic activated sludge treatment systems. The provision of aeration equipment generally represents less than 5% of the capital cost, but the energy consumption to run aeration represents approximately 25% of the annual operating costs (Griffiths et al, 1989), based on experiences from municipal wastewater treatment plants. Swine wastewater contains total nitrogen at a level normally one order of magnitude higher than that of municipal and/or domestic wastewater (Boursier et al, 2005; Zhang et al, 2006). The chemical oxygen demand (COD) equivalent of NH_3 that can be calculated from Equ. (3-1) equals 3.76, while the COD of a typical organic carbon compound such as glucose ($C_6H_{12}O_6$) is only 1.067 based on Equ. (3-2). This simple comparison attests that the oxygen demand of ammonium laden swine wastewater is much higher than an average municipal wastewater characterized mainly by its carbon content. From the above numbers, it’s fair to say that, as opposed to municipal wastewater treatment, much more air needs to be supplied in aeration systems to treat swine wastewater.

$$NH_3 + 2O_2 \rightarrow H_2O + H^+ + NO_3^- \qquad (3\text{-}1)$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad (3\text{-}2)$$

In order to reduce the energy cost incurred by aeration, several advanced methods have been studied to improve nitrogen removal, such as the partial nitritation treatment for anaerobic digestion liquor of swine wastewater using swim-bed technology (Yamamoto et al., 2006), the anaerobic ammonium oxidation (Anammox) and the partial denitritation in anaerobic nitrogen removal from piggery waste (Ahn et al., 2004). Regardless of what the next step is (it could be denitritation or Anammox), the first step is to produce an effluent with a significant amount of nitrite, which can be accomplished by a process called “shortcut nitrification” (also termed nitritation). Shortcut nitrification produces nitrite instead of nitrate from ammonium by controlling process parameters to allow the ammonia oxidizing bacteria (AOB) to grow faster than the nitrite oxidizing bacteria (NOB), thus omitting the step for nitrite to be oxidized to nitrate. It’s considered in favor of conventional nitrification with 25% otherwise required oxygen saved (Ruiz et al., 2003) and 33~35% less sludge produced (Turk et al., 1987). The controlling parameter can be temperature because at a higher temperature (> 25°C), AOB have a greater specific growth rate than NOB (Hellinga et al., 1998). It can also be the dissolved oxygen (DO) because at a lower DO, the AOB have a higher affinity for oxygen than NOB (Wiesmann, 1994). Free ammonia (FA, NH_3) and free nitrous acid (FNA, HNO_2) are found to have selective inhibition on NOB (Anthonisen et al., 1976), which means a certain combination of reactor type and feeding strategy benefiting FA and FNA production can also be used as a controlling parameter for shortcut nitrification.

Chapter 2 has confirmed the possibility of accumulating nitrite from swine wastewater by using a continuous feeding strategy in an activated sludge sequencing batch reactor (SBR), in which ammonium loads were increased from 0.04 g NH_4-N/ L·d to 0.7 g NH_4-N/L·d to generate an effluent with 13-23% of NH_4-N, 15-21% of NO_3-N, and 56-72% of NO_2-N. In this chapter, the nitrite production stability was tested using four different ammonium loading rates, 0.075, 0.062, 0.053, and 0.039 gNH_4/gMLSS·d in a 2-month running period under the 1.5-day HRT. In addition, a reducing load test in which ammonium loads were reduced from 0.081 to 0.011 g/gMLSS·d was conducted to qualitatively visualize the relationship between the gradually reduced ammonium loads and the nitrite production efficiencies.

3.3 Materials and Methods

Manure Source

Raw swine wastewater was collected once a week from a reception sump of a finishing barn at the University of Minnesota Southern Research and Outreach Center, where fresh manure in a shallow pit inside the barn was flushed out biweekly. The characteristics of the raw wastewater analyzed included pH, chemical oxygen demand (COD), NH_4-N, NO_2-N, and NO_3-N. If not used immediately, the collected manure was stored at 4°C or below.

Nitritation Sequencing Batch Reactor (NSBR) Set-Up

The same 12 L lab-scale SBR with a working volume of 10 L used in Chapter 2 (Fig. 2.1 and Fig. 2.2) was again employed here with the system setup similar to that presented early. Briefly, the SBR was operated at room temperature (20±3°C) without temperature control. The air was provided by a vacuum pressure pump (Barnant 60010-2392, USA) with the aeration intensity kept at 10 liters per minute (LPM) to keep the biomass completely mixed without using a mechanical stirrer, and the running time of the air pump was controlled by a timer (BH-94460-45, Cole-Parmer Instruments Co., USA). Two peristaltic pumps (MasterFlex 7550-30, USA) were used for feeding and discharging, respectively. A mixer (Servodyne 50003-20, Cole-Parmer Instruments Co., USA) was installed in the influent tank for complete mixing at a constant rate of 80 rpm during the feeding period of SBR. The pumps and the mixer were automatically controlled by a computer program WIN LIN V1.2 (Cole-Parmer Instrument Co., USA).

NSBR Operation

After the 1-month possibility test period reported in Chapter 2, the SBR was restarted and seeded with fresh residual sludge from Waseca municipal wastewater treatment plant. The start-up of the nitritation SBR was the same as described in Chapter 2. After start-up, the reactor was fed with the collected swine wastewater at different dilutions to obtain different ammonium levels. The dilution rate was calculated by dividing the sum of volumes of the swine wastewater and added tap water by the volume of the swine wastewater. During this 2-month test period of the NSBR, the 1.5-day HRT, 8-h cyclic operation mode was applied. This mode consisted of 4 h 38 min aerobic feeding, 1 h 22 min aerobic reaction, 30 min settling, 24 min withdrawal and 1 h 6 min idle. The nitrite production stability was tested using four different ammonium loading rates, 0.075, 0.062, 0.053, and 0.039 $gNH_4/gMLSS \cdot d$ by adjusting ammonium concentrations in the range of 498 mg/L to 1018 mg/L in the influent according to MLSS levels, which varied from 6000 mg/L to 11000 mg/L in this test period. Effluent samples were collected every two days in the 2-month running period. Five samples were collected for each of the four ammonium loading rates. Sludge withdrawal was minimally performed in order to maintain the proliferated biomass in the system during this period, obtaining an SRT greater than 20 days for autotrophic nitrifiers to achieve complete nitrification (Zimmerman et al., 2004). In this experiment, the independent variable was the ammonium loading rate and the dependent variable was the nitrite, nitrate and ammonium ratios in effluents.

After the data collection for stability analysis was done, a reducing load test was conducted. The influent ammonium concentration gradually decreased from 631 mg/L to 84 mg/L (631, 491, 384, 298, 204, 117 and 84 mg/L) and the corresponding ammonium load was decreased from 0.081 to 0.011 $gNH_4/gMLSS \cdot d$ (0.081, 0.059, 0.046, 0.035, 0.024, 0.016 and 0.011 $gNH_4/gMLSS \cdot d$). Under each influent concentration, the reactor was run for two days (6 cycles), in which 3 effluent samples were collected from the latter 3 cycles. Sludge withdrawal was conducted daily during this period in order to keep a relatively stable level of the mixed liquid suspended solids (MLSS) at 5400 ± 256 mg/L. The SRT, which was calculated by dividing the total solids in the reactor by total daily withdrawn solids, was around 30 days. In this experiment, the independent variable was the ammonium loading rate and the dependent variable was the percent nitrite dominance in the system.

Analytic Methods

Liquid samples were obtained every two days from influent and effluent in the reducing load test and stability periods. After centrifuging at 5000 rpm for 10 min, the supernatant was obtained for analyses of ammonium (NH_4^+–N), nitrite (NO_2^-–N) and nitrate (NO_3^-–N). Measurements of NH_4^+, NO_2^- and NO_3^- were performed for both influent and effluent liquid samples following the Hach DR2800 Spectrophotometer Manual (Hach, 2005). Nitrite (NO_2^--N) was measured by the diazotization method using TNT 839 vials (Hach, USA) and nitrate (NO_3^--N) by the cadmium reduction method using TNT 835 vials (Hach, USA). Ammonium (NH_4^+–N) was measured by the Nessler method using Nessler's reagent (Hach, USA). pH was measured by a portable pH meter (Orion 210A, Thermo Scientific, USA). MLSS were measured according to the standard methods (APHA, 2005). The FA and FNA concentrations are calculated using Equ. (2-1) and Equ. (2-2).

Statistical analysis was done by using SAS JMP V6.0. The comparison of different data sets was conducted by One-Way Analysis of Variance (ANOVA) and Tukey-Kramer HSD test embedded in JMP. The null hypotheses of no significant difference between data sets were rejected at 95% significance level when $p < 0.05$.

3.4 Results and Discussion

Analysis of Raw Swine Wastewater

The characteristics of raw swine wastewater collected from the finishing barn at Waseca were pH 8.21-8.33, 8000-11000 mg L^{-1} COD, 1400-1800 mg L^{-1} NH_4-N, NO_2-N and NO_3-N were negligible.

