Treatment of sludge from intensive whiteleg shrimp farming using a sequencing batch reactor

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ABSTRACT

The wastewater/sludge generated from the shrimp aquaculture industry contains high levels of nitrogen (N), phosphorous (P), and carbon (C). This study aimed to evaluate the efficacy of N, P, and C removal and recovery from sludge obtained during the siphoning process of intensive white leg shrimp farming by using a sequencing batch reactor (SBR) with two trials. In the first trial, reactors were operated aerobically (3 - 5 days) and anaerobically (4 - 6 days) in sequence, resulting in a total cycle time of 9 days. In trial 2, the reactors were run aerobically for the first 3, 4, & 5 days, respectively, succeeded by anoxic conditions until the end of the experiment on day 14. The results showed that the removal of total ammonia nitrogen and chemical oxygen demand was about 60 - 70%, but the treatment efficiencies of total N and P were extremely low. Moreover, the anaerobic mode improved the mineralization of P, while aerobic condition promoted nitrate production. Further studies are needed to improve the nutrient and organic removal performance of the SBR.

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1. Introduction

Vietnam has many advantages to develop brackish-water shrimp farming, especially in provinces of Mekong delta (MK). Particularly, brackish-water shrimp farming area is about 740,000 ha with shrimp production volume reaching over 900,000 tons (DOF, 2021). Shrimp farming contributes to income rising, employment generating, and poverty alleviation for people. However, the high development of shrimp farming, especially intensive shrimp farming (ISF) models, has led to the problem of environmental pollution.

Activities of ISF have generated wastewater, particularly sludge from siphon process (sludge), containing a wide range of organic and inorganic nutrients. Investigating 20 typical ISF ponds in the MK, Tran et al. (2022) reported that average levels (mg/L) of total suspended solid (TSS), chemical oxygen demand (COD), total ammonia nitrogen (TAN), total Kjeldahl nitrogen (TKN), total phosphorous (TP) in sludge were 3.423, 1.854, 101, 161, and 92, respectively. Compared to the QCVN 02-19:2014/BNNPTNT (MARD, 2014), concentrations of TSS and COD exceeded the permitted standards by over 34 and 12 times, respectively.

Conventional wastewater treatment methods can treat wastewater and sludge from the ISF ponds. Aerobic (activated sludge) and anaerobic (up-flow anaerobic sludge blanket, UASB) biological treatment process helps to reduce nitrogen and carbon concentration in the ISF effluents; however, these systems are costly to construct, operate and generate secondary waste (solid waste) requiring an area for disposal (Timmons et al., 1998; Boopathy et al., 2005).

Sequencing batch reactor (SBR), a variant of activated sludge, is seen as a potential solution to reduce nutrient concentrations in sludge from the ISF ponds (Boopathy, 2018). Unlike conventional activated sludge treatment technology that requires many reaction tanks, the SBR is a type of wastewater treatment facility based on suspended growth of microorganisms, in which there are two phases: an aerobic phase, where carbon oxidation (COD removal) and nitrification are combined into a single process, and an anaerobic phase where denitrification is accomplished (Boopathy et al., 2005; Boopathy et al., 2007; Boopathy, 2018). Therefore, the SBR can overcome most of the disadvantages of conventional aerobic - anoxic methods and biological filtration such as: no need for a settling tank after biological treatment; combine aerobic and anoxic processes in the same tank, thereby increasing nitrogen and carbon treatment efficiency (Boopathy et al., 2005; Boopathy et al., 2007; Boopathy, 2018). Moreover, anaerobic and aerobic sludge reactors can apply microbe to decompose the sludge into bioavailable nutrients and facilitates the nutrients recovery that can subsequently be used as algae/plant nutrition (Goddek et al., 2015; Goddek et al., 2016). The SBR systems have been used to treat many types of wastewater containing high nutrient and organic content such as slaughterhouse, swine manure, dairy, sewage, especially sludge from the ISF (Boopathy et al., 2005; Boopathy et al., 2007; Kundu et al., 2013). Regarding to the treatment of sludge from the ISF (raceway) by SBR, Boopathy et al. (2005) found that the treatment efficiencies of organic carbon and nitrogen compounds reached nearly 100% with the operating mode: aerobic phase for 3 days and anaerobic phase for 6 days.

