Effect of intermittent aeration mode on nitrogen concentration in the water column and sediment pore water of aquaculture ponds

Dantong Zhu1,2,3, Xiangju Cheng1,2, David J. Sample3,*, Mohammad Nayeb Yazdi3

1 State Key Laboratory of Subtropical Building Science, South China University of Technology, Guangzhou 510640, China
2 School of Civil Engineering and Transportation, South China University of Technology, Guangzhou 510640, China
3 Department of Biological System Engineering, Virginia Polytechnic Institute and State University, Virginia Beach 23455, United States

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ABSTRACT

Nitrogen in pond sediments is a major water quality concern and can impact the productivity of aquaculture. Dissolved oxygen is an important factor for improving water quality and boosting fish growth in aquaculture ponds, and plays an important role in the conversion of ammonium-nitrogen (NH4+-N) to nitrite-nitrogen (NO2-/NO3-N) and eventually nitrate-nitrogen (NO3-N). A central goal of the study was to identify the best aeration method and strategy for improving water quality in aquaculture ponds. We conducted an experiment with six tanks, each with a different aeration mode to simulate the behavior of aquaculture ponds. The results show that a 36 hr aeration interval (Tc = 36 hr: 36 hr) and no aeration resulted in high concentrations of NH4+-N in the water column. Using a 12 hr interval time (Tc = 12 hr: 12 hr) resulted in higher NO2-/NO3-N concentrations than any other aeration mode. Results from an 8 hr interval time (Tc = 8 hr: 8 hr) and 24 hr interval time (Tc = 24 hr: 24 hr) were comparable with those of continuous aeration, and had the benefit of being in use for only half of the time, consequently reducing energy consumption.

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Introduction

The rapid development of the aquaculture industry to satisfy demands of an increasing population has led to residual feed, metabolites, and excreta being deposited in sediments of aquaculture ponds. Nitrogen in the pore water within pond sediments stems from feed inputs and digestion byproducts (Dalsgaard and Pedersen, 2011), and is a major concern in aquaculture ponds. Nitrogen concentrations in pore water are often 50 to 500 times higher than those in the water column (Zhou et al., 2015). Nitrogen is a key growth-limiting element, that, when in excess, fosters toxic microcystis blooms during the summer, which could limit a pond’s primary productivity, and degrade its water quality (Burkholder et al., 2016; Suominen et al., 2017; Chaffin et al., 2018). Nitrogen could
impose a significant threat to the water environment if discharged, and negatively impacts aquaculture production, thus a number of practices have been developed to reduce or remove it (Ray et al., 2015; Xiao et al., 2017; Sacristán de Alva et al., 2018). Nitrogen released from pond sediments can promote autotrophic growth in freshwater ecosystems, seriously affecting the health of many aquatic species (Cheng et al., 2016; Wang et al., 2016).

Nitrification and denitrification in the water environment are tightly coupled, and the temporal scale of nitrate diffusion at the sediment-water interface (SWI) is thought to limit the role of sediment denitrification in ponds (Venterink et al., 2003; O’Connor and Hondzo, 2008). Denitrification is a process by which nitrogen (predominately nitrate-nitrogen) is reduced, ultimately leading to the production of N₂ and N₂O gas. Denitrification is used for mediating levels of bioavailable nitrogen in aquatic ecosystems; it occurs under anaerobic conditions and requires a suitable carbon source to act as an electron donor, and available nitrate in the water column (Cold et al., 2018). Dissolved oxygen (DO) is an important factor for improving water quality and boosting fish growth in aquaculture ponds, and plays an important role in the conversion of ammonium-nitrogen (NH₄⁺-N) to nitrite-nitrogen (NO₂⁻-N) and eventually, nitrate-nitrogen (NO₃⁻-N), which is performed primarily by ammonia-oxidizing bacteria (Ammonio-mox) and archaea (Methanomox and methanogenic Archaea) (Mulholland et al., 2008; Liu et al., 2013; Xia et al., 2017). Several studies have suggested that nitrification and denitrification may occur in the water column of some estuaries with high turbulence (Abell et al., 2010; Gao et al., 2012; Xia et al., 2017), but the dominant driver is thought to likely be from DO generated by turbulence. Artificial aeration is a popular water quality enhancement used in many aquaculture ponds (Abu Hasan et al., 2013; Brđjanovic et al., 1998; Harris et al., 2015; Regmi et al., 2014). However, most oxygenation equipment requires mechanical assistance, with consequent increases in maintenance costs and electrical consumption (Fan et al., 2013). Thus, operationally simple and cost-effective technologies and methods for improving the water quality of aquaculture ponds are needed.

