Denitrification performance of acclimated bio-floc in sequencing batch reactor

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Abstract. Bio-floc technology (BFT) is a new aquaculture models contain both aquaculture and water treatment. Nevertheless, there exists a universal phenomenon of nitrate accumulation in bio-floc system with zero water exchange. In this study, bio-floc was acclimated under 2 hours anaerobic conditions per day by sequencing batch method, which aims to enrichment culture denitrifying microorganisms. The results illustrated that NH4+-N and NO2--N concentration in effluent was remained below 0.5 mg/L and 0.1 mg/L respectively, coupled with NO3--N below 57 mg/L during 240 days cultivation and acclimatization. The nitrate concentration was far less than results reported in other bio-floc system with zero water exchange. After acclimation, the anaerobic denitrification performance of the bio-floc was tested and it exhibited high denitrification activity. Nitrate removal rate was approximately 100%, and special nitrate reduction rate was as high as 475.74 mg/(gVSS.d). This acclimation method made bio-floc could be used to nitrogen removal in aquaculture water treatment.

1. Introduction
Aquaculture, namely the farming of fish and shellfish in aquatic environments, makes an important contribution to resolve the contradiction of world population growth and food shortage [1, 2]. It was estimated that global aquaculture production was 64 million tons in 2010 and ever-increasing [3]. Despite its significant role in gratifying increasing food demand, the fast-developing aquaculture caused several environmental problems. Traditional aquaculture models discharge a great deal of organic matter such as residual bait and feces, along with nutrients, caused water pollution and eutrophication in natural waterbodies [4, 5]. Based on this, more and more attention is directed at aquaculture process in harmony with environment [6-8].
Bio-floc technology (BFT) is one of the new aquaculture models to reduce nutrients emissions. Using the experience of activated sludge process in wastewater treatment, with special characteristics of water quality, that is, of a great quantity of organic nitrogen contained in organic matter such as residual bait and feces. taking into account meanwhile, bacteria in bio-floc decompose organic and inorganic nitrogen in aquaculture water mainly through metabolic pathway of ammonification, nitrification and nitrogen assimilation and so on [9, 10]. In most instances, bio-floc technology utilized microbial assimilation as predominant metabolism pathway by adding carbohydrate. In this way, organic and inorganic nitrogen were converted to bacterial protein, which can be used as supplement feed of harvested target biomass. Nevertheless, a great quantify of carbohydrate addition is obviously uneconomical, and is considered to enhance production of CO2, one of the greenhouse gases that gave rise to global warming [11]. Based on this, some investigators acclimated chemolithoautotrophic. bio-floc by reducing carbohydrate addition [12]. However, this led to accumulation of NO3-N on account of anaerobic process deficiency. As a matter of fact, accumulation of NO3-N has become an ubiquitous phenomenon in situ treatment of aquaculture water based on bio-floc technology. Correia [13] reported that the maximum of NO3-N concentration can reach 420.8 mg/L. Apparently, high concentration of NO3-N not only revealed potential toxicity to harvested target biomass, but also contributed to water eutrophication [14, 15].

In order to reduce accumulation of NO3-N in bio-floc system, bio-floc was acclimated under 2 hours of anaerobic conditions per day in a sequencing batch reactor in this study, expected to enrichment culture denitrification microorganism. After acclimation, denitrification performance was investigated by testing nitrate reduction rate, COD consumption and alkalinity variation, etc. The cultivated bio-floc exhibited high denitrification activity and could be used to nitrogen removal in aquaculture water treatment.

2. Materials and Methods

2.1. Denitrifies enrichment culture
Bio-floc was inoculated from tilapia intensive culture system, and acclimated in a sequencing batch reactor (Figure. 1a). During acclimation the mixed liquid suspended solids was controlled below 5000 mg/L. And the sequencing batch reactor was operated by 22 h aerobic condition and 2 h anaerobic condition in each period. Follow the tilapia intensive culture systems, ensure nitrogen nutrient load of bio-floc system at 0.1 kgN/(kgMLSS·d). Additionally, brown sugar was added as carbon source to make C/N ratio above 7.9. Solid retention time was set at 20 d in the course of acclimation, pH was remained above 7.2 by adding sodium bicarbonate, and dissolved oxygen was not less than 4mg/L in aeration phase. The bio-floc was acclimated 240 days.