The application of SBR in the treatment and nutrient recovery of sludge from the ISF can be a potential technical alternative to current technologies in the management of sludge. The objective of this study was to evaluate the removal efficiencies and recovery performance of nitrogen, carbon, and phosphorus (P) in sludge from the intensive whiteleg shrimp ponds under different sequences of react periods (aerobic/anoxic).

2. Materials and Methods

2.1. Collecting sludge

Sludge was collected from the intensive whiteleg shrimp ponds at Phuoc An shrimp farm, Nhon Trach district, Dong Nai province (10,648 N; 106,941 E). Shrimps were intensively cultured according to a three-stage process with an initial stocking density of 1,000 fish/m^2 and gradually decreasing stocking density to about 400 - 500 shrimp/m^2 in stage 2 and about 150 - 200 shrimp/

m2 in stage 3 until harvesting. The sludge samples were collected through the siphon discharge pipe and put into a large plastic tank, then let it settle for 1 h, decant the clear water above to take only the settled sludge. When sampling, the samples were stirred well and sieved through a 2 mm sieve to remove peeled shrimp shells, dead shrimp, and large-sized matter. After screening through a sieve, the samples were kept in PE nylon bags (30 cm x 50 cm) and stored in a foam box with ice. The samples were then transported to the Toxicology Laboratory, Research Institute of Biotechnology and Environment, Nong Lam University, Ho Chi Minh City within 1 h. Salinity and pH in samples were in the range of 4 - 5‰ & 6.2 - 6.7, respectively.

2.2. Experimental procedure

2.2.1. Reactor configuration

In this experiment, a lab scale reactor was cylindrical in shape with 27 cm in diameter and 35 cm in height and made from plastic buckets. The reactor was painted black outside and equipped with an aeration system and stirring motor. Total working volume of the reactor was 20 L that just 5 L were used. Air was injected into the reactor by dispersing air bubbles through 2-mm holes in copper pipe (spacing 10 cm) placed on the reactor floor. An impeller positioned 10 cm from the bottom of the reactor (Figure 1). During aerobic phase, the reactors were continuously aerated and stirred at 100 rpm. In anaerobic phase, aeration and stirring were turned off (Boopathy et al., 2005).

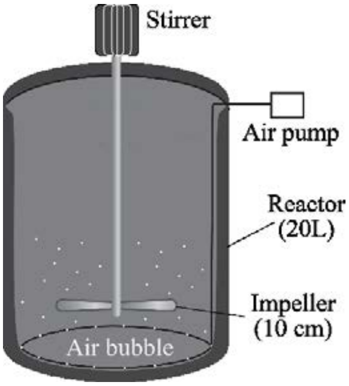

Figure 1. Reactor configuration.

2.2.2. Sequencing batch reactor operational conditions

The experiment consisted of two trials. The trial 1 was to evaluate the effectiveness of the SBR with the operating mode as reported by Boopathy et al. (2005) with slight modifications.

The trial 1 was carried out in three SBR reactors: reactor 1, 2 and 3 operating aerobically (DO > 3 mg/L) for the first 3, 4 and 5 days, respectively (Figure 2). In anaerobic phase, reactor 1, 2 and 3 were operated anaerobically

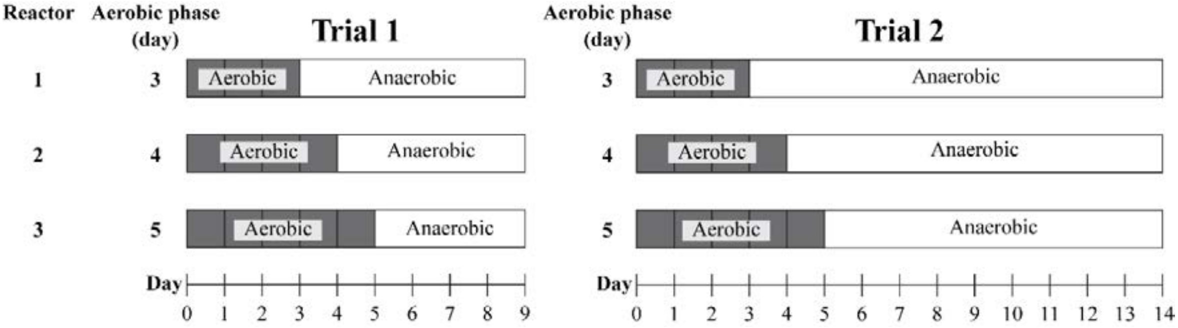

Figure 2. Sequencing batch reactor operational conditions for two trials.

