A two-step fed SBR for treating swine manure

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Abstract
Specially designed sequencing batch reactors (SBR) may be a solution to disposal of massive pig manure, rich in nitrogen (N) and phosphorus (P), produced by concentrated swine operations. In this study, the technical feasibility of simultaneous N and P removals for swine manure was investigated through a bench-scale SBR system operated in a cyclic fashion of anaerobic–anoxic–anoxic/aerobic using low-intensity aeration (1.0 L/(m³ s)), coupled with two-step influent feeding. The risks of emission of greenhouse gases (i.e., CH₄, N₂O and CO₂) were also investigated during the critical periods of operation. 97.5% of the total nitrogen (TN) in the treated manure was removed with only 15 mg N/L of the oxidized N (NO₃–N) left in the effluent. The reductions of total P (TP), chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD₅), and turbidity reached 95, 96, 100, and 95%, respectively. The changes of nutrients as well as soluble COD over time in a complete cycle demonstrated that the SBR system could accomplish multiple processes featuring simultaneous nitrification–denitrification and P removal under aeration with undetectable dissolved oxygen (DO) in the liquid. The unique features of DO, oxidation–reduction potential (ORP) and pH tracked throughout the SBR operation reflected biological changes associated with the nutrients and organic matter in the manure. The primary influent feeding in 75% of the total amount, accompanied by a rapid drop in ORP, stimulated CH₄ emission in a range from 990 to 1200 ppm; however, the secondary influent feeding in 25% of the total amount at the end of the first aeration, with combination of the previously accumulated NO₂–N, resulted in N₂O emission up to 13.9 ppm.

Keywords: Sequencing batch reactor (SBR); Nutrients; Swine manure; Two-step influent feeding

1. Introduction
Concentrated swine operations throughout the world are presently producing a considerable amount of manure with abundant nitrogen (N) and phosphorus (P) as well as organic matter. Liquid swine manure can be a resource for providing essential plant nutrients for maximum productivity. However, continuing land application for manure disposal could result in excessive nutrient loss from soil to water, causing eutrophication that deteriorates water quality [1,2]. Therefore, there is an acute need for development of novel technologies for removals of N and P from animal manure, for which sequencing batch reactors (SBR) using specific designs may hold promises.

Nitrification is generally considered autotrophic conversion of ammonia through nitrite to nitrate in the activated sludge process [3]. However, many observations showed that heterotrophic nitrification can constitute a significant fraction under favorable conditions, such as low DO and relatively high organic loading [4–6]. Compared to autotrophic nitrifiers, heterotrophic nitrifiers generally tend to grow more rapidly with higher yields but requiring lower DO concentrations (e.g., below 0.9 mg O₂/L) [7–9]. These findings imply that nitrification for biological N removal might be operated under low-intensity aeration, even without free oxygen in the bioreactor. Therefore, the energy cost related to SBR operation would be remarkably reduced.

Theoretically, the amount of ammonium in the influent fed into a reactor in the anaerobic stage would be mostly converted into nitrate at the end of aerobic stage under the classic anaerobic–aerobic operation of the SBR. Swine manure commonly contains a large load of nutrients with a total
were restarted when the activated sludge was fed with NO₃ for instance, activities of DNPAOs, such as anoxic P uptake, favorable conditions predominated in the bioreactor [20, 21]. Moreover, both the aerobic PAOs and kinds of PAOs could belong to the identical group based on microbiology of PAOs is not fully understood yet, these two accumulating organisms (DNPAOs) [19–22]. Although the capability of nutrient removal would thus be increased to ensure a low nitrate level in the final effluent.