Stability Test

The nitrite production stability was tested using four different ammonium loading rates, 0.075, 0.062, 0.053, and 0.039 $gNH_4/gMLSS \cdot d$ in a 2-month running period. The total inorganic nitrogen (TIN) composition in the effluent was calculated and the results are shown in Table 3.1. The NO_2-N in the effluent accounted for 65% of the TIN for daily ammonium loads of 0.075 and 0.053 and was slightly lower at 0.062 and 0.039 loading rates (63% and 60%), respectively. The NO_3-N content was about 16% for all but one loading rate (20% for the 0.039 loading rate). NH_4-N was about 20%, 21%, 19%, and 20% of the TIN for 0.075, 0.062, 0.053, and 0.039 daily loads, respectively. Statistical analysis was performed and the *p*-values of the one-way ANOVA were 0.0024, 0.001, and 0.689 for NO_2-N/TIN, NO_3-N/TIN, and NH_4-N/TIN, respectively, indicating significant difference existing for NO_2-N/TIN and NO_3-N/TIN but not for NH_4-N/TIN under the four loading rates. Further comparisons for all pairs using Tukey-Kramer HSD suggest that for NO_2-N/TIN and NO_3-N/TIN, the significant different data set is from the load 0.039 at 95% significance level (Fig. 3.1). The ranges of NO_2-N, NO_3-N, and NH_4-N under the four loading rates are shown in Table 3.2. Conclusively, the TIN composition in the effluent was not affected when the ammonium load was between 0.053 and 0.075 g NH_4/g MLSS·d, but under 0.039 g NH_4/g MLSS·d, the NO_2-N percentage declined a little accompanied by a slight rise in NO_3-N, suggesting that nitrite oxidizers were less inhibited when the ammonium load was kept under a certain threshold. Despite that, the process has accomplished the goal to make NO_2-N become the dominant component of TIN in the effluent under all loads during the entire running period, namely, successful nitrite accumulation from swine wastewater, which is the premise for conducting experiments in the second SBR using the high nitrite effluent as its influent.

Table 3.1 TIN composition in effluents under four different ammonium loads

Influent load (g NH_4/g MLSS d)	NO_2/TIN	NO_3/TIN	NH_4/TIN
0.075±0.005 (5*)	0.65±0.025	0.16±0.011	0.20±0.02
0.062±0.002 (5*)	0.63±0.011	0.16±0.02	0.21±0.022
0.053±0.001 (5*)	0.65±0.011	0.16±0.014	0.19±0.019
0.039±0.003(5*)	0.60±0.02	0.20±0.02	0.20±0.03

* sample number

Table 3.2 NO_2-N, NO_3-N and NH_4-N ranges under four different ammonium loads

g NH_4/g MLSS d	NO_2 (mg/L)	NO_3 (mg/L)	NH_4 (mg/L)
0.075	445-591	122-140	144-166
0.062	395-501	90-136	121-174
0.053	411-456	96-112	102-153
0.039	268-329	88-100	71-120

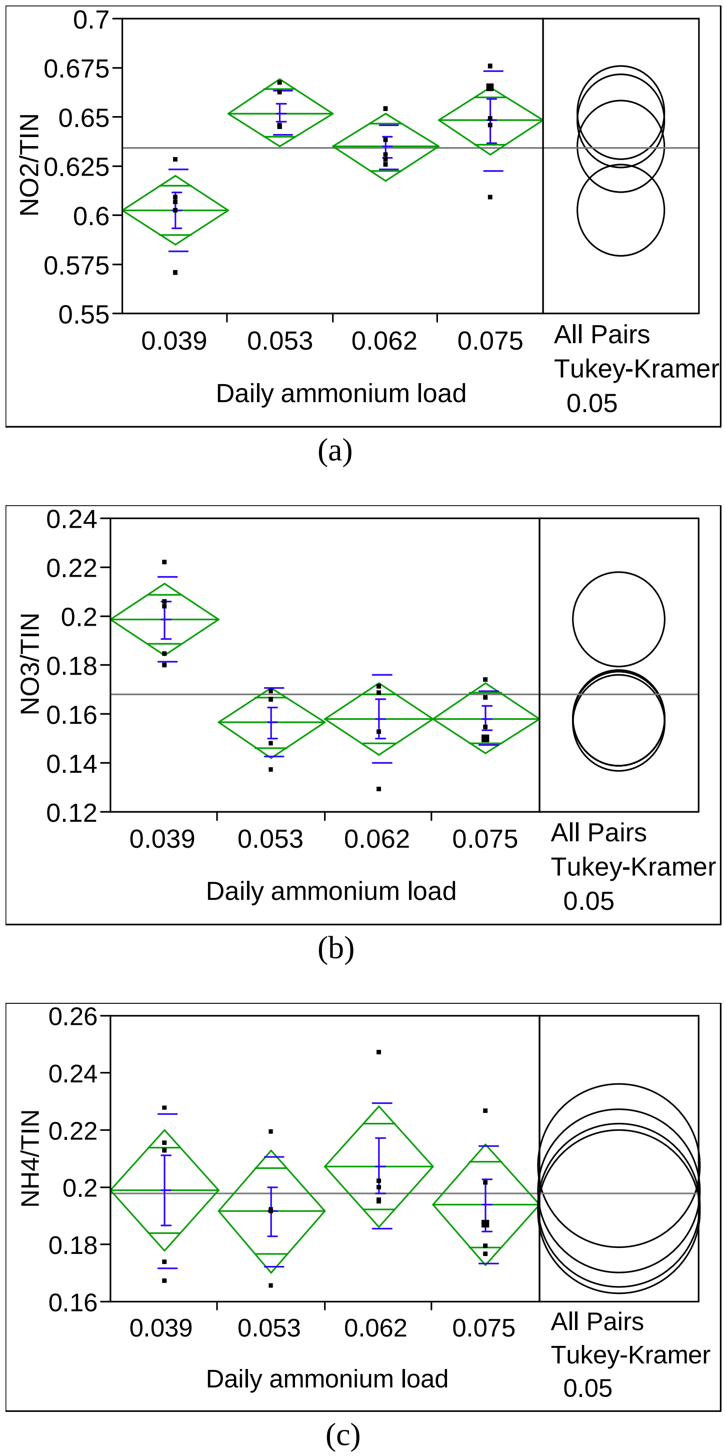


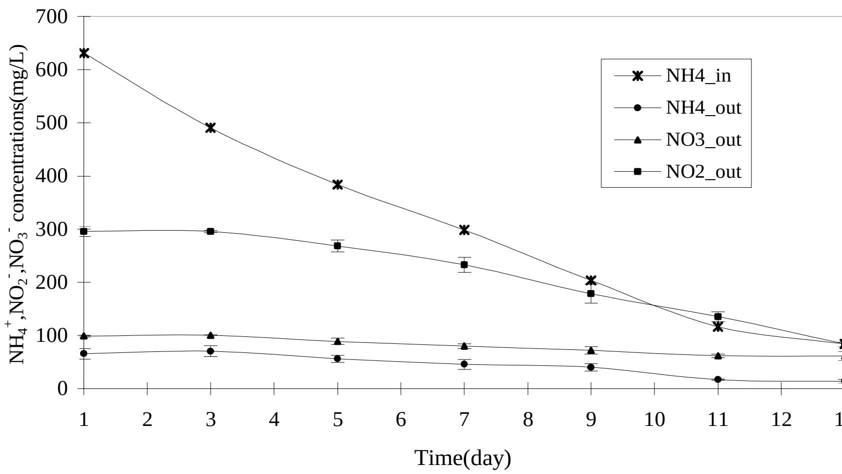
Fig. 3.1 (a)Oneway Analysis of NO_2/TIN by daily ammonium load; (b) Oneway Analysis of NO_3/TIN by daily ammonium load; (c) Oneway Analysis of NH_4/TIN by daily ammonium load The centerlines of the means diamonds are the group means. The top and bottom of the diamonds form the 95% confidence intervals for the means (Sall et al., 2005).

Reducing Load Test

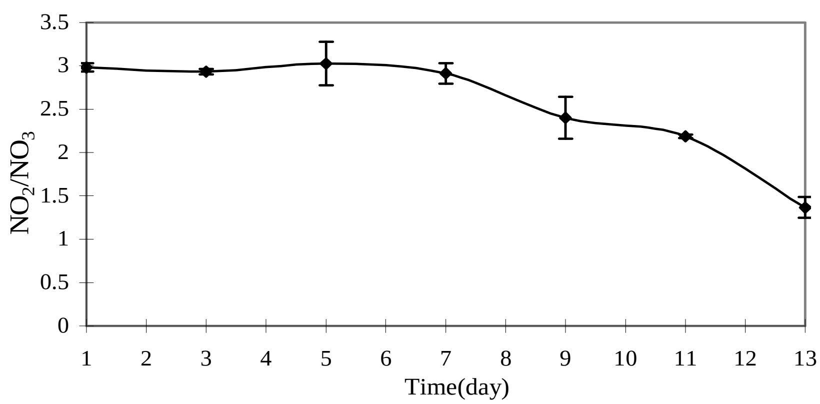
The 1.5-day HRT cycle was chosen for the reducing load test to qualitatively evaluate the relationship between the concentrations of free ammonia and free nitrous acid and the nitrite content in the effluent when the MLSS was kept at 5400±256 mg/L through the entire period. Fig. 3.2 (a) shows the profiles of influent NH_4^+ and effluent NH_4^+, NO_2^-, and NO_3^- concentrations during the test. The influent NH_4^+ was gradually reduced from 630.1 to 84.1 mg/L. The corresponding changes in NO_2^- production and the $NO_2^-/(NO_2^- + NO_3^-)$ ratio in the effluent are shown in Table 3.3. The evolution of NO_2^-/NO_3^- ratio in the effluent is shown in Fig. 3.2 (b). After day 7, the NO_2^-/NO_3^- ratio experienced a sharp decline from about 3.0 to 2.4, and finally to 1.36. This change of the NO_2^-/NO_3^- ratio may be explained by examining Fig. 3.2 (c) and (d). The large standard deviations in Fig. 3.2 (d) might be owing to the calculation stacking effect of deviations from both the NO_2^- concentration and the pH. When the NH_4^+ load dropped from 0.035 to 0.024 g/gMLSS·d, the value of pH climbed from 6.2 to 6.4, accompanied by an abrupt decline of FNA from 1.2 to 0.6. From that point forward, the nitrite dominance environment in the system was no longer existing. The FA concentration was always kept under 0.1 mg/L except in the final day, implying that FA was not the acting inhibitor on NOB until day 13 throughout the entire test period. The observations from this experiment demonstrate that the inhibition of FNA on NOB depends on its levels and would decrease under a certain level in a given system.