(DO < 0.5 mg/L) since day 4, 5 and 6 until the end of the trial on day 9. The experiment was repeated three times using a different initial sludge sample.

Sludge samples were collected in the beginning (day 0) and at the end of the aerobic (days 3, 4, & 5) and anaerobic phase (day 9) and were analyzed for parameters including nitrogenous compounds (TKN, TAN, nitrite, and nitrate), phosphorous (TP and soluble - PO_4^{3-}), and COD (total and soluble). Moreover, total solid (TS) and total volatile solid (TVS) were determined in the beginning and at the end of the experiment.

For trial 2, based on the performance of the reactors from the trial 1, the reactors 1, 2 and 3 were operated aerobically for the first 3, 4 and 5 days, respectively, and then operated anoxically until the end of the experiment on day 14 (Figure 2). The experiment was conducted without replication. Samples were collected daily and were analyzed for COD, TAN, and nitrate.

2.3. Analytical methods

Sludge from each reactor was sampled and centrifuged at 5,000 rpm for 10 min and the supernatant used for chemical analyses of TAN, nitrite, nitrate, PO_4^{3-} and soluble COD (sCOD). For the other parameters, sludge is used without centrifuge. All parameters were analyzed using standard methods (Baird et al., 2017).

2.4. Statistical analysis

All data were presented on mean ± standard deviation (SD). Levels of parameters in sludge were compared to the QCVN 02-19:2014/BNNPTNT standards (MARD, 2014). A Tukey test, along with one-way blocked analysis of variance (ANOVA) with replication as blocks was used to assess differences in levels of parameters in sludge during trial 1. Statistical analysis was done using the IBM SPSS Statistics for Windows, version 22 (Armonk, NY: IBM Corp). Significant level was evaluated as $P < 0.05$. Because of no replication in trial 2, data were shown in figures without statistical analysis.

The removal efficiency of the SBR was estimated using the equation:

$$Removal\ efficiency\ (\%) = (\Delta C_{in\text{-}out}/C_{in})\times 100$$

where, ΔC_{in-out}: inflow-outflow concentrations of parameters (mg/L); C_{in}: inflow concentrations of parameters (mg/L). For trial one, carbon and nitrogen removal was reported as the average of triplicate SBRs.

3. Results

For trial 1, average levels of parameters in sludge in the beginning and at the end of the aerobic and anaerobic phase were presented in Table 1. Initial sludge from siphon process contains very high concentrations of organic compounds (COD and TVS) and nutrients (TKN, TAN, nitrate, TP, and PO_4^{3-}). Particularly, COD levels exceeded the permitted standards of the QCVN 02-19:2014/BNNPTNT (COD ≤ 150 mg/L) (MARD, 2014) by 250-fold. But nitrite level in the initial sludge was very low (Table 1).

In general, the concentrations of organic compounds and nutrients during the experimental period tended to decline (Table 1). The initial TKN content of 1,803 ± 453 mg/L was reduced to 1,695 ± 430 mg/L (a decrease of about 100 mg/L) at the end of experiment with a removal efficiency of only 6%. Meanwhile, nitrification during the aerobic stage of SBR resulted in the decrease of TAN from 172 ± 41 mg/L to 68 ± 14 mg/L at day 5 with a relatively high treatment efficiency (60%) but increased in the anaerobic phase (86 mg/L ± 23 mg/L at the end of the experiment) (Table 1). Nitrate

Table 1. Concentrations (mean ± SD, n = 3) of parameters in sludge in the beginning and treatment phases in the trial 1

Day	Phase	TKN (mg/L)	TAN (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	TP (mg/L)
0		1,803 ± 453^{a}	172 ± 41^{a}	0.033 ± 0.011^{a}	86 ± 10^{ab}	651 ± 210^{a}
3	Aerobic	1,788 ± 450^{a}	138 ± 41^{a}	0.209 ± 0.297^{a}	151 ± 20^{a}	641 ± 217^{ab}
4	Aerobic	1,755 ± 431^{ab}	100 ± 37^{b}	0.044 ± 0.035^{a}	180 ± 64^{a}	634 ± 213^{ab}
5	Aerobic	1,721 ± 480^{ab}	68 ± 14^{b}	0.036 ± 0.007^{a}	183 ± 53^{a}	628 ± 208^{ab}
9	Anaerobic	1,695 ± 430^{b}	86 ± 23^{b}	0.062 ± 0.024^{a}	49 ± 12^{b}	589 ± 163^{b}