2.2. Anaerobic denitrification performance test
The acclimated bio-floc, taken from SBR reactor, was washed 5 times by tap water and separately transferred into three filter Erlenmeyer flasks with 2 L effective volume (Figure. 1b). Characteristics of the bio-floc was as follows: MLSS=2823 mg/L, MLVSS=1917 mg/L, SVI=51.95 mg/L, SV=14.67%, f=MLVSS/MLSS=68%. The bio-floc was subject to 12 h starvation treatment before denitrification test. Sodium nitrate was added subsequently as nitrogen source, the NO3-N mass concentration was kept at about 35 mg/L. Operation conditions of denitrification performance with acclimated bio-floc were as follows: stirrer speed was 200r/min, dissolved oxygen was less than 0.5 mg/L, initial C/N ratio was 16, pH was 7.43, as well as the temperature was not less than 25 degrees Celsius. Samples were collected during the 2 hours of denitrification test, and the concentration of NO2-N, NO3-N, pH, DO, COD and alkalinity were measure, according to standard methods as follows.
2.3. Analytical methods

2.3.1. Detection index. NH$_4^+$-N, NO$_2^-$-N, NO$_3^-$-N, COD, total alkalinity, MLSS, SV and VSS were measure by using standard procedures [16]. pH, DO and T: Hash HQ40D multi-parameter dissolved oxygen meter.

2.3.2. Calculation methods. NaRE (nitrate removal rate), %; NaRR (nitrate reduction rate), mg/(L·h); SNaRR (special nitrate reduction rate), mg/(gVSS·d) were individually calculated as follows [17]

\[
\text{NaRE} = \frac{[\text{NO}_3^- - \text{N}]_0 - [\text{NO}_3^- - \text{N}]_t}{[\text{NO}_3^- - \text{N}]_0} \times 100
\]

\[
\text{NaRR} = \frac{[\text{NO}_3^- - \text{N}]_0 - [\text{NO}_3^- - \text{N}]_t}{\text{t}_N}
\]

\[
\text{SNaRR} = \frac{([\text{NO}_3^- - \text{N}]_0 - [\text{NO}_3^- - \text{N}]_t) \times 24}{\text{MLVSS} \times \text{t}_N}
\]

Where: $[\text{NO}_3^- - \text{N}]_0$ (mg/L) is the initial concentration of NO$_3^-$-N at the beginning of the test; $[\text{NO}_3^- - \text{N}]_t$ (mg/L) is the remaining concentration of NO$_3^-$-N at the end of the test, mg/L; $\text{t}_N$ (h) is the reaction time; MLVSS (mgVSS/L) is the mixed liquor volatile suspended solids.(Note: NO$_3^-$-N drops to 0 mg/L as the final time of the calculation equation, 24: the reaction time (h) is converted to day (d)).

![Figure 1. Device diagram of each test.](image)

a 1. heating bar 2. aeration device 3. air pump 4. inlet and outlet of bio-floc/water 5. multi-parameter water quality analyzer, b 1filtrer Erlenmeyer flasks 2. heating bar 3. magnetic stirrer 4. B11 Magnetic stirrer 5. multi-parameter water quality analyzer 6 sampling tube.

3. Results and Discussions

3.1. Denitrifies cultivation and acclimation in sequencing batch reactor

Biomass accumulation is the inevitable outcome of nutrients effective degradation. As illustrated in Figure. 2, the mass of mixed liquid suspended solids increased approximately follows the exponential law during denitrification microorganism’s cultivation and acclimation in sequencing batch reactor. After 240 days of acclimation, the mass of MLSS was reached to 5088 g.

Variation of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N concentration during acclimation was demonstrated in Table 1, it can be seen that NH$_4^+$-N and NO$_2^-$-N accumulated at the beginning of acclimation period, and degraded soon afterwards. The concentration of NH$_4^+$-N and NO$_2^-$-N was increased to 12.70 mg/L and 2.34 mg/L at the first day, and subsequently decreased to below 1 mg/L and 0.5 mg/L after 15 d of cultivation. In the middle and later period, the concentration of NH$_4^+$-N was kept stable below 0.5 mg/L and NO$_2^-$-N kept below 0.1 mg/L. Through 240 days of cultivation and acclimation, maximum
concentration of NO$_3$-N was under 57 mg/L. Samocha [18] reported that NO$_3$-N concentration was increased to 220±84 mg/L within 180 days in intensive raceways system with zero water exchange for white shrimp culture. Compared with the results got by Samocha, NO$_3$-N concentration reduced significantly after acclimation. SVI was maintained below 66.3 ml/g during 240 d of acclimation, illustrated the bio-floc had high activity and good sedimentation property.

Figure 2. The total mass change of bio-floc.