Table 3.3 The relationship between NH_4^+ loads and NO_2^- production

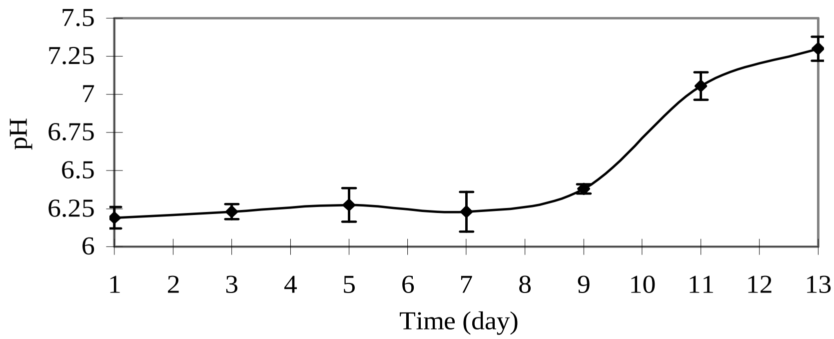
Time (day)	Influent NH_4^+ (mg/L)	NH_4^+ load (g/gMLSS·d)	NO_2^- production (g/L ·d)	NO_2^-/(NO_2^-+ NO_3^-)
1	630.98	0.081	0.20	0.75
3	491.02	0.059	0.20	0.74
5	383.85	0.046	0.18	0.75
7	298.4	0.035	0.16	0.74
9	203.6	0.024	0.11	0.70
11	116.58	0.016	0.09	0.69
13	84.11	0.011	0.06	0.58



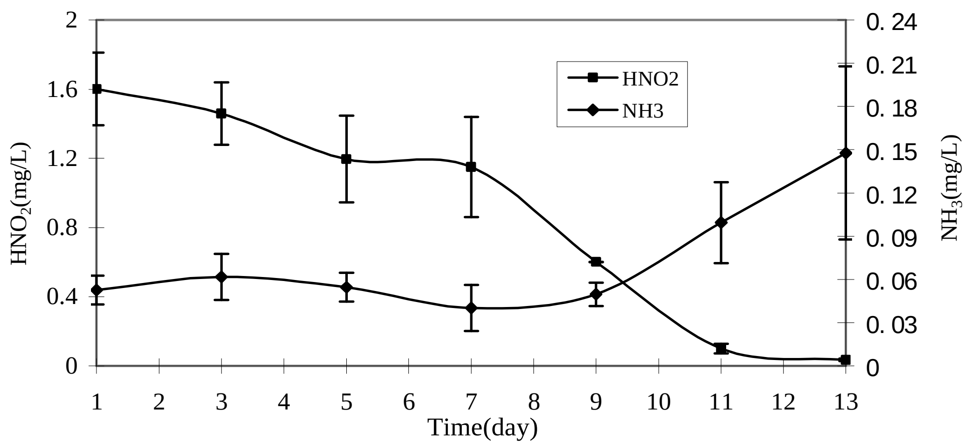
(a)



(b)



(c)



(d)

Fig. 3.2 (a) Profiles of influent NH_4^+ and effluent NH_4^+, NO_2^-, NO_3^- during the reducing loads test period. The standard bars on the effluent NH_4^+, NO_2^-, NO_3^- lines are from three samples. (b) Changes of NO_2/NO_3 ratio over the period. The standard bars are from three samples. (c) Changes of pH over time. The standard bars are from three samples. (d) Changes of Free ammonia (NH_3) and free nitrous acid (HNO_2). The standard bars are from three samples.

3.5 Conclusions

Shortcut nitrification and denitrification is a process based on the intermediate, i.e., nitrite, which exists in both nitrification and denitrification processes to treat wastewaters containing ammonium, especially meaningful for high ammonium wastewaters. Through the combination of an activated sludge SBR and a continuous feeding strategy, an environment favoring the nitrite accumulation from swine wastewater was established.

The nitrite production stability was tested using four different ammonium loading rates, 0.075, 0.062, 0.053, and 0.039 gNH_4-N/gMLSS·d in a 2-month running period. The TIN composition in the effluent was not affected when the ammonium load was between 0.053 and 0.075 g NH_4-N/g MLSS·d (64% NO_2-N, 16% NO_3-N, and 20% NH_4-N). Under 0.039 g NH_4-N/g MLSS·d, a little more NO_2-N was transformed to NO_3-N with an effluent of 60% NO_2-N, 20% NO_3-N, and 20% NH_4-N.

The reducing load test has revealed the relationship between a declining FNA concentration and the decreasing nitrite production, implying that the inhibition of FNA on NOB depends on its levels and would decrease under a certain concentration in a given system. The NH_4^+ load was gradually decreased from 0.081 to 0.011 g/gMLSS·d. When the NH_4^+ load was between 0.081 and 0.035 g/gMLSS·d, the ratio of NO_2^-/(NO_2^-+ NO_3^-) was kept stable around 0.75. When the NH_4^+ load dropped from 0.035 to 0.024 g/gMLSS·d, the ratio dropped to 0.70, accompanied by an abrupt decline of FNA from 1.2 to 0.6. From that point forward, the nitrite dominance environment in the system was no longer existing.

Combining the results from both reducing load and stability tests, it is concluded that an ammonium loading rate around 0.035 gNH_4-N/gMLSS·d is the lower threshold for producing a nitrite dominance effluent from the activated sludge SBR.

Chapter 4.

Nitrogen Removal from Swine Wastewater by Shortcut Nitrification and Denitrification in a Sequencing Batch Reactor System

4.1 Abstract

Shortcut nitrification and denitrification (SND) is a novel nitrogen removal process that has drawn significant researchers' attention lately. In this study, the application of SND in swine wastewater treatment was investigated using two sequencing batch reactors (SBRs) connected in series in order to reduce the needs for energy and carbon dosage. The first SBR produced a nitrite rich effluent that was fed to the second SBR where both nitrogen and phosphorus removal were achieved. Three COD/NO_x-N ratios (3.6, 4.8 and 6) were selected to test the influence of carbon availability on the TIN reduction and phosphorus removal efficiencies for the second SBR. It was observed that COD/NO_x-N ratios of 4.8 and 6 could achieve 97% and 98% TIN removals while 3.6 only resulted in 84% removal. The COD/NOx-N ratio has no significant influence on dissolved phosphorus (DP) removal and the mean value of DP removal was about 52%. Two solid retention times (SRTs) were selected to test the effect of SRT on nitrogen and phosphorus removal. No significant difference on TIN removal was observed under SRT 16 and 23 days, but DP removal was much better under SRT 23 days than under 16 days (around 67% vs. 38%).

4.2 Introduction

Swine wastewater, generated in confined swine operations and characterized by high contents of nitrogen and phosphorus, has been mostly applied to cropland as fertilizer for many years (Karlen et al., 2004). Although deemed to be a good source for replenishing nutrients in soil (Choudhary et al., 1996), the amount of manure generated was indeed over abundant when taking both its unmatched N-P-K ratio and the soil carrying capacity into account (Jackson et al., 1996; Shepard, 2000; Karlen et al., 2004). A six-year field study with liquid swine wastewater showed that for continuous corn production, 16% of the applied N was lost through subsurface drainage (Karlen et al., 2004), which is one reason why agriculture has been implicated as a major contributor to non-point sources of pollution (Dinnes et al., 2002).

Biological methods combining high-rate anaerobic and aerobic unit processes are considered superior to other physicochemical methods for treating swine wastewater considering its efficiency and cost (Oleszkiewicz, 1985). Therefore, studies to treat swine wastewater using bioreactors featuring anaerobic and/or aerobic settings have come onto the stage recently (Zhang et al., 2006, Ahn et al., 2004, Yamamoto et al., 2006).

Swine wastewater is characterized by a high fraction (42−84%) of inert COD (Boursier et al., 2005) and a level of total nitrogen normally one order of magnitude higher than that of municipal wastewater (Boursier et al, 2005; Zhang et al, 2006), which normally results in incomplete denitrification when treated in biological systems. To overcome this issue, shortcut nitrification and denitrification (SND) is becoming a novel nitrogen removal process that has drawn growing attention among researchers because it can theoretically cut the oxygen consumption by 25% in aerobic stage and electron donor (ED) requirement by 40% in anoxic stage (van Kempen et al., 2001; Ruiz et al., 2003).

Shortcut nitrification (also termed nitritation) is the first step for implementing the SND process owing to the need for nitrite as substrate for denitrification (Philips et al. 2002). Whereas nitrite accumulation can be obtained by changing operational conditions favoring the growth of ammonium oxidizing bacteria (AOB) while inhibiting nitrite oxidizing bacteria (NOB).

As for denitrification, the type of the ED, which is often expressed as chemical oxygen demand (COD), and the ratio of the ED to oxidized nitrogen, NO_x-N, are critical for successful complete denitrification. Denitrification from nitrite instead of nitrate (named shortcut denitrification) eliminates the ED requirement in the reduction of nitrate to nitrite, greatly lowering the COD/N ratio for complete denitrification. For example, the COD/N ratio required for full denitrification from NO_3-N to N_2 is about 7.6 g O_2/g N by the elemental balance analysis of microbial growth on glucose, sodium acetate, and methanol (Sobieszuk et al., 2006), while a COD/NO_2-N ratio greater than 2.5 g O_2/g N may be sufficient for complete denitrification in the shortcut process as reported (Abeling and Seyfried, 1992; Hellinga et al., 1998). However, the actual value of a proper COD/N ratio will depend on operating conditions of the system and the type of ED used for denitrification (Zhang et al., 2007). Although many types of short chain carbohydrates such as acetate, lactate and glucose can be used as the carbon source for denitrification, Cuervo-Lopez et al. (1999) found that sludge settleability and reactor stability would be affected by different carbon sources. The use of acetate did not influence the sludge settleability whereas sludge flotation was associated with lactate and foaming with glucose (Cuervo-Lopez et al., 1999). In a word, the selection of a proper carbon source and its dosage is essential for achieving complete denitrification, under which a low total inorganic nitrogen (TIN) discharge can be guaranteed.