Day	Phase	PO_4^{3-} (mg/L)	COD (g/L)	sCOD (g/L)	TS (g/L)	TVS (g/L)
0		10.3 ± 13.2^{a}	37.5 ± 11.3^{a}	6.14 ± 1.67^{a}	135 ± 47^{a}	34.1 ± 0.^{5}a
3	Aerobic	6.07 ± 3.31^{a}	34.3 ± 8.8^{ab}	4.75 ± 1.99^{ab}	-	-
4	Aerobic	4.01 ± 4.77^{a}	31.0 ± 7.0^{b}	3.75 ± 1.82^{bc}	-	-
5	Aerobic	3.87 ± 2.41^{a}	29.7 ± 8.3^{b}	3.18 ± 1.39^{bc}	-	-
9	Anaerobic	16.1 ± 14.9^{a}	24.2 ± 7.4^{c}	2.33 ± 0.94^{c}	125 ± 40^{a}	29.3 ± 4.0^{a}

Mean values in the same column with different superscript letters differ significantly (One-way ANOVA, Tukey test, $P < 0.05$).

TKN: total Kjeldahl nitrogen; TAN: total ammonia nitrogen ; TP: total phosphorus; COD: chemical oxygen demand; sCOD: soluble chemical oxygen demand; TS: total solid; TVS: total volatile solid.

concentrations increased with time in the aerobic phase, from an initial concentration of 86 ± 10 mg/L to 183 ± 53 mg/L on day 5 and decreased in the anoxic phase (49 mg/L ± 12 mg/L at the end of the experiment) (Table 1). The concentrations of TKN and TAN after the SBR treatment were statistically significantly lower than those of the initial ($P < 0.05$); but there was no statistically significant difference in the content of nitrite and nitrate between day 0 and 9 ($P > 0.05$) (Table 1).

It seems there was a steady decline in TP during the experimental period, from an initial concentration of 651 ± 210 mg/L to 589 ± 163 mg/L on day 9 with a removal efficiency of about 10% (Table 1). Concentrations of PO_4^{3-} were decreased with time in the aerobic phase, from the initial concentration of 10.3 ± 13.2 mg/L to 3.87 ± 2.41 mg/L at day 5 (removal efficiency of 62%) and increased in the anaerobic phase (concentration reached 16.1 ± 14.9 mg/L at the end of the experiment), attributing to the release of soluble P from the sludge. Differences in TP concentrations between sampling days were statistically significant ($P < 0.05$), whereas differences in PO_4^{3-} levels among sampling days were not statistically significant ($P > 0.05$) (Table 1).

The initial COD and sCOD concentrations of 37.5 ± 11.3 g/L and 6.14 ± 1.67 g/L, respectively, were decreased slowly in both aerobic and anaerobic phases. Levels of these COD were removed more in the oxic phase than in the anoxic phase. Levels of COD and sCOD at day 9 dropped to 24.2 ± 7.4 g/L and 2.33 ± 0.94 mg/L, respectively. The removal efficiencies of COD and sCOD were 36 and 62%, respectively. Significant differences in COD and sCOD concentrations between day 0 and day 9 were found ($P < 0.05$) (Table 1).

During the SBR treatment, the initial contents of TS (135 ± 47 g/L) and TVS (34.1 ± 0.5 g/L) were dropped to 125 ± 40 g/L and 29.3 ± 4.0 g/L at the end of the trial with relatively low removal efficiencies of 7.3 and 14%, respectively. Differences in TS and TVS levels in the beginning and at the end of the experiment were not significant ($P > 0.05$) (Table 1).

Figure 3 presented changes in concentrations of TAN, nitrate, and COD during the trial 2. From this figure, the initial TAN level of 130 mg/L were gradually decreased to 58.5 mg/L, 37.0 mg/L, and 16.4 on days 3, 4, and 5, respectively, during the aerobic phase of operation (Figure 3). At the same time, the nitrate concentrations were increased correspondingly with a decline in TAN in the reactors implying nitrification. Particularly nitrate levels increased from 281 mg/L at the beginning of the trial to a maximum of 523 mg/L in reactor 3 on day 5. When the reactor 1 was operated anoxically the nitrate level decreased slowly and finally reached 218 mg/L on day 14. While the SBR also treated the COD during both aerobic and anaerobic steps of the reactor operation (Figure 3), which slowly dropped from 45.8 g/L at the beginning of SBR operation to 12 g/L at the end of the trial on day 14, leading to 73% removal of COD in the SBR. More organic compounds were eliminated in the aerobic mode than the anaerobic mode.