Table 1. Water quality during acclimation.

| Time/d Index | 1d (First day) | 2-15d | 16-90d | 91-240d |
|--------------|----------------|-------|--------|---------|
| NH$_4^+$-N(mg/L) | 12.7           | ≤6    | ≤1     | ≤0.5    |
| NO$_2$-N(mg/L)   | 2.34           | ≤2    | ≤0.5   | ≤0.1    |
| NO$_3$-N(mg/L)   | No record      | No record | ≤41    | ≤57     |
| SVI(ml/g)        | 66.3           | 55-68 | ≤63    | 41-60   |

3.2. Denitrification performance test

3.2.1. Nitrate removal and nitrite accumulation. Denitrification performance of bio-floc was showed in Figure. 3a. NO$_3$-N concentration decreased from 34.19 mg/L to 0 mg/L within 1.3 h, coupled with NaRE up to nearly 100%, and SNaRR up to 475.74 mg/ (gVSS d). The results indicated that bio-floc had excellent denitrification activity after acclimation. However, there was a phenomenon of NO$_2$-N accumulation appeared in the early stage of reaction. As NO$_3$-N concentration decreased to approximately 0 mg/L at initial 0.9 h, NO$_2$-N concentration increased to a maximum of 15.63 mg/L. But then it was quickly dropped down to 0.15 mg/L at 1.3 h, and maintained closed to 0 mg/L until the end of the reaction. Researchers have depicted that denitrification is the four – step model as the pathway of NO$_3$→ NO$_2$→ NO→ N$_2$O→ N$_2$, which indicates that each step is catalyzed by different enzymes [19]. As ASM-ICE model proposed by Pan, the four – step enzymatic reduction is not mutually independent process but compete for electronics with each other, which makes it possible to accumulate NO$_2$-N during denitrification [20]. The results were similar with Ge and Peng et al [21]. They studied the effects of different COD/NO$_3$-N ratios on NO$_2$-N accumulation, and also found that when COD/NO$_3$-N up to 25, NO$_2$-N accumulation increased substantially to a value of 17.19 ± 2.08 mg/L. In this test, the initial ratio of COD/NO$_3$-N was 26.39, and the maximum concentration of NO$_2$-N was 15.63 mg/L. Additionally, a vast amount of studies suggested that factors influenced NO$_2$-N accumulation included microorganism species, carbon source, pH and C/N ratio, etc.

As Figure. 3b shown, regression function of NO$_3$-N concentration and reaction time was established to further investigate the removal rate of NO$_3$-N. A linear curve was obtained in the time of NO$_3$-N concentration decreased to nearly 0 mg/L, and the correlation coefficient $R^2$ was 0.992. On
this basis, NaRR of acclimated bio-floc was calculated as 42.34 mg/ (L.h), along with SNaRR was 528.57 mg/ (gVSS.d). Compared with 86.4 mg/ (gVSS.d) of SNaRR obtained by Quan [22], who used hydrolysed molasses as an external carbon source in biological nitrogen removal, acclimated bio-floc exhibited better denitrification performance.

![Graph of NO\textsubscript{3}-N, NO\textsubscript{2}-N, and NaRE over time](image)

**Figure 3.** Denitrification performance of bio-floc after acclimation.

### 3.2.2. Variation of alkalinity and COD

Alkalinity increased about 75.85 mg/L in the course of denitrification (Figure. 3c). Combined with the NO\textsubscript{3}-N removal rate, it can be calculated that the production of alkalinity was about 2.22 g per gNO\textsubscript{3}-N. COD/NO\textsubscript{3}-N ratio was set at about 26.39 to ensure adequate carbon source for denitrification, and COD declined from 902.52 mg/L to 546.33 mg/L at 2 h.

### 4. Conclusion

In this paper, bio-floc was acclimated under anaerobic conditions by sequencing batch method. Bio-floc showed stable nitrogen removal performance in the middle and late acclimation stage. The concentration of NH\textsubscript{4}+ -N in effluent was remained below 0.5 mg/L, NO\textsubscript{2}-N concentration was below 0.1 mg/L, and NO\textsubscript{3}--N concentration was less than 57 mg/L within 240 days of cultivation and acclimation.

After cultivation and acclimation, bio-floc exhibited high denitrification activity. In denitrification test, NO\textsubscript{3}--N concentration decreased from 34.19 mg/L to approximately 0 mg/L within 1.3 h, NaRE was approximately 100%, and SNaRR was up to 475.74 mg/ (gVSS.d). This acclimated method made a good prospect for bio-floc to nitrogen removal in aquaculture water treatment.
Acknowledgments
This work was financially supported by Central Public-interest Scientific Institution Basal Research Fund, South China Sea Fisheries Research Institute, CAFS (2016TS07, 2017YB15); The strategic emerging industries and future industrial development of special funds projects in Shenzhen (201608081135); Natural Science Fund of Guangdong (2017A03031 3147).

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