Numerous studies have elucidated the diversity of P accumulating organisms (PAOs), capable of accumulating excess amounts of polyphosphate, in the enhanced biological P removal (EBPR) process for wastewater [17, 18]. The population of PAOs typically consists of two groups: one group capable of utilizing only oxygen as the final electron accepter under a classic anaerobic and aerobic sequence (aerobic PAOs), and the other group accumulating P by using nitrate/nitrite as the electron acceptor instead of oxygen, called denitrifying P-accumulating organisms (DNPAOs) [19–22]. Although the microbiology of PAOs is not fully understood yet, these two kinds of PAOs could belong to the identical group based on rRNA analyses [12, 18]. Moreover, both the aerobic PAOs and DNPAOs would be individually reactivated once the respective favorable conditions predominated in the bioreactor [20, 21]. For instance, activities of DNPAOs, such as anoxic P uptake, were restarted when the activated sludge was fed with NO₃ at the end of the anaerobic stage in SBR [20, 23]. The DNPAOs were reported to be in preference to aerobic PAOs, principally because of the savings of organic substrate and energy (aeration) and the anoxic phosphate removal occurring simultaneously with denitrification in the same reactor [15, 23]. Based on these findings, it may be hypothesized that under the condition of low-intensity aeration, free oxygen would be largely utilized by autotrophic and/or heterotrophic nitrifiers for nitrification; the newly produced nitrate would simultaneously be utilized as the electron acceptor by DNPAOs for P uptake. Therefore, the multiple processes, including nitrification–denitrification and P storage in sludge, could be simultaneously carried out during the phase of aeration. Additionally, if the anoxic stage is introduced in the middle of the aerobic phase of an anaerobic–aerobic SBR coupled with secondary manure feeding, P release will be inhibited while P uptake by DNPAOs will continue to proceed due to the remaining oxidized N.

A variety of methanogenic archaea, including Methanobrevibacter sp., Methanocorpusculum sp., and Methanoculleus sp., widely exist in swine manure storage [24], which presents a potential capacity of converting 95% volatile solids into CH₄ in prevailing anaerobic conditions [25]. Normally, nearly half a cycle of the SBR operation is conducted under the anaerobic stage along with influent feeding, leading to an apparent tendency for CH₄ emission. The mechanisms of nitrous oxide emission commonly include processes of (1) nitrification, utilizing nitrite as an alternative electron acceptor, thereby reducing it to N₂O; (2) dissimilatory nitrate reduction (denitrification); and (3) assimilatory nitrate reduction [26]. Combination of nitrification–denitrification is proven to be the dominating process for simultaneous removal of nutrients in an SBR system, which may accompany with emission of N₂O.

Presently, cost-effective engineering technologies at a massive scale for animal manure treatment as seen in industrial and/or domestic wastewater plants are not readily available. In this study, the technical feasibility of simultaneous N and P removals for swine manure was investigated through a bench-scale SBR system. Two-step influent feeding was adopted to minimize the concentration of oxidized N in effluent. In order to reduce energy cost and stimulate heterotrophic nitrification, the aerobic stage was maintained by low-intensity aeration. Therefore, this particular SBR was run in a mode of anaerobic–aerobic/anoxic–anaerobic–anoxic/aerobic. In addition, three kinds of biogas, i.e., CH₄, N₂O, and CO₂, were also investigated during the critical periods of operation to assess the risk of greenhouse gas emissions.

2. Materials and methods
2.1. Swine manure and characteristics

Raw swine manure was collected 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 test manure for the SBR was sampled from a concrete manure tank where the solids-separated liquid (sieve opening: 2.5 mm) was stored for normally less than one week before further treatment. Prior to loading the influent tank, the collected manure was stored at 4 °C or below. The properties of the influent manure are shown in Table 1.

2.2. System configuration

The reactor body was fabricated from a transparent Plexiglas cylinder (19.0 cm in diameter), with a total volume of 11.0 L, and a working volume of 8.0 L. It was equipped with a number of subsystems, including influent feeding/effluent discharging, air supply, and online monitoring and data acquisition systems (Fig. 1). Briefly, two peristaltic pumps (MasterFlex L/S 7550-30) in charge of influent feeding and effluent discharging, respectively, and a mechanical mixer (Servodyne 5003-20) with a lab-made, four-blade paddle (15.2 cm length × 12.0 cm height) were programmed through a Linkable Instrument...
Network for Windows (WIN LIN V1.2) in a series of predefined control time sequences or ‘block’. Air was provided by a vacuum-pressure pump (Barnant 60010-2392), run on a timer (Cole-Parmer BH-94460-45), through five separate gas-diffusing stones (Fisher Brand) evenly placed at the bottom of the reactor. DO in the liquid was measured using an oxygen meter (Extech, Model 407510) that was inserted into a 30 mL chamber, where the liquid drawn 7 cm below the surface was passed through at a flow rate of 4.8 mL/s by a peristaltic pump (MasterFlex 7518-10). The data acquisition system (Campbell 21X) was used for the continuous recording of pH and oxidation-reduction potential (ORP) (Campbell CSIM11) at an interval of one minute.