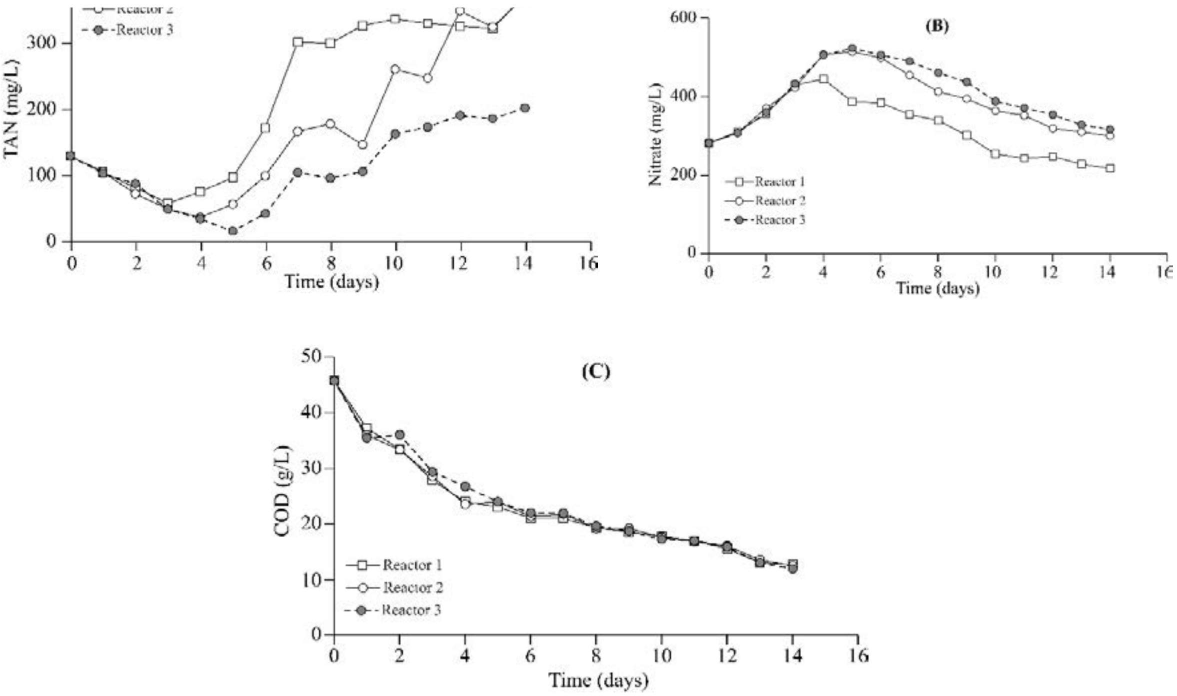

Figure 3. Changes in concentrations of total ammonia nitrogen (TAN; A), nitrate (B), and chemical oxygen demand (COD; C) during the trial 2.

4. Discussion

Previous publications have revealed that the ISF wastewater (including wastewater from water exchange and sludge from siphoning) from intensive and super-intensive shrimp farming often contains high levels of biodegradable organic compounds and dissolve nutrients (Boopathy, 2018; Nguyen et al., 2022; Tran et al., 2022). Average COD levels were in the 466 - 1,854 mg/L range; while concentrations of TAN, TKN, and TP were in a typical range of 29.8 - 101 mg/L, 9.63 - 161 mg/L, & 17.3 - 92 mg/L, respectively (Boopathy, 2018; Tran et al., 2022; Nguyen et al., 2022). The sludge characteristics in this study were much higher than the results of previous studies. It could be due to the difference in sludge sampling methods. In this study, the sludge from siphoning process was settled for 1 hour and the clear water decanted, thus the sludge sample would be denser. Meanwhile, the studies of the above-mentioned authors sampled sludge without settling.