Adopting an intermittent aeration (IA) strategy, where periods of aeration and non-aeration (NA) alternate, creating aerobic and anoxic conditions, has been explored as a way to facilitate nitrogen removal from wastewater, biological sludge and landfill waste (He and Shen, 2006; Lim et al., 2012; Maekawa et al., 1995; Sang et al., 2009). In an IA system, high DO concentrations during the aeration period enable aerobic nitrifying bacteria to oxidize NH₄⁺-N to NO₂⁻-N and then to NO₃⁻-N. During the subsequent NA period, the DO concentration declines to a sufficiently low enough level so that NO₂⁻-N and NO₃⁻-N species are transformed to nitrogen gas mediated by anoxic denitrification (Lim et al., 2012). Depending on the DO level in the water, some microbes may shift to alternatives to oxygen as their electron acceptor, resulting in metabolic changes (Quadie et al., 2014). The advantages of IA include: (1) a reduction in operational costs (Liu et al., 2013); (2) a reduction in the amount of carbon required for denitrification (Lim et al., 2012); (3) a single reactor is sufficient due to the coexistence of nitrifier and denitrifier microbial communities that can perform simultaneous nitrification-denitrification (SND) (Moura et al., 2012); (4) lower biomass production which ultimately must be addressed; (5) SND pH trends offset, thus moderating swings in pH (Quadie et al., 2014), and; (6) less N₂O is released than in continuous aeration (CA) methods (Béline and Martinez, 2002).

To maintain DO concentrations in the water column of aquaculture ponds, farmers typically leave the aerator running continuously. Although this practice ensures sufficient DO levels in the aquaculture water, it also substantially increases the amount of external energy required. If an IA could maintain the DO concentration of the water and also minimize the concentration of nutrients that were released from sediments into the water column, the cost of the external energy needed for aeration would be reduced, while simultaneously, aquaculture water quality would be improved or at least maintained. However, IA operating modes can vary greatly, so developing an optimized scheme that balances the need to maintain and improve water quality with output of fish cultivation in aquaculture ponds is difficult. Research focused on how nutrient concentrations change in the water column and in the pore water in pond sediments under varying IA conditions have not been conducted to date. The objectives of this study were to address these research gaps by investigating how NH₄⁺-N, NO₂⁻-N and NO₃⁻-N concentrations in the water column and sediment pore water vary during each aeration mode. The best aeration mode for the aquaculture ponds considering energy consumption, and improving aquatic habitat will be identified. Our assessment will be conducted through use of a physical model, which consisted of six identical experimental tanks with sediment collected from an aquaculture pond. We monitored DO concentrations periodically in each tank. Water quality within the tanks were similar initially, but were subjected to different patterns aerobic and anaerobic conditions. Several water samples were collected from different points within each tank during the experimental period, and were analyzed immediately to measure concentrations of NH₄⁺-N, NO₂⁻-N and NO₃⁻-N, DO, pH, and temperature. The results of this study can help guide design of aquaculture ponds to operate more effectively, and simultaneously provide a better habitat for fish, and reduce pollution from aquaculture ponds.

### 1. Materials and methods

#### 1.1. Experimental setup and procedure

The experimental setup is illustrated in Fig. 1; the section within the dashed-line represents the aeration system. Six identical experimental tanks were constructed, each with a unique aeration time interval. Each tank was made of a plexiglass box, 35 cm high, 50 cm long and 25 cm wide, divided into three internal sections along its base using two 2 cm thick plates, as shown in Fig. 1. Sediment was taken from an aquaculture pond in the Pearl River Fisheries Research Institute of China (Guangzhou, Guangdong Province), placed in a sealed, polyethylene plastic barrel, and returned to the lab the same day. Stones, debris, leaves, benthos and garbage were discarded and the sediments stirred thoroughly to ensure a uniform composition with consistent physicochemical properties. Each tank represents a physical model of an
aquaculture pond. In Fig. 1, P1 represents the sampling location of the water column. Once the sediments were loaded into the tank, Rhizon samplers were installed at points P2, P3 and P4, located at 1, 3 and 6 cm below the SWI, respectively. Using a Rhizon sampler (2.5 mm, Rhizosphere Research Products, Netherlands) to extract pore water has many advantages, such as low cost, minimal disturbance, avoiding sample contact with air, and preserving the chemical components of the sample (Seeberg-Elverfeldt et al., 2005; Shotbolt, 2010). Distilled water was then poured into the side of each tank slowly to minimize suspension of sediment. Each tank was left undisturbed for seven days before the experiment commenced. Each experimental tank included a movable cover with a 2 cm-diameter hole for measuring DO concentration and collecting water column samples; this cover could be removed if needed. The cover and tank were fully wrapped in tinfoil to reduce light and potential algae growth in each tank.