2.3. SBR operation

The activated sludge, obtained from a local municipal wastewater treatment plant, was inoculated in the reactor for start-up. The SBR had been operated under alternating anaerobic–aerobic conditions at a temperature of 20 ± 1°C over 3 months to reach steady state before the start of experiment.

The SBR system was operated in an 8.0-h cycle mode with two separate influent feedings. The 8-h cycle consisted of 1 h 15 min anaerobic, 2 h 45 min anoxic/aerobic, 1 h 30 min anaerobic, 2 h anoxic/aerobic, and 30-min settling. The primary feeding (600 mL in 4 min) always occurred at the start of each 8-h cycle. The secondary feeding (200 mL in 2 min) was conducted at the beginning of the second anaerobic stage. Effluent of 800 mL was discharged within the last 15 min of settling in each cycle. Therefore, the hydraulic retention time (HRT) was 3.3 days. According to the preliminary tests, aeration with intensity of 1.0 L/(m³ s) could maintain the liquid in anoxic stage during the two individual phases of aeration. Suspended solids (SS) in the SBR at the end of the test were maintained at 0.75 ± 0.10% by withdrawing a comparable amount of excess sludge. Excess sludge volume of 140–180 mL containing SS levels of 1.74 ± 0.15% was discharged from the reactor manually twice a day 5 min before the end of settling, by which the solids retention time (SRT) could be determined at 23 ± 0.4 days. The volume loss (due to discharge of excessive sludge and water evaporation induced by aeration process) was replenished with water to keep the volume constant inside the reactor. The agitation speed during the first half hour of anaerobic periods and the periods of aeration was controlled at 25 rpm using the lab-made, four-blade paddle mixer.

![Diagram of the sequencing batch reactor (SBR).](image-url)
2.4. Sampling and analytical 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 200 mL each were taken from the effluent tank when one cycle was completed. For on-line chemical measurement, 10 mL of mixed liquid was drawn during mixing at an interval of 0.5 h by a 25 mL syringe then injected into a 15 mL centrifuge tube. After centrifuging at 7000 rpm for 6 min, the supernatant was obtained for analyses of ammonium nitrogen (NH\textsubscript{4}–N), nitrate nitrogen (NO\textsubscript{3}–N), nitrite nitrogen (NO\textsubscript{2}–N), chemical oxygen demand (COD) and soluble phosphorus (SP). Measurements of pH, total solids (TS), suspended solids (SS), volatile suspended solids (VSS), total phosphorus (TP), soluble phosphorus (SP), total Kjeldahl nitrogen (TKN) and 5-day biochemical oxygen demand (BOD\textsubscript{5}) for both influent and effluent liquid samples were performed following the standard methods [27]. NH\textsubscript{4}–N, NO\textsubscript{2}–N, NO\textsubscript{3}–N, TKN and 5-day biochemical oxygen demand (BOD\textsubscript{5}) were measured following the DR/3000 spectrophotometer manual [28]. Concentrations of total nitrogen (TN) were the sum of TKN and NO\textsubscript{3}–N. Percent reductions for those parameters are calculated by dividing the difference between the influent and effluent concentrations for each 8-h cycle by the influent concentration, then multiplied by 100.

The gas sampling port was permanently located 10 cm above the liquid surface inside the reactor. After flushing twice, a gas sample was collected using a 15 mL syringe and injected into a 5 mL vial with suitable over-pressure for preventing gas leaking. Gas samples were collected at points of particular interest (such as at the start/end of feeding, aeration, and mixing; during the rest of the anaerobic stage mainly because CO\textsubscript{2} (produced by microorganisms’ respiration) was resolubilized into the liquid alkalinity inside the reactor after influent feeding [30]. Subsequently, a sharp drop in pH was observed during the occurrence of positive values of ORP basically coincided with the liquid was detected until about half an hour before the end of the second aeration phase (Fig. 2). Meanwhile, the occurrence of positive values of ORP basically coincided with the appearance of measurable DO. Therefore, the first aeration phase was actually an anoxic phase, during which the oxygen delivered by low-intensity aeration was instantaneously consumed by the autotrophic organisms and/or existing reductive compounds.