Solid retention time (SRT) is one of the most important control parameters in biological nutrient removal (BNR) processes. Typical values of SRT used for design of conventionally loaded treatment systems are in the range of 4 to 10 days. The values are prolonged to the range of 15 to 30 days if extended aeration is applied (Rittmann and McCarty, 2001). A sequencing batch reactor (SBR) with high nutrient removal efficiencies has the features of an extended aeration unit and thus has an SRT of 15 days or greater, which ensures good nitrification. Considering phosphorus removal, it has been shown in practice that good P removal is possible at SRTs ranging from 3 to 60 days (Randall, 1992). Okada et al. (1992) determined that an SRT of more than 20 days was necessary to achieve higher efficiency of biological P removal. Tremblay et al. (1999) obtained the maximum phosphorus removal at SRTs of 20 and 25 days among five SRTs tested ranging from 10 to 30 days. Observations of the influent BOD to phosphorus removal ratio for a number of wastewater treatment plants as a function of their designed SRT showed that processes with a longer SRT had less phosphorus removal for a given BOD (Grady et al., 1999). At long SRTs, the phosphorus accumulating organisms (PAOs) are in a more extended endogenous phase, which will deplete more of their intracellular storage products (Stephens and Stensel, 1998). Therefore, a moderately long SRT may balance good phosphorus removal and the system stability.

In this study, the shortcut nitrification and denitrification was investigated for treating high ammonium containing swine wastewater using two SBRs, one performing partial nitrification and the other aiming at TIN and phosphorus reduction. In the first SBR, the nitrite accumulation was realized by applying a continuous feeding strategy. The denitrification and phosphorus removal happening in the second SBR was enhanced through introducing a small stream of unoxidized swine wastewater mixed with a certain amount of sodium acetate. A two-step fed mode was applied to the second SBR. The investigation focused on the influences of three COD/N loading ratios and two SRTs on nitrogen and phosphorus removal efficiencies.

4.3 Materials and Methods

Swine Wastewater Source

Raw swine wastewater was collected every 2 to 3 weeks from a reception sump of a barn at the University of Minnesota Southern Research and Outreach Center, where fresh manure in a shallow pit inside the barn was flushed out biweekly. Prior to loading the influent tank, the collected manure was stored at 4°C or below. The strength of the influent was adjusted using different dilution ratios according to the difference between the raw wastewater and the test concentration needed. The characteristics of the raw wastewater analyzed included pH, total solids (TS), total volatile solids (TVS), suspended solids (SS), volatile suspended solids (VSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), NH_4-N, total Kjeldahl nitrogen (TKN), total phosphorus (TP) and dissolved phosphorus (DP).

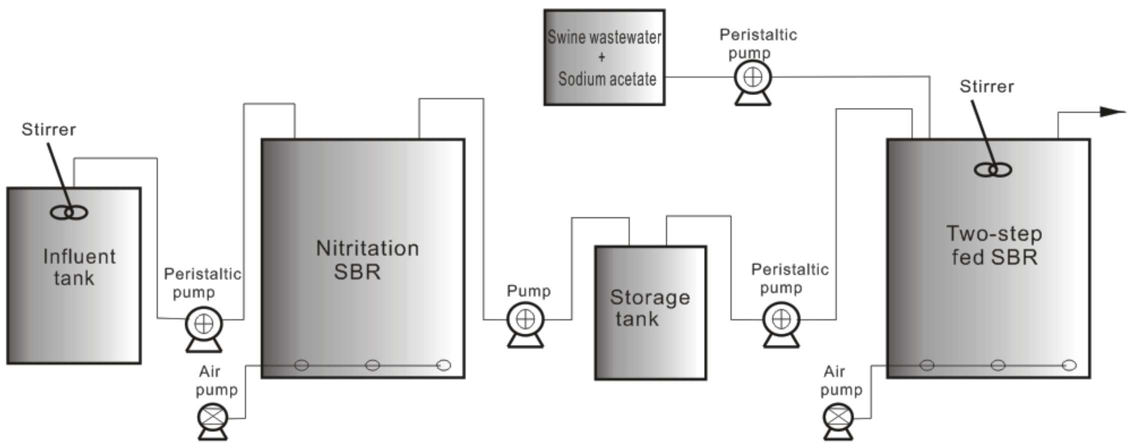
SND System Set-Up

Two activated sludge SBRs, one with a working volume of 10 L, and the other 8 L, were used in this study. The SBR system diagram is shown in Fig. 4.1. The first SBR was operated as a nitrite accumulation (nitritation) reactor with a significant amount of COD removed simultaneously, while the second one with a two-step fed mode could substantially reduce nitrogen and phosphorus. Both SBRs consisted of influent feeding, effluent discharging and air supply subsystems. The whole system was operated in ambient environment (20±3°C) without temperature control. Peristaltic pumps (MasterFlex 7550-30, USA) were used for feeding and discharging. The air was provided by vacuum pressure pumps (Barnant 60010-2392, USA) while the aeration intensity was kept at 10 L min^{-1} for the first SBR and 1 L min^{-1} for the second one. One mixer (Servodyne 50003-20, Cole-Parmer Instruments Co., USA) was installed in the influent tank for complete mixing at a constant rate of 80 rpm during the SBR feeding period and the other in the second SBR operating continuously except settling and withdrawal. The pumps and the mixer for the first SBR were automatically controlled by a computer program WIN LIN V1.2 (Cole-Parmer Instrument Co., USA) and the running time of the first air pump was controlled by a timer (BH-94460-45, Cole-Parmer Instruments Co., USA). The pumps and the mixer in the second SBR were programmed through the control software, Campsci PC400 (Campsci Co., USA). The seed sludge was obtained from Waseca municipal wastewater treatment plant.

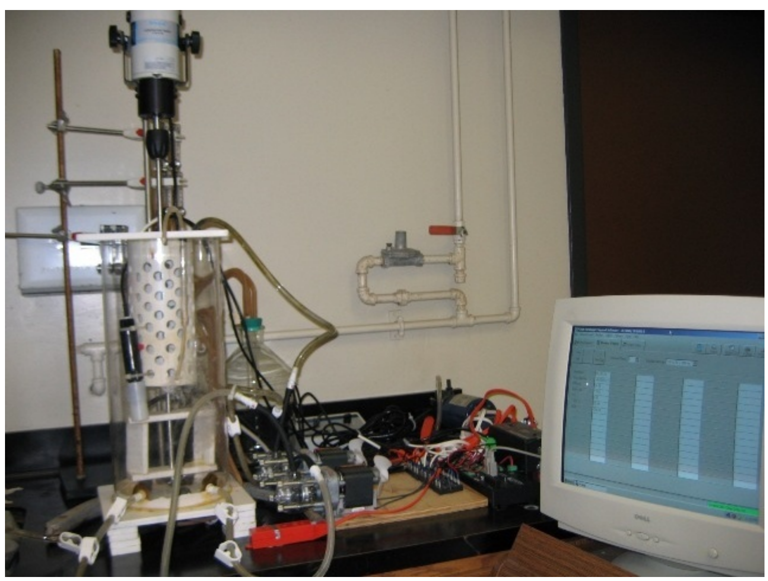
System Operation

The 10 L nitritation SBR (NSBR) was operated in an 8-h cycle mode, consisting of 4h 38 min aerobic feeding, 1h 22 min aerobic reaction, 30 min settling, 24 min withdrawal and 1h 6 min idle. Influent of 2.22 L was added to the reactor for each cycle so the hydraulic retention time (HRT) was kept at around 1.5 days.

The two-step fed SBR was operated in a 4-h cycle mode with two separate feedings. The cycle consisted of 30 min anaerobic, 1h 15 min aerobic, 30 min anaerobic, 1h 15min aerobic and 30 settling and withdrawal. The primary feeding including 680 ml NSBR effluent, 67 ml raw swine wastewater and a certain amount of sodium acetate was conducted in the first 2 min of the first anaerobic phase. The second feeding including the same three components at their half amounts occurred in the first 1 min of the second anaerobic phase. The amount of acetate added was calculated by subtracting the COD in the feeding raw swine wastewater from the COD required by the tested COD/NOx-N ratio. Effluent of 1120 mL was discharged within the last 5 min of settling in each cycle. Therefore, the hydraulic retention time (HRT) was 1.19 days. Solid retention times were maintained by withdrawing a comparable amount of excess sludge. The volume loss (due to discharge of excessive sludge and water evaporation induced by aeration) was replenished with water to keep the volume constant inside the reactor. The agitation speed was controlled at 25 rpm. A full factorial experimental design was employed with three feeding COD/NO_x-N ratios (6, 4.8, and 3.6) and two SRTs (23 and 15 days) as independent variables (resulting in 6 combinations with 2 replicates for each combination) to examine the treatment effect on nitrogen and phosphorus removal efficiencies. The dependent variables were the removal efficiencies of TIN and phosphorus of the step-fed SBR.



(a)



(b)

Fig. 4.1 (a) Schematic diagram of the full process; (b) Real step-fed SBR.