The air flow rate was measured by an air discharge meter, and corresponded to the size of the aquaculture pond being simulated and the tank, air flow rate was set at 1.0 L/min. The aerobic and anaerobic periods chosen for each batch experiment are provided in Table 1. The supply of air was controlled and was provided intermittently for each of the assigned time intervals. Each experiment took 6 d and various alternating aerobic/anaerobic periods were tested, where $T_{aerobic}$ is the time of aeration and $T_{anaerobic}$ is the time of non-aeration. The experiment was conducted with six similar tanks, two were maintained as being continuously aerobic ($T_{aerobic} = 0$) and anaerobic ($0: T_{anaerobic}$), while the other four were intermittent aerobic and anaerobic ($T_{aerobic}: T_{anaerobic}$). The cycle time for the other four tanks consisted of alternating aerobic and anaerobic periods with individual tanks using sequences of 8 hr: 8 hr, 12 hr: 24 hr, 24 hr: 36 hr, respectively.

### 1.2. Sample collection and analysis

Water samples were collected from the four sampling points of each tank every day throughout the experiment, and were analyzed immediately to determine their NH$_4$-$N$, NO$_2$-$N$ and NO$_3$-$N$ concentrations. Miniaturized photometrical methods developed by Laskov et al. (2007) and Tu et al. (2010) were used for rapid analysis. The indophenol method was used to estimate NH$_4$-$N$ concentrations; the copper cadmium reduction method was used to estimate NO$_3$-$N$ concentrations; and the Gris chromogenic reagent was used to estimate NO$_2$-$N$ concentrations. Each sample was divided into three subsamples and analyzed separately, then the average of the three analyses was taken as the result. DO, water temperature and pH were measured in situ every day after collecting water samples using a YSI ODO meter (model#605404, YSI Incorporated, USA) and a pH meter (PH-801/902, ADVICS, China), respectively, each was measured three times and the average of the three was used as the result.

### 1.3. Data analysis

Statistical tests were conducted to assess whether there was a statistically significant difference between groups. All statistical methods were analyzed using SPSS Statistics 26 (IBM). An $\alpha = 0.05$ was used for all significance testing. First, a test for normality was conducted using the Shapiro-Wilk goodness-of-fit test, with the null hypothesis, $H_0$: the distribution of monitoring data followed a normal distribution. Next, data groups were assessed to determine if they were different, i.e., the means of the two groups were not different. The test used depended upon whether the data were dependent, or paired (i.e., from the same tank), or independent, i.e., unpaired (from different tanks). Paired data were assessed using the Student’s $t$-test. Since the Student $t$-test requires that the underlying distribution be normal, in the case of non-normal data, the nonparametric Wilcoxon Signed-rank test was used. In the case of unpaired data, if the data followed a normal distribution, Welch’s $t$-test with unequal variance and independent samples was used to assess the same null

### Table 1 – Experimental conditions.

<table>
<thead>
<tr>
<th>Tank No.</th>
<th>$T_{aerobic}$ (hr)</th>
<th>$T_{anaerobic}$ (hr)</th>
<th>Cycle times during tests</th>
<th>Air Flow (L/min)</th>
<th>Total aeration time (hr)</th>
<th>Total anaerobic time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>144</td>
<td>None</td>
<td>None</td>
<td>0</td>
<td>144</td>
</tr>
<tr>
<td>2</td>
<td>144</td>
<td>0</td>
<td>None</td>
<td>1.08</td>
<td>144</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>1.00</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>1.10</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>24</td>
<td>3</td>
<td>1.07</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>36</td>
<td>2</td>
<td>1.07</td>
<td>72</td>
<td>72</td>
</tr>
</tbody>
</table>
hypothesis used for paired data (Lucke and Nichols, 2015). In the case of unpaired data that did not follow a normal distribution, the Mann-Whitney U test was used (Burant et al., 2018). If the p-value is less than the indicated significance level (0.05), the null hypothesis can be rejected, and it is likely that the populations are distinct.

The average rate of change in NH₄⁻N, NO₂⁻N and NO₃⁻N in the water column can be calculated by:

\[
v = \frac{C_f - C_0}{n}
\]

where, \(v\) is the average rate of change (mg/L/day); \(C_0\) (mg/L) and \(C_f\) (mg/L) represent the concentrations of the nutrient species at the beginning and ending of the experiment, respectively; \(n\) (day) is the incubation days.

The observed NO₃⁻N concentrations in the water column were fit to Eq. (2), which is a form of a logistic curve, which are commonly used for paired data (Lucke and Nichols, 2015). The equation was chosen because of its shape, mathematical properties, and accuracy of fit.

\[
C = A_2 + (A_1 - A_2)/(1 + (t/x_0)^P)
\]

where, \(C\) (mg/L) is the concentration of NO₃⁻N, \(A_1\) (mg/L) is the initial value, \(A_2\) (mg/L) is the final value, \(x_0\) is a centering parameter, and \(P\) is a power term. The equation was linearized and was fit using linear regression and an \(\alpha = 0.05\).