The pH change over time has some unique features. First, the pH increased rapidly when influent was fed into the reactor. This phenomenon is believed to be caused by the rapid increase in the liquid alkalinity inside the reactor after influent feeding [30]. Subsequently, a sharp drop in pH was observed during the rest of the anaerobic stage mainly because CO\textsubscript{2} (produced by microorganisms’ respiration) was resolubilized into the solution [30] and the biological P was released from PAOs [20]. After aeration was initiated, the pH increased again with time, which could be attributed to CO\textsubscript{2} stripping from the system [20,30]. The subsequent decrease of pH in the aeration

3. Results and discussion

3.1. Quality of effluent

Table 2 shows the concentrations of solids, nutrients and organic matter as well as turbidity in the discharged effluent along with the corresponding reductions after an 8-h cycle during SBR operation.

TKN reduction reached 99.1% with a final level in effluent of 8.0 mg N/L, while NH\textsubscript{4}+–N was reduced by 100%. The difference between TKN and NH\textsubscript{4}+–N is believed to be the non-degradable organic nitrogen [27] that has less impact on water quality than ammonium. 97.5% of the TN reduction was realized, and levels as low as 15 mg N/L of NO\textsubscript{3}–N and no NO\textsubscript{2}–N were found in the effluent. Although the treatment is not able to remove all NO\textsubscript{3}–N in the effluent, it will help reduce the potential risk of blue baby syndrome (23 mg N/L) if the treated liquid somehow makes its way to public waters [14]. The reductions of TP and SP reached over 95%, with final concentrations of 2.1 and 1.5 mg P/L in the effluent, respectively. This is similar to the results from tests using low-strength synthetic wastewater generally containing TP in influent less than 20 mg P/L [9,20]. It can thus be concluded that the remarkable performance of this two-step feeding SBR in removing nutrients in the treated manure makes it possible to meet the stringent environmental requirements in terms of protecting natural water resources.

Nearly, 100% of BOD\textsubscript{5} was broken down in this experiment. The remaining COD likely consists of refractory compounds [15], such as humate, cellulose, lignin and/or non-degradable polysaccharide. Over 92% of VSS was biodegraded and the reduction of turbidity reached as high as 95%, although the TS reduction was relatively low in this study. The predominant component of solids remaining in the reactor could be oxidized iron minerals and/or soluble ‘refractory’ organic matter, the latter probably contributing to most of the remaining COD in the effluent.

3.2. Profiles of DO, ORP and pH

The unique features of DO, ORP and pH tracked throughout the operation of SBR may indirectly reflect specific chemical, biochemical, and biological changes over time [9,20,29–31].

Since low-intensity aeration was selected, no free oxygen in the liquid was detected until about half an hour before the end of the second aeration phase (Fig. 2). Meanwhile, the occurrence of positive values of ORP basically coincided with the appearance of measurable DO. Therefore, the first aeration phase was actually an anoxic phase, during which the oxygen delivered by low-intensity aeration was instantaneously consumed by the autotrophic organisms and/or existing reductive compounds.

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stage was mainly a result of the release of H⁺ from nitrification until a pH valley was eventually reached. The valley was reported as the pH breakpoint that coincided with the end of NH₄⁺–N conversion to NO₃⁻/Cₐ–N/NO₂⁻/C₀–N [30]. The rebound of pH occurred following the valley, probably because more CO₂ was stripped than produced in the system. The characteristics of the pH curve over time after the secondary feeding were not markedly different.

The primary feeding with 75% of the total amount reduced ORP tremendously at the beginning, but an 'elbow' at ORP of around −250 mV was observed, which was believed to be the end of denitrification and the start of biological P release [9,20,30]. From that point forward, the decrease of ORP was slowed down. Although free oxygen in the liquid was not detected during the first aeration phase, ORP displayed a rapid increase in the first 15 min, followed by a slow increase afterwards. After the secondary feeding, however, the ORP showed a rapid decrease in the first 10 min, reaching a value of −110 mV (with an average decrease ratio of ORP per minute, i.e., dORP/dT, of −11.4 mV/min), then decreased at a lower rate in the next 30 min (average dORP/dT of −1.3 mV/min), finally reached another faster decreasing rate again in the rest of time (average dORP/dT of −2.8 mV/min). After the rapid rebound of ORP due to start of the second aeration, ORP reached a relative plateau value in the remaining one hour, which indicated that the SBR reached an oxidized state with little biodegradable carbon left and the aeration should be terminated [20,30]. The cyclic features of pH and ORP in a period of 24 h are presented in Fig. 3, showing the comparable stability of the test SBR system. These particular behaviors of pH and ORP in this study with specific features can be used in combination with the characteristic curves of nutrient concentration in the reactor (discussed below) as guidelines to monitor status of operation and perform problem-solving diagnosis.