Analytic Methods

For the nitritation SBR, liquid samples were collected from both influents and effluents in test days during the experiments. Effluent samples were collected and NH_4^+,

NO_2^-, NO_3^-, and COD were determined a day before the experiment on the step fed SBR was conducted. Measurements of NH_4^+, NO_2^-, NO_3^-, and COD were performed for liquid samples following the Hach DR2800 Spectrophotometer Manual (Hach, 2005). Influent and effluent samples were also collected from the step fed SBR. For typical 4-h cycles of the step fed SBR, liquid samples were obtained within the cycle at intervals shown in Fig. 4.3. TP, DP, TS, SS, VSS, TKN, BOD_5, mixed liquid suspended solids (MLSS), and pH for the raw wastewater and liquid samples were measured according to the standard methods (APHA, 2005). Measurements of COD, NH_4^+, NO_2^-, and NO_3^- were performed for liquid samples following the Hach DR2800 Spectrophotometer Manual (Hach, 2005). The FA and FNA concentrations were calculated using Equ. (2-1) and Equ. (2-2) as described early.

Statistical analysis was done by using SAS JMP V6.0. The comparison of different data sets was conducted by One-Way Analysis of Variance (ANOVA) and Tukey-Kramer HSD test embedded in JMP. The null hypotheses of no significant difference between data sets were rejected at 95% significance level when $p < 0.05$.

4.4 Results and Discussion

Swine Wastewater Characteristics

The means and standard deviations of raw wastewater properties are presented in Table 4.1.

Table 4.1 The characteristics of the raw swine wastewater (based on 4 samples in the full system running period)

Item	Value
pH	8.2±0.7
COD (mg/L)	12500±1798.1
BOD_5 (mg/L)	7368.8±778.2
TKN (mg/L)	2558.5±579.8
NH_4^+ (mg/L)	1998.0±131.0
TP (mg/L)	440.1±57.2
P (mg/L)	225.5±43.1
TS (%)	0.93±0.14
TVS (%)	0.45±0.09
SS (%)	0.33±0.05
VSS (%)	0.28±0.04
COD/TKN	5.1±1.4
COD/TP	28.9±6.9

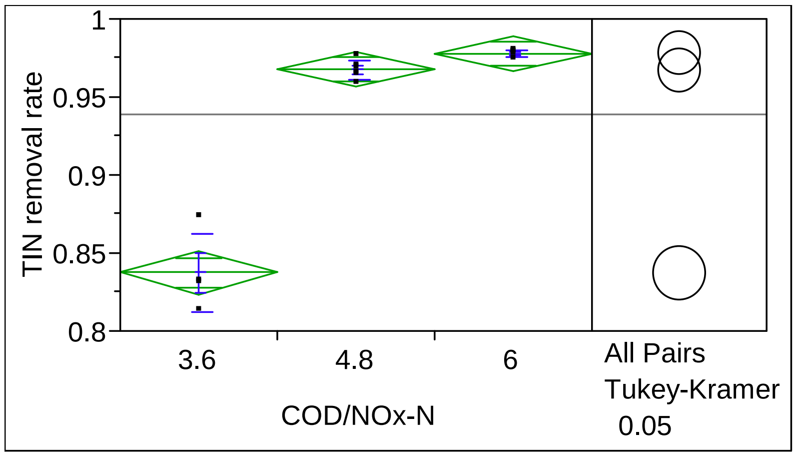
The NSBR Effluent as the Step-fed SBR Influent

To facilitate discussions, the related results from the previous chapters are reiterated here. In Chapter 3, it is confirmed that an effluent with a relatively stable TIN composition could be obtained under different ammonium loading rates from the stability test, which is the premise for conducting contrast experiments in the step-fed SBR. It was found that a HNO_2 concentration of 0.13 mg/L could be the toxicity threshold for nitrite to denitrifying bacteria (Abeling and Seyfried 1992). Therefore, too high a nitrite loading concentration may pose adverse effect on microorganisms in the SBR. From Equ. (2-2) and the neutral pH (assuming 7.5) of the step-fed SBR, safely speaking, the nitrite loading concentration cannot exceed 450 mg/L, without considering the dilution capacity of the SBR. As seen from Table 3.4, the loading rate of 0.039 $gNH_4/gMLSS\cdot d$ was chosen to produce the influent for the second SBR. The nitrite, nitrate and ammonium loading concentrations applied were in the range of 246 - 302 mg/L, 92-117 mg/L, and 71-88 mg/L, respectively. The relatively higher nitrate concentration (23%) in the TIN composition compared to the case in the nitritation SBR effluent (Table 3.3) might be owing to the possibility that some nitrite was oxidized to nitrate in the storage tank between the two SBRs.

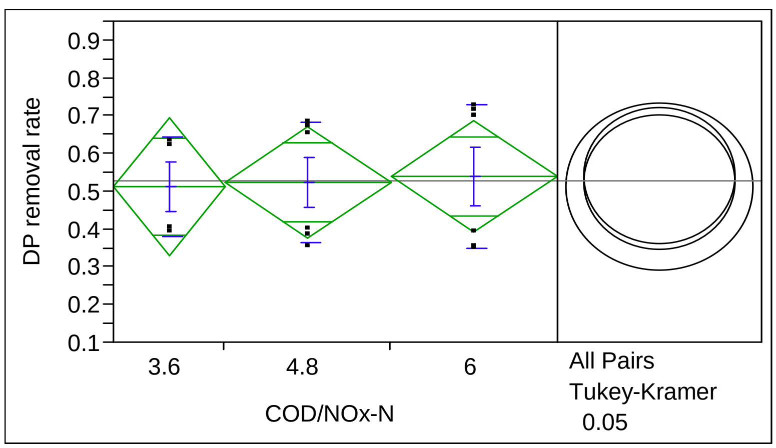
Effect of Different COD/NOx-N Ratios on Nitrogen and Phosphorus Removal

Since the COD/N ratio for full denitrification of NO_3-N and NO_2-N is 7.6 (Sobieszuk et al., 2006) and 2.5 (Hellinga et al., 1998), respectively, three COD/NO_x-N ratios, i.e., 3.6, 4.8, and 6, were selected to test the influence of carbon availability on the TIN reduction and phosphorus removal efficiencies. The ANOVA test of the main effect of feeding COD/NO_x-N ratios on TIN reduction gave a p-value <0.0001, indicating that COD/NO_x-N ratio has a significant influence on TIN removal. Further analysis using Tukey-Kramer HSD test shows that COD/NO_x-N ratios of 4.8 and 6 have no significant difference with 97% and 98% removal rates while 3.6 is an exception with an 84% removal rate (Fig. 4.2 (a)). Both feeding COD/NO_x-N ratios of 4.8 and 6 have achieved an effluent TIN under 20mg/L with mostly nitrate. However, under the feeding COD/NO_x-N ratio of 3.6, there were 10.1±0.38 mg/L ammonium and 56.8±8.9 mg/L nitrate present in the effluent. The nitrate level greatly exceeded 23 mg N/L, a threshold level for blue baby syndrome (Knobeloch et al., 2000). The presence of the large amount of nitrate and elevated amount of ammonium indicates that the denitrification was not sufficient due to the shortage of carbon source, leading to nitrate accumulation in the system. The elevated ammonium could be attributed to this high nitrate environment that dampened nitrification during the aerobic phases with incomplete ammonium oxidation as a result.

Hong et al. (1982) have recommended an influent soluble BOD/P ratio of at least 15:1 to achieve low dissolved phosphorus (DP) concentrations in the effluent from anaerobic-aerobic systems with relatively short SRTs. A moderately efficient process, such as a nitrifying Anaerobic/Aerobic (A/O) or Anaerobic/Anoxic/Aerobic (A^2/O) process, requires 20-25 mg of BOD_5 (34-43 mg of COD) to remove one mg of phosphorus and the quantity becomes even larger for longer SRTs (Grady et al., 1999). The DP concentration fed into the second SBR was between 42 and 60 mg/L. Although the COD/DP ratio in the influent remained in the range of 36-68, most biodegradable COD could be consumed by denitrifiers rather than phosphate accumulating organisms (PAOs), which might affect the removal of DP. The ANOVA test of the effect of the feeding COD/NO_x-N ratio on DP removal gives a *p*-value of 0.966, indicating that COD/NOx-N ratio has no significant influence on DP removal. Further analysis using the Tukey-Kramer HSD test presents three highly overlapped circles (Fig. 4.2 (b)), providing evidence that there is no significantly different effect on DP reduction for different COD/NOx-N loading ratios. The mean value of DP removal is about 52% with 13-28 mg/L DP left in the effluent. According to the U.S. National Pollutant Discharge Elimination System (NPDES) for Concentrated Animal Feeding Operations (CAFOs), if the treated wastewater at the above DP level does not meet the facility's nutrient management plan, chemical precipitation treatments must be considered to further reduce the DP concentration.