1.4. Nitrogen cycle in water and sediment

A conceptual schematic for the sediment-water nitrogen cycle is shown in Fig. 2. NH₄⁺-N is shown diffusing upward out of the sediment, and oxidizing in the water column. NO₂⁻-N also is shown diffusing into sediment pore water, and denitrifying in the sediment due to anoxic conditions. It is likely that NO₂⁻-N is an intermediate product of nitrification/denitrification.

2. Results and discussion

2.1. Environmental factors

DO concentration remained high in all tanks, the sole exception was the non-aeration condition, which means that no matter whether CA or IA is applied, DO in the water column was maintained at a satisfactory level (Fig. 3a). The pH value was lowest at NA, CA was lower than intermittently aeration modes (Fig. 3b). The temperature of the tanks was similar, ranging from 26 to 27 °C, NA was a little higher (Fig. 3c).

2.2. Variability in nitrogen

NH₄⁺-N content has a significant impact on the water quality of an aquaculture pond. NH₄⁺-N can be directly used by harmful algae such as cyanobacteria (Glibert et al., 2016), and is toxic to some aquatic macroinvertebrates (El-Sheikh et al., 2018; Gooddy et al., 2016). An analysis of NH₄⁺-N concentration in deep sediments (3 and 6 cm) for the various aeration modes (unpaired data) was conducted with either Welch’s t-test if data are normally distributed, or the Mann-Whitney U test if not. Corresponding p-values for the Shapiro-Wilk statistic for each aeration mode are shown in Table 2. Of all treatments, only the 8 hr: 8 hr, 24 hr: 24 hr, and 36 hr: 36 hr modes at P4 were found not to be normal distributed.

A comparison of the means of each aeration mode with all other modes was conducted using either the t-test or Wilcoxon signed-rank test, with the specific test depending upon the aforementioned normality criteria results. The p-values from the Welch’s t-test and Mann-Whitney U test for P4 and P3 are shown in Table 3 and Table 4, respectively. The mean of the NA mode was found to be significantly different than the means of the CA mode, \(T_e = 8\) hr: \(8\) hr mode and \(T_e = 24\) hr: \(24\) hr mode for NH₄⁺-N concentration at P4. As expected, the lowest p-value result was between the CA and NA modes. There was no significant difference between all other aeration modes for NH₄⁺-N concentration at P4. Aeration appears to have only a limited effect on NH₄⁺-N concentration in deep (6 cm) sediments; mainly during continuous or more frequent aeration cycles.

At P3, the mean for the NA mode was not significantly different than the mean for only the 8 hr: 8 hr modes (continuous aeration and the mode with the most frequently changing aeration/non-aeration). The mean for the CA mode was found not to be significantly different than the mean for the \(T_e = 8\) hr: \(8\) hr mode. The means for the \(T_e = 24\) hr and \(T_e = 36\) hr modes (the two groups with the longest interval times) were found not to be significantly different. The means of all other aeration modes were significantly different for NH₄⁺-N concentration at P3. At this depth, the effects of aeration on the NH₄⁺-N concentration are beginning to show.

The mean concentrations of NH₄⁺-N between all aeration modes at P3 and P4 are shown as a histogram (Fig. 4). The NA mode exhibited the lowest mean concentration among all groups, which means aeration caused the concentration of NH₄⁺-N in deep sediment pore water to increase. Compared with the NA mode, continuous aeration or frequently changing aeration, non-aeration cycles did not increase NH₄⁺-N in deep sediment pore water, while infrequently changes resulted in greater improvement. However, it did not increase as the time interval became larger, \(T_e = 12\) hr: \(12\) hr mode had the highest concentration of NH₄⁺-N at P3. The results show that NH₄⁺-N at P3 was released to the shallow layer and water column because of the concentration gradient, and the NH₄⁺-N in water column nitrified rapidly and accelerated the release of NH₄⁺-N in the sediment. But NH₄⁺-N in the sediment pore

![Fig. 2 – Nitrogen cycle in water and sediment.](image-url)
Sánchez-García et al. (2015) showed that the NH₄⁺-N in pore water originates mainly from the degradation of organic matter and the mineralization of organic nitrogen. Additionally, since biological denitrification and ammonification dominate under low redox conditions, even more NH₄⁺-N will be released from the sediment into the pore water. Thus, a 12 hr interval of intermittent aeration had the largest effect on release of NH₄⁺-N from sediment pore water.

The variability of NH₄⁺-N concentrations in the shallow sediment (P2) pore water is shown in Fig. 5. Three curves were fit to the experimental data as a function of time (t, days). As P2 is close to the sediment-water interface (SWI), it was found to be susceptible to disturbances caused by aeration. As shown in Fig. 5, NH₄⁺-N concentrations of the NA and 8 hr:8 hr modes increased with time. Because of the frequently alternating aeration/non-aeration modes, a large amount of NH₄⁻-N was released from sediments and transformed to NO₂⁻-N and NO₃⁻-N rapidly through nitrification. NH₄⁺-N could exist in large quantities only in the NA mode with its lower DO content. For all aeration modes, NH₄⁺-N concentrations were lower than 1 mg/L, except for the 36 hr:36 hr mode. Although the NH₄⁻-N released from sediments could be consumed by oxygen during the aeration stage, the non-aeration stage was 36 hr, so only part of the available NH₄⁻-N reacted with oxygen. Since aeration was conducted for only half the time (4 intermittent aeration modes), the results indicate that longer internal exchange times lowered the efficiency of removing NH₄⁻-N in shallow sediments pore water.