According to the literature, wastewater and sludge from the ISF might be treated by activated sludge method, UASB, filtration system and sludge management; yet, these treatment methods can be costly to construct and operate (Timmons et al., 1998; Boopathy et al., 2005). On the contrary, the SBR reactors are very simple in design and operation, using only one reactor for many treatment phases. The SBR effectively removed organic matters and nutrients in sludge come from intensive shrimp recirculating aquaculture systems in the US (Boopathy et al., 2005; Boopathy, 2009; Boopathy, 2018). Regarding to the treatment of sludge from an intensive shrimp raceway system by the SBR (3 days aerobic phase, 6 days anaerobic phase), Boopathy et al. (2005) found that the initial TAN concentration in sludge of 91 mg/L was decreased to 5.3 mg/L on the third day of the oxic phase. While, in the aerobic phase, the nitrite concentrations were increased from 237 to 403 mg/L on the second day and decreased sharply in the anaerobic phase (to 3.0 mg/L on day 9). At the same time, the levels of nitrate increased, and the highest nitrate level was 91.8 mg/L on day 3. Then in the anoxic phase, the nitrate content declined steadily and dropped to 1 mg/L at the end of the trial. The initial COD content of 1,602 mg/L was slowly decreased in both aerobic and anaerobic phases and the removal of organic matter in oxic condition was higher than in anoxic condition. Level of COD decreased to 69 mg/L on day 9 (Boopathy et al., 2005). Remarkably, the addition of Bacillus consortium in the SBR can be effective in controlling the growth of shrimp pathogen, *Vibrio harveyi* in the sludge (Boopathy, 2018). According to these authors, the sludge of the ISF already contained many different groups of bacteria that perform nitrification and denitrification reactions as well as carbon metabolism (Boopathy et al., 2005; Boopathy et al., 2007; Boopathy et al., 2018). The results of this study showed that nitrate levels increased due to nitrification in oxic stages and reduced due to denitrification in anoxic stages. While carbon removal occurred in both aerobic and anaerobic phases (Table 1).

No study has evaluated the potential of treating P in sludge/wastewater from the ISF by the SBR. But the SBR has proven to be able to remove P from slaughterhouse wastewater with a removal efficiency of 85 - 89% (Fongsatitkul et al., 2011). According to Dutta & Sarkar (2015), P was removed by a special group of bacteria known as polyphosphate accumulating organisms (PAO). Under anoxic phase, PAOs utilize carbon source, mostly volatile fatty acids and change them to polyhydroxyalkanoates (PHAs). The energy for this process was taken chiefly through the intracellular hydrolysis of poly-P compounds, leading to the regeneration of ortho-phosphate into the water. Under oxic phase, PAOs can uptake surplus P and convert into intracellular poly-P, biomass growth, and glycogen replenishment by oxidizing stored PHAs (Dutta & Sarkar, 2015). In this study, the increased ortho-phosphate content in the anaerobic phase could be due to the mineralization of the precipitated P compounds (such as calcium phosphate, calcium sulphate, calcium carbonate, etc.) in the SBR when the pH dropped under 6 as stated by Delaide et al. (2019). Goddek et al. (2018) also reported that the UASB worked best at mineralizing and mobilizing P (7 - 26%) from fish sludge, whereas the aerobic reactor showed the least mineralization performance (-5%). Levels of P from these UASB effluents were close to the lettuce hydroponic solution (Goddek et al., 2018).

The removal efficiencies of nutrient and organic matter (in range of 60 - 70% at the highest) in this work were lower than those of reported by the aforesaid authors (nearly 100%) (Boopathy et al., 2005; Boopathy, 2009; Boopathy et al., 2018). It could be due to the concentrations of nutrients and organic matter in the sludge of this experiment was higher, so the treatment time of 9 or 14 days might be not enough. In addition, the C/N ratio in this study (20/1) was higher than the optimal C/N ratio for SBR tanks of 10/1 (Fontenot et al., 2007; Roy et al., 2010). Moreover, addition of Bacillus consortium could be to enhance the performance of SBR for the treatment of sludge (Boopathy et al., 2015).

5. Conclusions

The SBR was relatively effective in treating TAN and COD in the sludge from three-stage ISF. However, the removal efficiencies of TN, TP, TS, and TVS were very low. Our findings obviously imply that anaerobic mode improves the mineralization performance of P, but aerobic condition enhances nitrate production. Further studies are needed to improve the treatment efficiencies of nutrients and organic matter in sludge/wastewater from the ISF and other aquaculture species: prolonging the treatment time in aerobic and anaerobic phases, optimizing the C/N ratio, adding microorganisms, etc. Generally, the use of SBR in wastewater/sludge treatment from aquaculture can be a simple, low-cost technical solution that supports current technologies in waste management from aquaculture.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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