(a)



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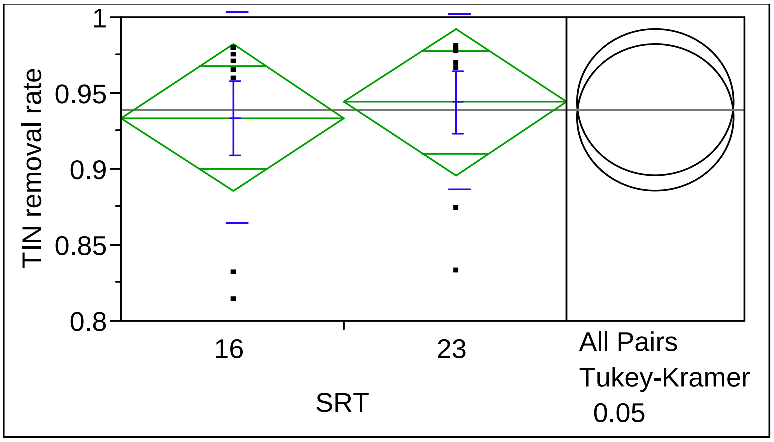
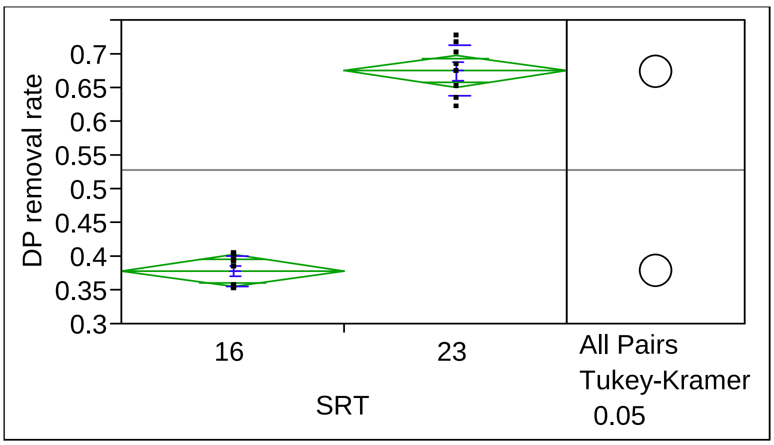


(c)



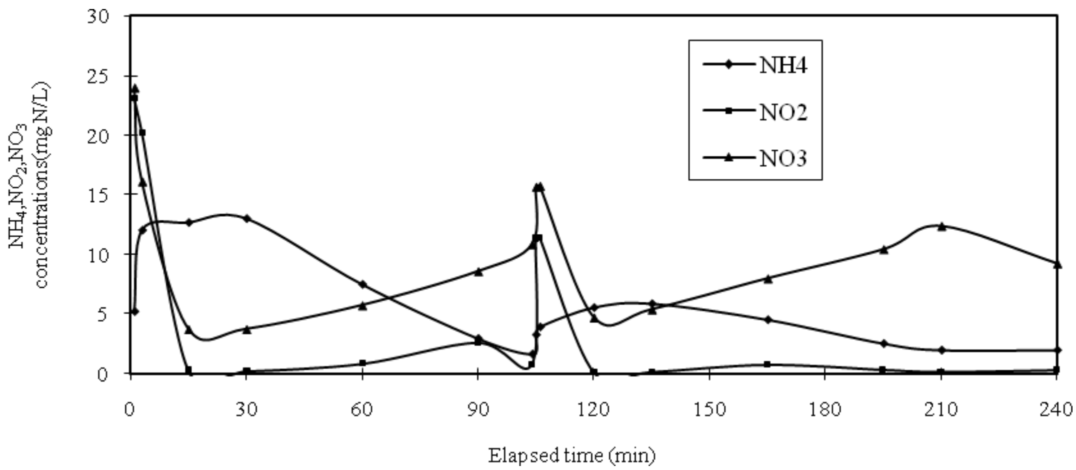
(d)

Fig. 4.2 (a) Oneway Analysis of TIN removal By COD/NO_x-N. (b) Oneway Analysis of DP removal By COD/NO_x-N. (c) Oneway Analysis of TIN removal By SRT. (d) Oneway Analysis of SRT removal By SRT. The center lines of the means diamonds are the group means. The top and bottom of the diamonds form the 95% confidence intervals for the means (Sall et al., 2005).

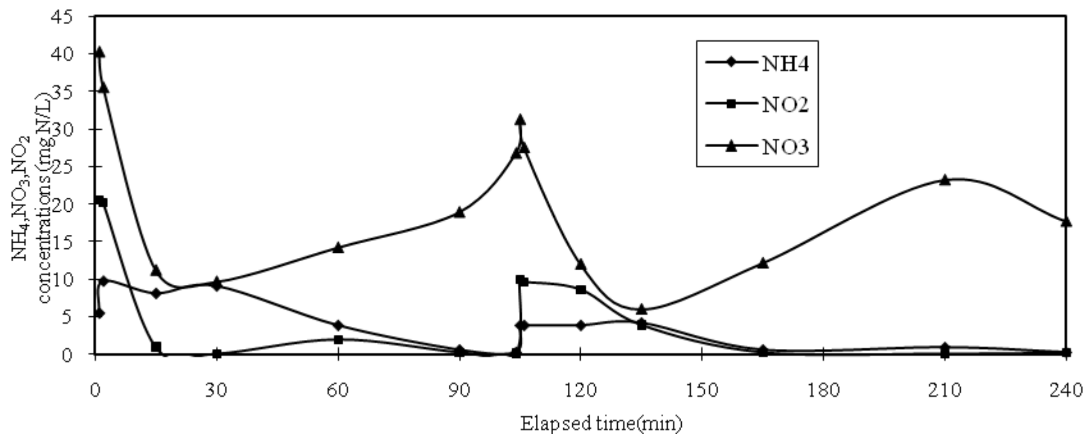
Effect of Different SRTs on Nitrogen and Phosphorus Removal

An SRT that can reconcile nitrification, denitrification and phosphorus removal with one another might be in the range of 15 to 25 days, according to Rittmann and McCarty (2001) and Tremblay et al. (1999), so two SRTs, 16 days and 23 days, were selected to test the effect of SRT on nitrogen and phosphorus removal. Since the microorganisms that can perform denitrification generally belong to the group of fast-growing heterotrophs and do not require a very long retention time, significant differences on TIN removal are not observed between the two SRTs (*p*-value = 0.750) because both SRTs are much longer than the SRT (2 to 4 days) required for solubilization and metabolism of particulate organic matter (Grady et al., 1999). Further analysis using the Tukey-Kramer HSD test gave the same result (no significant difference) for both SRTs with a removal rate of around 94% (Fig. 4.2 (c)).

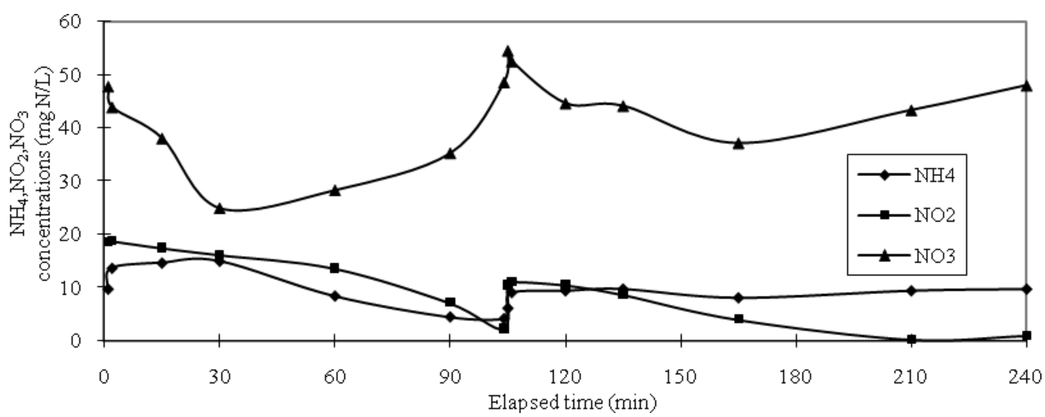
The ANOVA and Tukey-Kramer HSD tests for the effect of SRT on DP removal are shown in Fig. 4.2 (d). DP removal was much better for SRT 23 days than for 16 days (around 67% vs. 38%). For slow-growing phosphate-accumulating organisms (PAOs), a too short SRT would not allow adequate growth of PAOs. Therefore, SRT is a critical factor determining the phosphorus removal efficiency (*p*-value <0.001). Edeline (1993) found that longer SRT related to a risk of sludge bulking and the older sludge might contain dead cells and cellular debris that flocculated badly and were difficult to decant. However, there was no sludge settleability deterioration observed in our system when the SRT was changed from 16 days to 23 days, which was evidenced by the SVI value always ranging from 65 to 97 mL/g. An SVI that does not exceed the value of 150 mL/g is an indicator of good settling properties of the sludge (Janczukowicz et al., 2001).


NH4
NO2
NO3
NH_4,NO_2,NO_3 concentrations(mg N/L)
Elapsed time (min)

(a)


NH4
NO2
NO3
NH_4,NO_3,NO_2 concentrations (mg N/L)
Elapsed time(min)

(b)


NH4
NO2
NO3
NH_4,NO_2,NO_3 concentrations (mg N/L)
Elapsed time (min)

(c)

Fig. 4.3 (a) The trend of nitrogenous components over a typical cycle under SRT of 23 days and COD/NO_x-N ratio of 6; (b) The trend of nitrogenous components over a typical cycle under SRT of 23 days and COD/NO_x-N ratio of 4.8; (c) The trend of nitrogenous components over a typical cycle under SRT of 23 days and COD/NO_x-N ratio of 3.6.