![Graph showing DO concentration, pH value, and temperature changes under different aeration modes.](image)

**Fig. 3** – (a) DO concentration of different aeration modes, (b) pH value of different aeration modes, (c) Temperature of different aeration modes.

<table>
<thead>
<tr>
<th>p-value</th>
<th>Aeration: Non-aeration Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>P4</td>
<td>0.1219</td>
</tr>
<tr>
<td>P3</td>
<td>0.4053</td>
</tr>
</tbody>
</table>

* p-value > 0.05 means the null hypothesis is accepted, i.e., the data are normally distributed.
The average concentration of nitrite (NO\textsubscript{2}-N) at different positions is shown in Fig. 6. There was higher oxygen content in the shallow sediment pore water and the water column than in deep sediments regardless of aeration mode. Thus, the concentration of NO\textsubscript{2}-N gradually increased from P4 to P1, except for the \(T_c = 12\) hr: 12 hr mode, which exhibited relatively high NO\textsubscript{2}-N concentration in the shallow sediment pore water and the water column. NO\textsubscript{2}-N is an intermediate product of NH\textsubscript{4}+-N converted to NO\textsubscript{3}-N during nitrification. It is well known that NO\textsubscript{2}-N is very unstable and easily converted to NO\textsubscript{3}-N and NH\textsubscript{4}+-N depending upon the DO content and redox conditions. However, the \(T_c = 12\) hr: 12 hr mode was able to maintain relatively high NO\textsubscript{2}-N concentrations. Nitrite is toxic, directly impacting the survival of aquatic animals (Xian et al., 2011) and the productivity of aquaculture (Krishnan et al., 2012; Yun et al., 2019). Maintaining low concentrations of NO\textsubscript{2}-N in aquaculture water is a key target for successful operation.

The deep sediment pore water oxygen content was low in all modes (Fig. 7), thus nitrate (NO\textsubscript{3}-N) concentrations were very low. NO\textsubscript{3}-N was mainly found in the water column. Once NO\textsubscript{3}-N diffused into the sediment pore water, it was consumed by microbial respiration, resulting in denitrification, decomposition, and mineralization (Qiao et al., 2018). In sediments, NO\textsubscript{3}-N content was low, below 0.1 mg/L, and ranged from 0.06 to 0.09 mg/L. Compared to the relatively large concentration of NH\textsubscript{4}+-N, it is unlikely that aeration affected NO\textsubscript{3}-N in deep sediments.

Aeration can indeed cause turbulence within surface sediments. Except for the NA and \(T_c = 36\) hr: 36 hr modes, NO\textsubscript{3}-N concentrations of all other aeration modes showed no significant difference between P1 and P2 (Table 5). Both the shallow sediment pore water and the water column were affected by turbulence. The NA mode not only made the oxygen inaccessible to the shallow sediment, but also restricted the release of nitrogen from the sediment to the water column. Longer time intervals of intermittent aeration (36 hr)

### Table 3 – Welch’s t-test and Mann-Whitney U test of NH\textsubscript{4}+-N between different aeration modes at P4.

<table>
<thead>
<tr>
<th>Aeration mode</th>
<th>p-value</th>
<th>Aeration: Non-aeration Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8 hr: 8 hr</td>
</tr>
<tr>
<td>NA</td>
<td>Welch’s</td>
<td>NA</td>
</tr>
<tr>
<td>CA</td>
<td>Welch’s</td>
<td>M-W U</td>
</tr>
<tr>
<td>8 hr:8 hr</td>
<td>Welch’s</td>
<td>M-W U</td>
</tr>
<tr>
<td>12 hr:12 hr</td>
<td>Welch’s</td>
<td>M-W U</td>
</tr>
<tr>
<td>24 hr:24 hr</td>
<td>Welch’s</td>
<td>M-W U</td>
</tr>
<tr>
<td>36 hr:36 hr</td>
<td>Welch’s</td>
<td>M-W U</td>
</tr>
</tbody>
</table>

\* p-value < 0.05 means the H\textsubscript{0} is rejected, i.e., it is likely that the means of the respective groups are different.