### 3.3. Profiles of nutrient concentration and organic matter

Fig. 4 shows the trend of nitrogenous compounds over the complete cycle. The primary influent feeding caused a drastic increase of NH₄⁺–N immediately, then at a slower pace during the rest of anaerobic stage, which was regarded as the process of ammonification [3]. Meanwhile, nitrate drastically decreased to almost zero, indicating completion of denitrification, which coincided with the change of pH (Fig. 2). Afterwards, nitrification of NH₄⁺–N to NO₃⁻–N (temporarily to NO₂⁻–N as the intermediate product) occurred during the first 2 h 45 min
The net/apparent rates of ammonium removal, nitrification, denitrification, P release and P uptake obtained during the 8-h SBR operation are shown in Table 3. These rates were measured during the anaerobic stage, where nitrate/nitrite increased and NH$_4^+$--N decreased constantly. The secondary influent feeding contributed to some increase of NH$_4^+$--N while the denitrification process resulted in most of the NO$_3^-$/NO$_2^-$ being removed. The change of mass balance of total nitrogen in liquid phase (sum of NH$_4^+$--N and NO$_3^-$/NO$_2^-$) over time experienced a similar tendency to that of NH$_4^+$--N concentration. The subsequent aeration process achieved complete removal of NH$_4^+$--N in the liquid before sludge sedimentation.

Fig. 5 shows the concentrations of P during each reaction phase. The increase of SP concentrations during the anaerobic stage 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 [32] and/or Acinetobacter spp. [15] by utilizing low molecular weight intermediates (particularly acetate) as their carbon and energy sources [19,32]. The amount of phosphate released in the anaerobic stage was proportional to the simultaneous decrease in the measured free oxygen was not found in the reactor (Fig. 2). Although the secondary feeding caused an apparent decrease of ORP (Fig. 2), no P release was found in the second anaerobic stage. P was continuously reduced at a lower pace in the second aeration stage.

It is observed that the variations of ORP and pH over time are generally coincident with the changes of N and P, as well as COD in this SBR system, indicating that the two-step feeding pattern is feasible for simultaneous removals of nutrients and organic matter in swine manure. Notably, nitrification was carried out under the conditions of nearly no free oxygen and negative ORP in this study, clearly indicating the existence and capability of both the autotrophic and heterotrophic nitrifiers in the SBR. It was commonly found that the total levels of NO$_3^-$--N and NO$_2^-$--N at the end of aeration were about half of the consumed NH$_4^+$--N from the feed, particularly for the first aeration, which strongly suggested the successful coexistence of nitrification and denitrification processes in this low-intensity aeration period. The major working force in this scenario would be DNPAOs, because distinct P uptake occurred during the anoxic stage featuring abundance of oxidized N as the rate of P uptake slowed down (Figs. 4 and 5). The observations of continuous P uptake rather than P release (Fig. 5) and the stable decline of oxidized N since the cease of the first aeration, combined with secondary feeding, also proved the existence of DNPAOs. Additionally, simultaneous nitrification and denitrification (SND) could be achieved within the activated sludge flocs where nitrification was restricted to the outer oxic zone whereas denitrification occurred mainly in the inner anoxic zones [33,34]. Undeniably, both nitrification and denitrification of aerobically treated swine manure could be sources of nitrous oxide [35]. Since DO was not detected and ORP remained in negative during the stage of aeration, accumulation of NO$_3^-$--N over time was observed (reaching about half of the NO$_3^-$--N amount). Therefore, incomplete nitrification may contribute to the emission of nitrous oxide.