Typical Cycles of the Step-fed SBR

Fig. 4.3 (a), (b) and (c) show the trend of the three inorganic nitrogen component over typical cycles under SRT 23 days and COD/NO_x-N ratios of 6, 4.8 and 3.6 respectively for the step-fed SBR. The first sampling point was conducted after the feeding of the NSBR effluent was done but before the feeding of a mixture of raw swine wastewater and a certain amount of sodium acetate, namely, the carbon sources. Under COD/NO_x-N ratios of 6 and 4.8, the carbon source feeding caused a small abrupt increase of NH_4^+-N, then at a slower pace during the rest of the first anaerobic phase, which was regarded as the process of ammonification (Grady et al., 1999). Both nitrate and nitrite drastically decreased to almost zero in the first 15 minutes, indicating that the carbon matter present in the system was sufficient for the completion of denitrification in both cases. Afterwards, nitrification of the influent NH_4^+-N to NO_3^--N (temporarily to NO_2^--N as the intermediate product) occurred during the first 1 h 15 min aeration phase, where nitrate/nitrite increased and NH_4^+-N decreased constantly. The secondary influent feeding of the NSBR effluent contributed to increases of all three nitrogenous components, NH_4^+-N, NO_3^--N and NO_2^--N, followed by quick drops of both NO_3^--N and NO_2^--N concentrations due to the denitrification process after the carbon source being added resulting in most of the NO_x-N being removed. Nitrification of the remaining NH_4^+-N to NO_2^--N and finally to NO_3^--N and oxidation of the residual NO_2^--N from the previous anoxic phase occurred during the second 1 h 15 min aeration phase, resulting in the gradually increasing NO_3^--N and decreasing NH_4^+-N. Further removal of NO_3^--N was observed in both cases during the settling period before withdrawal, indicating that there was still some biodegradable organic carbon present in the supernatant that could be used for denitrification. Finally, the NH_4^+-N, NO_3^--N and NO_2^--N concentrations were 1.95 mg/L, 9.25 mg/L, and 0.3 mg/L and 0.32 mg/L, 17.7 mg/L, and 0.25 mg/L respectively for COD/NO_x-N ratios of 6 and 4.8. As shown in Fig. 4.3 (c) when the COD/NO_x-N ratio of 3.6 was applied, the large amount of nitrate accumulation was observed due to the shortage of available carbon source. The curves are characterized by insufficient denitrification with incomplete nitrification, which might relate to the inhibitory effect of high concentrations of NO_3^--N and NO_2^--N present through the entire cycle. The NO_2^--N concentration was averaged 9.98 mg/L through the cycle period which could reduce the ammonium oxidation activity according to Fig. 4.4. The mechanism is that nitrite acts as a protonophore that stimulates basal electron transport, inhibits ATP (adenosine triphosphate) synthesis, stimulates ATP hydrolysis, and inhibits various exchange reactions catalysed by the ATP-ase (Sijbesma et al., 1996; Rottenberg, 1990).

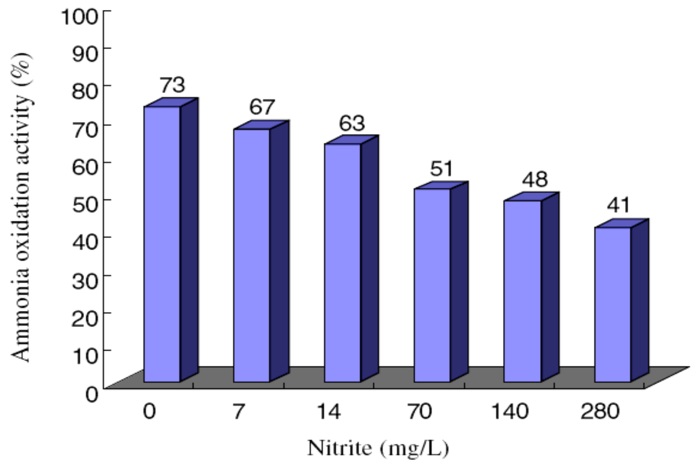
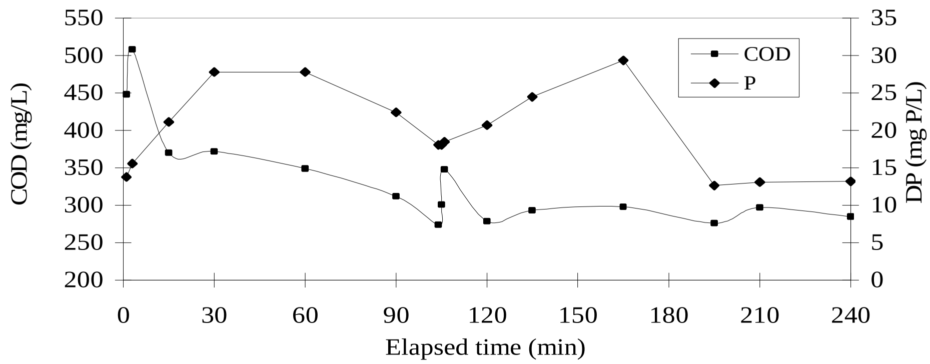


Fig. 4.4 Ammonia-oxidizing activity relative to the initial activity in *Nitrosomonas europaea* cells exposed to nitrite after a 24-h incubation (pH 8) in the absence of ammonium under aerobic conditions (Stein and Arp 1998)

Fig. 4.5 (a) , (b) and (c) show the concentrations of COD and DP in each cycle under SRT 23 days and COD/NO_x-N ratios of 6, 4.8 and 3.6 respectively for the step-fed SBR. Under the COD/NO_x-N ratios of 6 and 4.8, the general rule for phosphorus release and uptake seems tenable. Specifically, the increase of DP concentrations during the anaerobic phase was basically the result of phosphate release by PAOs, which was believed to be the work of bacterial species, belonging to the *Proteobacteria beta* subclasses (Jeon et al., 2003) and/or *Acinetobacter* spp. (Obaja et al., 2003) by utilizing low molecular weight intermediates (particularly acetate) as their carbon and energy sources (Wang et al., 1998; Jeon et al., 2003). The amount of phosphate released in the anaerobic phase was accompanied by the simultaneous decrease soluble COD and extended to the beginning period of aerobic phase. Afterwards, DP was taken up in later part of the aerobic phase. There seems no regular rule to follow for the trend of DP under the COD/NO_x-N ratios of 3.6 possibly due to the disturbance of high nitrate or nitrite and insufficient carbon sources available in the system. However, the removal of DP was just slightly lower (63%) under the COD/NO_x-N ratio of 3.6 than 4.8 (67%) and 6 (71%). The statistical analysis suggests that SRT rather than COD/NO_x-N ratio is the significant factor affecting DP removal.



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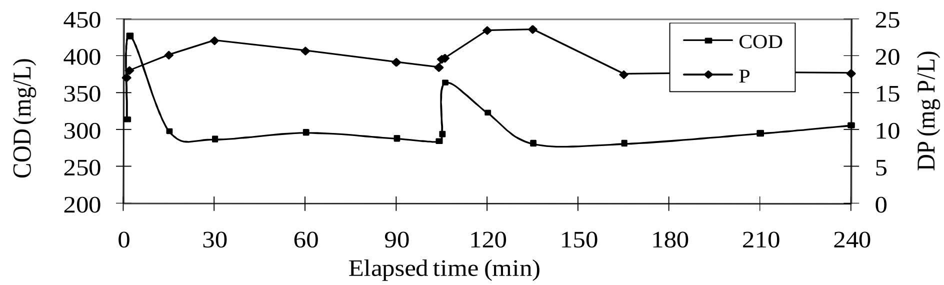

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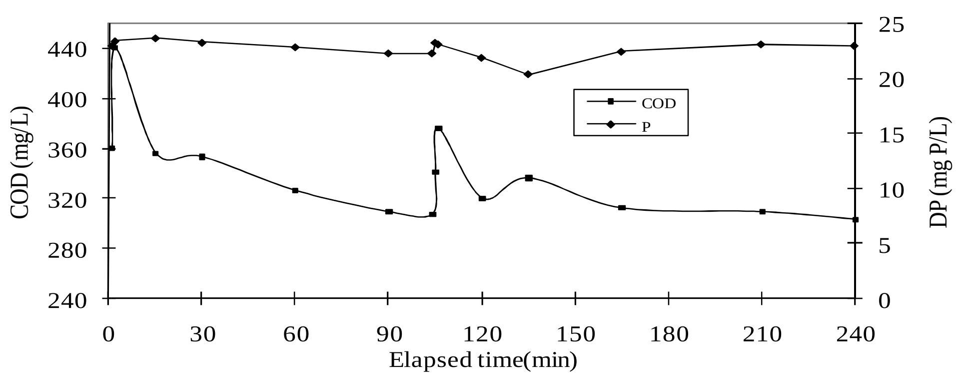

(c)

Fig. 4.5 (a) The trend of COD and DP over a typical cycle under SRT of 23 days and COD/NO_x-N ratio of 6; (b) The trend of COD and DP over a typical cycle under SRT of 23 days and COD/NO_x-N ratio of 4.8; (c) The trend of COD and DP over a typical cycle under SRT of 23 days and COD/NO_x-N ratio of 3.6.

Simple Models for Estimation of Easily Biodegradable Carbon

The concepts for building models were borrowed from activated sludge models (ASMs) developed by International Water Association (IWA). Models were only built for estimation of easily biodegradable carbon (S_S) used by denitrifiers in anaerobic stages using software AQUASIM. The variables and processes involved in the model are listed in Table 4.2. The values of the stiochiometric and kinetic coefficients (Table 4.3) are adopted from literature. Besides S_S, there was another variable, X_H (the amount of microorganism performing denitrification), that was unknown in the model so the trial-and-error method was used for estimation of X_H. When a value of X_H was attempted in AQUASIM, the S_S used for denitrification from NO_2^--N or NO_3^--N could be estimated by the parameter estimation function in the softeware. The trail was stopped when the total S_S estimated approached the amount of COD reduction in the corresponding period within a cycle. The model could not estimate S_S for some cycles with no distinct features, i.e., cycles when COD/NO_x-N of 3.6 was applied. The results estimated for the first anaerobic phase under SRT of 23 days and COD/NO_x-N of 6 showed that for about the same amount of NO_2^--N and NO_3^--N removal (20 mg/L), a lot more S_S was needed for NO_3^--N (88 mg/L S_S) than for NO_2^--N (32 mg/L S_S). However, for the second anaerobic phase under SRT of 23 days and COD/NO_x-N of 4.8, just a little more S_S was needed for NO_3^--N (36 mg/L S_S) than for NO_2^--N (25 mg/L S_S) to remove about the same amount of

NO_2^--N and NO_3^--N (10 mg/L). The unstable performance of this model might be owing to the two simplified equations that could not include some possible reactions such as the carbon take-up by PAOs, anoxic storage of S_S and etc. In addition, the stiochiometric and kinetic coefficients directly adopted from literature might not be representative for use in simulation of biological activities in an SBR treating swine wastewater.