### Table 4 – Welch’s t-test of NH\textsubscript{4}+-N between different aeration modes at P3.

<table>
<thead>
<tr>
<th>Aeration mode</th>
<th>Aeration: Non-aeration Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CA</td>
<td>0.2048</td>
</tr>
<tr>
<td>8 hr: 8 hr</td>
<td>0.5299</td>
</tr>
<tr>
<td>12 hr: 12 hr</td>
<td>0.0002\textsuperscript{*}</td>
</tr>
<tr>
<td>24 hr: 24 hr</td>
<td>0.0115\textsuperscript{*}</td>
</tr>
<tr>
<td>36 hr: 36 hr</td>
<td>0.0015\textsuperscript{*}</td>
</tr>
</tbody>
</table>

\* p-value < 0.05 means the H\textsubscript{0} is rejected, i.e., it is likely that the means of the respective groups are different.

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**Fig. 4** – Average concentration of NH\textsubscript{4}+-N of different aeration modes at P3 and P4.
inhibited nitrogen release, except for the $T_c = 12 \text{ hr}: 12 \text{ hr}$ mode. No matter whether the subspecies was NO$_2$-N or NO$_3$-N, this mode always had the highest concentration at P1 and P2. NH$_4$-N had the highest concentration at P3 for this mode (Fig. 4), and decreased gradually at P2 (Fig. 5). It appears that NH$_4$-N was easily released from sediment to pore water, and was rapidly converted to NO$_2$-N and NO$_3$-N in the $T_c = 12 \text{ hr}: 12 \text{ hr}$ mode, indicating that 12 hr of time interval had the greatest effect on nitrogen release.

2.3 Influence of aeration mode on nitrogen transformation

The concentration and fate of nitrogen has an important influence on the growth of aquatic biota. Regeneration and transformation of nitrogen from sediments to the water column are affected by the chemical and biological stability of the sediments (Wu et al., 2001). We analyzed the concentration and fate of nitrogen in the water column for each aeration mode. The variation of NO$_2$-N and NO$_3$-N concentrations in the water column as a function of time are shown in Fig. 8. For the NA mode, DO in the water column declined gradually, and NO$_2$-N and NO$_3$-N concentrations were very low. Due to the instability of NO$_2$-N, its concentration remained at or close to zero, and NO$_3$-N was always lower than 0.2 mg/L.

For all aeration modes, NO$_3$-N concentrations gradually rose due to the presence of DO concentration in the water column. NO$_3$-N concentration in the water column increased for all aeration modes, and was highest for the $T_c = 12 \text{ hr}: 12 \text{ hr}$ mode, which was higher even than the CA mode. The maximum concentration of NO$_2$-N exceeded 0.15 mg/L; and for most of the incubation time was above 0.05 mg/L, while other aeration modes were all lower than 0.05 mg/L during the entire experiment. The results show that an interval of 12 hr for alternating aeration/non-aeration had the greatest effect.
on the nitrogen exchange and transformation between the water column and the sediment.

Experimental data were fit to Eq. (2), and assessed using linear regression. Eq. (2) fit the data well, i.e., $R^2$ values were all above 0.93 and the null hypothesis (no relationship) was rejected at the $a = 0.05$ level (Table 6). From the curve fit, it can be concluded that $P > 1$, $A_1 < A_2$, and the NO$_3$-N concentration gradually approaches $A_2$ with time, and the minimum and maximum nitrate concentrations were observed during the $T_c = 36 \text{ hr}$ and the $T_c = 12 \text{ hr}$ modes, respectively.

NH$_4^+$-N, NO$_2$-N, and NO$_3$-N in the sediment and water column are part of the nitrogen cycle, and the nitrification and denitrification processes occurred simultaneously. DO and NO$_3$-N concentrations in the sediments were very low, thus the NO$_3$-N in the water column originated mainly from nitrification, resulting in a concentration gradient between the sediment and the water column. Part of the NO$_3$-N diffused into sediment pore water where denitrification proceeded rapidly. Because of the large concentration gradient, a large amount of NH$_4^+$-N was released from the sediments to the water column, and portion was nitrified to form NO$_2$-N and NO$_3$-N, and the remainder controlled the change of NH$_4^+$-N concentration in the water column. Xia et al. (2017) reported that during incubation experiments, ammonification, anammox, dissimilatory nitrate reduction to ammonium (DNRA), and inorganic nitrogen uptake may occur simultaneously in addition to nitrification and denitrification processes. The variability of NO$_3$-N concentration was likely affected by nitrification, DNRA, and denitrification. DNRA has been found in many organic rich sediments, e.g., during toxic-anoxic oscillations (Abril et al., 2010).