### 3.4. Quality of biomass

Based on the changes of nutrients over time (Figs. 4 and 5), the net/apparent rates of ammonium removal, nitrification, denitrification, P release and uptake, both as the average and maximum, were calculated in Table 3 in terms of the efficiency of biomass. Since these net values were obtained from the operating system with several processes coexisting, such as nitrification, denitrification, and/or denitrifying P uptake, it is not surprising that these values were relatively lower than those derived from sludge batch experiments controlled by a single factor [15]. The efficiency of DNPAOs for P uptake by utilizing oxidized N was commonly lower than that of aerobic PAOs by a factor of 1.13 to 1.26 mg N/gSS h in this study (Table 3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Anaerobic (8:00–9:15)</th>
<th>Anoxic (9:15–12:00)</th>
<th>Anoxic/anaerobic (12:00–13:30)</th>
<th>Anoxic/aerobic (13:30–15:30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Maximum</td>
<td>Average</td>
<td>Maximum</td>
</tr>
<tr>
<td>NH$_4^+$--N removal</td>
<td>mg N/gSS h</td>
<td>–</td>
<td>–</td>
<td>3.06</td>
<td>4.46</td>
</tr>
<tr>
<td>Nitrification</td>
<td>mg N/gSS h</td>
<td>–</td>
<td>–</td>
<td>1.93</td>
<td>3.68</td>
</tr>
<tr>
<td>Denitrification</td>
<td>mg N/gSS h</td>
<td>16.09</td>
<td>16.09</td>
<td>1.13$^a$</td>
<td>–</td>
</tr>
<tr>
<td>P release</td>
<td>mg P/gSS h</td>
<td>3.70</td>
<td>8.19</td>
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<td>–</td>
</tr>
<tr>
<td>P uptake</td>
<td>mg P/gSS h</td>
<td>–</td>
<td>–</td>
<td>1.56</td>
<td>4.12</td>
</tr>
</tbody>
</table>

$^a$ Note: on the assumption that the amount of removed ammonium N is the sum of the oxidized N by nitrification and the emitted N by denitrification.
The overall observed biomass yield reached approximately 2.56 g SS per day under operation with an SRT of 23 days, which was equal to a conversion constant of 0.18 g SS/g of COD fed in influent, according to biomass analysis in this study. The total P level in sludge (expressed as a ratio of P to volatile suspended solids in the mixed liquor sampled close to the end of secondary aeration) was found around 8.6%. Typically, the P content in biomass in the enhanced biological phosphorus removal process was reported on the order of 5–7%, with some values higher than 15% of cell dry weight [3,18]. Methods commonly used to handle dewatered sludge generally may include composting (provided that heavy metal content is acceptable), incineration, and landfill in a full-scale system.

3.5. Risk of emission of greenhouse gases

Five minutes after primary influent feeding, the amount of methane (CH₄) in the headspace of the SBR (emitted from the liquid) quickly reached over 990 ppm (Fig. 6), 600 times the atmospheric background (1.65 ppm), due to abundant external input of organic matter accompanied by a rapid decrease in ORP (Fig. 2). The CH₄ level continued to increase and reached approximately 1200 ppm at the end of the anaerobic stage. Meanwhile, the CO₂ concentration also gradually climbed because of microbial respiration. When aeration started, the levels of both CH₄ and CO₂ in the liquid and/or sludge were dramatically reduced, leaving behind two very transient peaks (Fig. 6). Since the stringent anaerobic state was shifted to anoxic by low-intensity aeration, the activity of methanogenic organisms was suppressed, which directly contributed to the reduction of the CH₄ concentration to background levels. On the contrary, CO₂ continuously remained at a level three times the background (1.65 ppm), showing the bloom of biological activity in the bioreactor. Although a slight rebound of CH₄ was observed shortly after the secondary feeding, the absolute level of CH₄ was significantly lower than observed for the primary feeding, probably because of the presence of ample nitrate/nitrite in the next 2-h period (Fig. 4), which likely inhibited the activity of the methanogens [36,37]. Again, generation of CH₄ was minimal during the second stage of aeration, along with the apparent decrease of CO₂, simply because the easily biodegradable COD in substrate had mostly been consumed (Fig. 5, Table 2). Therefore, to minimize CH₄ generation, measures, such as addition of ferric-containing chemicals should immediately follow primary influent feeding.