Table 4.2 Stiochiometric matrix

Variable / Process	S_S	S_{NO2}	S_{NO3}	X_H
Anoxic growth of heterotrophs on nitrate $\eta_{NOX}*\mu_H*S_S*X_H/(K_S+S_S)*S_{NO3}/(S_{NO3}+K_{NOx})$	$-\frac{1}{Y_H}$		$-\frac{1-Y_H}{2.86Y_H}$	1
Anoxic growth of heterotrophs on nitrite $\eta_{NOX}*\mu_H*S_S*X_H/(K_S+S_S)*S_{NO2}/(S_{NO2}+K_{NOx})$	$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{1.71Y_H}$		1
Decay of heterotrophs b_H*X_H				-1

PS: the concentrations of all nitrogen species are given as nitrogen. Consequently, two conversion factors (1.71, 2.86) must be used in the matrix to convert nitrite and nitrate nitrogen, respectively, to an equivalent COD basis for calculation.

Table 4.3 Stiochiometric and kinetic coefficients

Parameter	Definition	Value	Dimension	References
K_{NOx}	oxidized nitrogen (nitrate and nitrite) half saturation constant for denitrification	0.5	g NOx-N m^{-3}	Henze et al. (2000)
K_S	Saturation constant for S_S	40	gCOD m^{-3}	Brenner. (2000)
η_{NOX}	Anoxic reduction factor	0.63		Henze et al.(2000)
b_H	decay coefficient of heterotrophs	0.05	d^{-1}	Henze et al.(2000)
μ_H	maximum specific growth rate of heterotrophs	4	d^{-1}	Rittmann and McCarty (2001)
Y_H	Yield coefficient of heterotrophs	0.18	g VSS $g^{-1}BOD_L$	Rittmann and McCarty (2001)

4.5 Conclusions

Shortcut nitrification and denitrification was attempted for high ammonium swine wastewater in two SBRs. The first SBR produced a nitrite rich effluent while the second performed both nitrogen and phosphorus removal. The performance of the second SBR was extensively investigated in this chapter for its denitrification efficiencies with respect to two factors, COD/NO_x-N and SRT.

Data show that COD/NO_x-N ratios of 4.8 and 6 have no significant difference in TIN removal rate (97% vs. 98%), while 3.6 has a much lower removal rate(84%). Both feeding COD/NO_x-N ratios of 4.8 and 6 have achieved an effluent TIN under 20mg/L with mostly nitrate; however, under the feeding COD/NO_x-N ratio of 3.6, there was significant accumulation of nitrate, which could pose adverse impact on natural waters if discharged directly.

The COD/NO_x-N ratio has no significant influence on DP removal. The mean value of DP removal is about 52% with 13-28 mg/L DP left in the effluent. Most biodegradable COD could be consumed by denitrifiers rather than phosphate accumulating organisms (PAOs), which might affect the removal of DP. Chemical precipitation treatments could be considered to further reduce the DP concentration.

Significant differences on TIN removal are not observed between the two SRTs (p-value = 0.750) because both SRTs are much longer than the SRT (2 to 4 days) required for solubilization and metabolism of particulate organic matter (Grady et al., 1999). DP removal was much better under SRT of 23 days (around 67%) than under 16 days (38%), which coincided with the result by Okada et al. (1992) who determined that an SRT of more than 20 days was necessary to achieve higher efficiency of biological phosphorus removal.

Considering both nitrogen and phosphorus removal efficiencies and the economic dosage, the best combination would be COD/NO_x-N ratio of 4.8 and SRT of 23 days for the step-fed SBR, which could achieve 97% TIN removal and 67% DP removal.

Chapter 5. Conclusions and Recommendations for Future Research

5.1 Summary and Conclusions

Based on an extensive literature review that has indentified the problems associated with the practice in wastewater handling and disposal in swine production and the ineffectiveness of the related treatment technologies currently in use, this thesis has investigated a new nitrogen removal process, shortcut nitrification and denitrification (SND), for treating swine wastewater characterized by high nitrogen and phosphorus but low biodegradable organic carbon. The research took a stepwise method by first looking at the possibility of generating an effluent rich in nitrite in a sequencing batch reactor (SBR) and then examining the performance of the subsequent SBR receiving the effluent from the first SBR in removing both nitrogen and phosphorus. In the first step, the results of the possibility study showed that nitrite accumulation from swine wastewater could be achieved by using a continuous feeding strategy for the SBR, which could produce free ammonia and free nitrous acid at inhibitory levels on nitrite oxidizers. Ammonium loadings were increased from 0.04 kg NH_4-N/m^3/d to 0.7 kg NH_4-N/m^3/d, accompanied by nitrite production from 0.03 kg NO_2-N/m^3/d to 0.4 kg NO_2-N/m^3/d. The nitrite/nitrate ratio (NO_2/NO_3) in the effluent lay mainly within the range of 3~4, producing an effluent with 13-23% of NH_4-N, 15-21% of NO_3-N, and 56-72% of NO_2-N. Two cyclic modes with HRTs of 3 and 1.5 days were employed. Lower HRT means larger daily feeding volume, which is always preferred if the same treatment efficiency can be obtained. Our results showed that HRT didn't affect the composition of effluent nitrogenous compounds significantly so a longer feeding time would lead to a greater nitrite production rate. The cycle comparison between the two modes with different HRTs shows that there is no big difference with regard to the whole nitritation process, which is characterized by continuous conversion of loaded ammonium to nitrite and nitrate in the aerobic feeding period and no further conversion after the loading was terminated, resulting in relatively stable levels for all the three nitrogen components in the entire cycle. Compared to the 3-day HRT mode, the 1.5-day HRT cyclic mode has doubled daily output in volume, so it was applied in all of our later experiments.

In order to better understand the process of nitrite accumulation, more bench experiments were performed including an effluent nitrogen composition stability test and a reducing load test. The nitrite production stability was tested using four different ammonium loading rates, 0.075, 0.062, 0.053, and 0.039 $gNH_4/gMLSS \cdot d$ in a 2-month running period. The TIN composition in the effluent was not affected when the ammonium load was between 0.053 and 0.075 g NH_4/g $MLSS \cdot d$ (64% NO_2-N, 16% NO_3-N, and 20% NH_4-N). Under 0.039 g NH_4/g $MLSS \cdot d$, a little more NO_2-N was transformed to NO_3-N with an effluent of 60% NO_2-N, 20% NO_3-N, and 20% NH_4-N. The reducing load test has revealed the relationship between a declining FNA concentration and the decreasing nitrite production. The NH_4^+ load was gradually decreased from 0.081 to 0.011 $g/gMLSS \cdot d$. When the NH_4^+ load was between 0.081 and 0.035 $g/gMLSS \cdot d$, the ratio of $NO_2^-/(NO_2^- + NO_3^-)$ was kept stable around 0.75. When the NH_4^+ load dropped from 0.035 to 0.024 $g/gMLSS \cdot d$, the ratio dropped to 0.70, accompanied by an abrupt decline of FNA from 1.2 to 0.6. From that point forward, the nitrite dominance environment in the system was no longer existing. Combining the results from both the reducing load and stability tests, it is concluded that an ammonium loading rate around 0.035 is the lower threshold for producing a nitrite dominance effluent from the activated sludge SBR.

In the second step, two activated sludge SBRs were connected to achieve SND, one performing partial nitrification to produce nitrite rich effluent and the other aiming at TIN and phosphorus reduction using the nitrite rich effluent as feedstock. Denitrification and phosphorus removal efficiencies were evaluated in the second step-fed SBR. The supplement of easily accessible carbon, sodium acetate, and the step-fed mode were combined to treat the effluent from the nitritation SBR. Three COD/NO_x-N ratios (3.6, 4.8 and 6) were selected to test the influence of carbon availability on the TIN reduction and phosphorus removal efficiencies under two solid retention times (SRT, 16 and 23 days). It was observed that COD/NO_x-N ratios of 4.8 and 6 could achieve 97% and 98% TIN removals with an effluent TIN under 20 mg/L (mostly nitrate) while 3.6 only resulted in 84% removal. Analysis on typical running cycles also showed that nitrate was significantly accumulated when COD/NO_x-N ratios of 3.6 was applied, indicating that the denitrification was not sufficient due to the shortage of carbon source. The COD/NOx-N ratio has no significant influence on dissolved phosphorus (DP) removal. For both SRTs, there was no significant difference observed on TIN removal, but DP removal was much better under SRT of 23 days than under 16 days (around 67% vs. 38%). For this combined SBR system with nitrite accumulation ability to efficiently remove both nitrogen and phosphorus, the best operating condition for the step-fed SBR would consist of a COD/NO_x-N ratio of 4.8 and an SRT of 23 days to achieve 97% TIN and 67% DP removals.

5.2 Contributions

The main contributions of this research include:

(1) Introduced a new treatment process that could lead to energy-saving in treatment of swine wastewater;

(2) Developed an easily operating and reliable system to achieve SND;

(3) Determined the ammonium loading rates that could generate a nitrite dominant effluent under the current setup;

(4) Determined the optimal COD/NOx-N ratio and SRT for the step-fed SBR, under which an effluent with TIN < 20 mg/L could be obtained.

5.3 Recommendations for Future Research

In order to improve this technology for actual applications, further research should focus on the following:

(1) In this study, only one aeration intensity for the nitritation SBR was used, which might not be the most economic. Further effort is needed to identify the best aeration rate for nitrite accumulation.

(2) The phosphorus removal was not as good as nitrogen. If biological method is considered as the further treatment, several factors need to be evaluated, e.g., the ratio of carbon to phosphorus instead of carbon to nitrogen, the

concentrations of nitrite and nitrate in the system at different levels. Otherwise, chemical precipitation may have to be used to further reduce phosphorus in the SBR effluent.

(3) Modelling can be a useful and powerful tool in understanding different biological processes, and in developing and designing efficient SBR systems at the minimal experimental cost. Due to the limitations of instrument (respirometer) and background knowledge, model development is not extensively investigated in this research. This can be an area for future endeavour as well.

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