According to Ritzkowski and Stegmann (2005), the ratio of nitrite to nitrate, or $[\text{NO}_2^\text{-N}]/[\text{NO}_3^\text{-N}]$ (NNR) is an indication of the nitrification process. If the NNR does not remain at an

<table>
<thead>
<tr>
<th>Statistic</th>
<th>NA</th>
<th>CA</th>
<th>$T_c = 8 \text{ hr}$</th>
<th>$T_c = 12 \text{ hr}$</th>
<th>$T_c = 24 \text{ hr}$</th>
<th>$T_c = 36 \text{ hr}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S–W p-value of P1</td>
<td>0.23*</td>
<td>0.18*</td>
<td>0.31*</td>
<td>0.32*</td>
<td>0.15*</td>
<td>0.47*</td>
</tr>
<tr>
<td>S–W p-value of P2</td>
<td>0.36*</td>
<td>0.32*</td>
<td>0.48*</td>
<td>0.41*</td>
<td>0.79*</td>
<td>0.76*</td>
</tr>
<tr>
<td>Student’s $t$-test p-value</td>
<td>0.03</td>
<td>0.04*</td>
<td>0.73*</td>
<td>0.76*</td>
<td>0.95*</td>
<td>0.00</td>
</tr>
<tr>
<td>Average value of P1</td>
<td>0.12 $\pm$ 0.03</td>
<td>0.24 $\pm$ 0.12</td>
<td>0.26 $\pm$ 0.12</td>
<td>0.37 $\pm$ 0.21</td>
<td>0.24 $\pm$ 0.12</td>
<td>0.24 $\pm$ 0.09</td>
</tr>
<tr>
<td>Average value of P2</td>
<td>0.09 $\pm$ 0.02</td>
<td>0.26 $\pm$ 0.13</td>
<td>0.24 $\pm$ 0.09</td>
<td>0.33 $\pm$ 0.18</td>
<td>0.25 $\pm$ 0.10</td>
<td>0.10 $\pm$ 0.03</td>
</tr>
</tbody>
</table>

* $p$-value $> 0.05$ means $H_0$ is accepted, i.e., the data is normally distributed; $b$ $p$-value of $t$-test $> 0.05$ means $H_0$ is accepted, i.e., there is no significant difference in the means of the respective groups.

Fig. 8 – Variation of NO$_3$-N and NO$_2$-N concentrations with time in the water column at various aeration modes.
elevated level, it is an indication of denitrification. As is shown in Fig. 8, the NNR increased first and then decreased for all aeration modes. The nitrification process involved two biochemical reactions, which were the oxidation of ammonium to NO2-N by ammonia-oxidation bacteria and the oxidation of NO2-N to NO3-N by nitrite-oxidizing bacteria (Nag et al., 2016). Since both reactions occurred during the aeration periods within both bioreactors, therefore, nitrite and nitrate concentration increased. As the experiment progressed, NO3-N continued to increase, but the opposite was the case for NO2-N. Additionally, the ratio decreased. During the non-aeration period, the concentrations of NO3-N and NO2-N changed only slightly, and Te = 12 hr: 12 hr for exchange aeration/non-aeration was the most effective for nitrification because the NNR was at its highest level of all modes.

2.4. Rate of change of nitrogen in water column

When nitrogen in aquaculture water is present in the form of either ammonia or nitrite, it is highly toxic to fish and other aquatic organisms. The nitrogen in the water column stems mainly from the sediments. Whatever the source of the nitrogen, it is most important to note that many of its forms are toxic to aquatic animals. The rate of change of nitrogen in the water column is an important index for aquaculture water quality.

Assessing the change in nitrogen concentrations in the water column with incubation time as the main focus of the analysis, the rate of change for each aeration mode is shown in Fig. 9. Results were computed using the concentrations at the start and end of the experiment (Eq. (1)). Each tank had a complete cycle of aeration: non-aeration. While the DO concentration was higher than 5 mg/L in the water column for the NA mode, the nitrification rate was relatively slow, and the concentration gradient of NH4-N between the sediment and the water column caused a large release of NH4-N, increasing to a rate of 0.05 mg/L/day. NH4-N concentrations also unexpectedly increased for the Te = 36 hr: 36 hr mode instead of decreasing as it did for all other aeration modes (Fig. 9a). The NH4-N content that released from the sediment to the water column after a long duration (36 hr) was higher than the content from nitrification, thus resulting in an increase in NH4-N concentration in the water column. At other aeration modes, concentrations fluctuated over time, but on average, without the long non-aeration stage, NH4-N concentrations decreased due to the presence of sufficient DO. The rate of change of NH4-N at the Te = 8 hr: 8 hr mode and the Te = 24 hr: 24 hr mode was almost the same as that for the CA mode, indicating that, although the aeration time was only half of the CA mode, but with the short intermittent cycle times of aeration, the DO concentrations in the water column were sufficient to cause nitrification to be greater than release. In the tank set for the Te = 12 hr: 12 hr mode, the rate of the change of NH4-N concentration was obviously higher than during other modes. The 12 hr intermittent aeration had a positive effect on the removal of NH4-N in the water column, as shown in Fig. 9a.