The characteristics of N₂O release over time (Fig. 7) were different from those of CH₂ and CO₂. The concentrations of N₂O emitted after the primary feeding were slightly elevated (0.41–1.78 ppm) compared to atmospheric background levels (0.33 ppm). Levels of N₂O tended to increase during the first aeration accompanied by increases in nitrate/nitrite (Fig. 4). A maximum level of N₂O up to 13.94 ppm, 42 times the background, was recorded before descending during the second anaerobic phase. Shortly after the start of the second aeration, the concentration of N₂O emission drastically decreased, nearly reaching the background level at the end of aeration. The decreasing trend of N₂O emission during the second aeration was mainly due to the increase in DO up to 3.0 mg O₂/L (Fig. 2), which is consistent with a previous report where little N₂O emission was found through nitrification under a higher level of aeration with DO of 3.8 mg O₂/L using an ¹⁵N tracer [35]. However, simultaneous nitrification-denitrification under low-intensity aeration without free oxygen in the first aeration period (Fig. 4) might contribute to the ascending trend of N₂O emission.

In comparison to CH₄, the key period of N₂O emission occurred in the period of secondary feeding instead of primary feeding in this study. Two factors could be responsible for that phenomenon. First, the reduction of N₂O to N₂ is generally catalyzed by nitrous oxide reductase (Nos), and the elevated level of nitrite (over 2 mg N/L) could inhibit Nos, leading to the accumulation and thus emission of N₂O in the off-gas [33,38]. A range from 7.75 to 1.63 mg N/L of nitrite within the 2-h reaction phase was observed after the secondary feeding, while no nitrite was found after the primary feeding (Fig. 4). Second, both the average and maximum rates of denitrification were much higher after the primary feeding than after the secondary feeding (Table 3), mainly because the ratio of influent to effluent was set at 3:1, with the secondary feeding performed immediately after the end of the first aeration. This is why a rapid ORP decrease occurred after the primary feeding, but without 'transitional changes' similar to those observed after the second feeding (Fig. 3). It is implied that a low rate of
denitrification with abundant nitrite present tends to promote N₂O emission, since one of the N₂O generation mechanisms is closely associated with the course of dissimilatory nitrate reduction through denitrification [26].

4. Conclusions

The specific SBR system with a two-step influent feeding, i.e., 75% of the total amount for the primary and 25% for the secondary, coupled with low-intensity aeration (1.0 L/(m² s)), can effectively remove nutrients and promote biodegradation of organic matter for swine manure treatment.

The 8-h per cycle SBR with alternating anaerobic–anoxic–anaerobic/anoxic/aerobic conditions realized the reductions of TN, TP, COD, BOD₅ and turbidity by about 98, 95, 96, 100, and 95%, respectively. The concentrations of NH₄⁺–N and SP were also reduced by about 100 and 97%.

The changes of nutrients, as well as soluble COD, over time in a complete cycle demonstrated that this particular SBR system could accommodate multi-process functions with simultaneous nitrification–denitrification and P removal. Both nitrifiers, autotrophs and heterotrophs, were believed to convert NH₄⁺–N to NO₂⁻/NO₃⁻–N under low-intensity aeration with no measurable DO and negative ORP. DNPAOs used the oxidized N as electron acceptors in both anoxic and anoxic/aerobic stages to achieve P uptake and denitrification. The unique features of DO, ORP and pH tracked throughout the SBR operation reflected the biological changes associated with the nutrients and organic matter in the manure. Periodic behaviors of pH and ORP were nicely repeated in a period of 24 h, showing the comparable stability of the test SBR system.

Primary influent feeding with a relatively abundant external carbon source caused a rapid increase in ORP, which stimulated CH₄ emission and resulted in a range of CH₄ concentrations from 990 to 1200 ppm in the SBR headspace. Corresponding preventive measures should be considered to minimize CH₄ emission, such as addition of ferric-containing chemicals. Accumulation of NO₂⁻–N during the first aeration together with insufficient organic matter provided by the secondary feeding resulted in N₂O emission up to 13.9 ppm. The low rate of denitrification in the anaerobic phase and incomplete nitrification during aeration were the major ‘catalyzers’ for N₂O emission in this SBR system.

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References