As an intermediate product of nitrification, NO2-N is very unstable, the rate of change was very low in water column at each mode due to the sufficient DO, except for the Te = 12 hr: 12 hr mode (Fig. 9b), other modes were less than 0.003 mg/L/day. However, NO3-N concentration was relatively high because of the strong nitrification at the Te = 12 hr: 12 hr mode, but still very low; compare to the rate of change of NO3-N, NO2-N accounted for only 11.3%. It can be seen in Fig. 9c that, although some DO in the water column was retained without aeration, the concentration of NO3-N exhibited only a marginal change. On the other hand, denitrification of NO3-N and NO2-N likely increased with sufficient carbon sources in the sediments (Tanner and Kadlec, 2003). Therefore, the NO3-N growth rate was very low. While at aeration conditions, the concentration of NO3-N had different levels of growth, the rate of change of 12 hr interval aeration was higher than that of other aeration conditions. Under this condition, reducing of NH4-N is much higher than the other tanks, indicating that 12 hr interval aeration can promote nitrification in the water column. There was no obvious difference between other aeration conditions, the rate of change was relatively low at the Te = 36 hr: 36 hr mode, in addition to the above factors of nitrification, according to the concentration difference between P3 and P4 in Fig. 6, partly due to NO3-N being diffused into the pore water.

![Fig. 9 – The average rate of change of nitrogen in water column for each aeration mode.](image-url)
2.5. Correlation between every two indexes

Correlation between NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N, DO, and pH are shown in Fig. 10. Scatter plots are presented in the lower panel, correlation coefficients ($r$) with $p$-value are located in the upper panel, and histograms are presented in the diagonal (Fig. 10). The absolute value of $r$ was categorized in four groups: negligible correlation ($r < 0.30$), low correlation ($0.30 < r < 0.50$), moderate correlation ($0.50 < r < 0.70$) and high correlation ($r > 0.70$). Our results indicate that there was an inverse relationship between NH$_4^+$-N and DO (high correlation $r = 0.77$); DO promotes nitrification and NH$_4^+$-N can be transformed to NO$_2^-$-N (Uysal et al., 2017). Low correlation was observed between NH$_4^+$-N and pH ($r = 0.41$).

There was a direct relationship between NO$_3^-$-N and pH (moderate correlation $r = 0.69$), and NO$_2^-$-N and pH (low correlation $r = 0.37$), the relationships between pH, NO$_3^-$-N and NO$_2^-$-N are similar to results reported by Wang et al. (2017). Temperature had direct effect on NO$_3^-$-N and DO (low correlation $r = 0.42$, and $-0.41$, respectively). Since, pH was correlated with all constituents (except temperature), it seems, when there is sufficient DO, pH plays an important role in the level of NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N. Nitrifying bacteria were affected by pH in three ways: activation and deactivation of nitrifying bacteria; nutritional effect, connected with alkalinity; and inhibition through free ammonia and free nitrous acid. Villaverde et al., 1997 found that pH could influence the nitrification efficiency, which showed a linear increase of 13% for a pH increase from 5.0 to 8.5. Correlation between NO$_3^-$-N and NO$_2^-$-N was 0.60, and there was little to no correlation observed between NO$_3^-$-N and DO, the latter is due to the presence of sufficient DO. Oxygen can promote the NH$_4^+$-N conversion to NO$_2^-$-N and NO$_3^-$-N, but as aeration time increases, DO content was maintained at a very high level, thus the correlation did not reflect actual conditions. Experimental conditions are quite different from natural conditions, primarily due to aeration. No matter whether the nitrogen content changes or not, oxygen concentrations were always maintained at a high level; in contrast with natural conditions in which oxygen will decrease markedly. Since we did not control pH in this experiment, it varied from 5.5 to 6.7, pH exhibited an obvious correlation with these four indexes. Further experiments with pH control are recommended topics for future research.

3. Conclusions

We conducted an experiment involving six identical tanks to simulate the effects of aeration on nitrogen in aquaculture
ponds, using a variety of aeration modes. The results showed that the effects of aeration were observed mainly in the shallow sediment pore water and the water column. The $T_e = 36 \text{ hr}$ and NA modes had higher $\text{NH}_4^+ - \text{N}$ concentrations at over 2 mg/L. The $T_e = 12 \text{ hr}$ had much greater $\text{NO}_2^- - \text{N}$ concentration than other aeration modes. Ammonia and nitrite are toxic, and can directly impact the survival of biota and the productivity of aquaculture. The $T_e = 8 \text{ hr}$ and $T_e = 24 \text{ hr}$ modes had similar nitrogen concentrations to the CA mode, but were used with less than half of the aeration time. These modes may be a better option than the CA mode. pH is an important factor affecting aquaculture quality with intermittent aeration. $\text{NO}_3^- - \text{N}$ concentration in the water column with aeration can be described using a logistic dose response function. While focused on aquaculture ponds, these results are meaningful for water quality of ponds.

Acknowledgments

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References


