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Simultaneous ammonia and nitrate removal in an airlift reactor using poly(butylene succinate) as carbon source and biofilm carrier

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HIGHLIGHTS

- Simultaneous ammonia and nitrate removal was achieved in one airlift reactor.
- DNRA and sulfate reduction were inhibited by intermittent aeration treatment.
- Denitrification rate was improved by aeration compared with anoxic condition.
- Heterotrophic nitrification was considered a potential ammonia metabolic pathway.

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ABSTRACT

In this study, an airlift inner-loop sequencing batch reactor using poly(butylene succinate) as the biofilm carrier and carbon source was operated under an alternant aerobic/anoxic strategy for nitrogen removal in recirculating aquaculture system. The average TAN and nitrate removal rates of 47.35 ± 15.62 $gNH_4–N\ m^{-3}\ d^{-1}$ and 0.64 ± 0.14 $kgNO_3–N\ m^{-3}\ d^{-1}$ were achieved with no obvious nitrite accumulation (0.70 ± 0.76 mg/L) and the dissolved organic carbon in effluents was maintained at 148.38 ± 39.06 mg/L. Besides, the activities of dissimilatory nitrate reduction to ammonium and sulfate reduction activities were successfully inhibited. The proteome KEGG analysis illustrated that ammonia might be removed through heterotrophic nitrification, while the activities of nitrate and nitrite reductases were enhanced through aeration treatment. The microbial community analysis revealed that denitrifiers of *Azoarcus* and *Simplicispira* occupied the dominate abundance which accounted for the high nitrate removal performance. Overall, this study broadened our understanding of simultaneous nitrification and denitrification using biodegradable material as biofilm carrier.

1. Introduction

The indoor recirculating aquaculture system (RAS) is a promising alternative to traditional aquaculture practice in outdoor systems due to its environmental sustainability (Martins et al., 2010). However, intensive feeding input always results in increasing accumulation of contaminants, i.e., ammonium, nitrite and nitrate in RAS. Therefore, the removal of residual nitrate-nitrogen is indispensable to achieve rigorous wastewater discharge standards. Traditional RAS use a bio-filter to convert ammonia and nitrite to nitrate through nitrification since ammonia and nitrite are much more toxic than nitrate (Gutierrez-Wing and Malone, 2006). Thus high nitrate accumulation was commonly detected in intensive RAS practice with low water exchange, which creates a long-term threat to aquatic animals and environmental eutrophication (van Rijn et al., 2006). Among various nitrate removal methods, biological denitrification conducted by heterotrophic microorganisms that eliminate nitrate to nitrogen gas (N_2) was proved to be a promising solution to RAS wastewater treatment (Hamlin et al., 2008). Such denitrification systems require an external carbon source to act as electron donors because of the low C/N ratio in typical RAS wastewater. During the last decades, various biodegradable polymers (BDPs) acted as simultaneous carbon source and biofilm carrier have been implied and achieved acceptable performance for RAS effluent nitrate removal under anoxic

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conditions (Boley et al., 2000; Shen and Wang, 2011; Gutierrez-Wing et al., 2012; Zhu et al., 2015).

Traditional denitrification is mainly performed under anoxic conditions since oxygen can highly inhibit the activity of denitrification enzyme and cause nitrite or nitrous oxide accumulation (Kampschreur et al., 2009). However, in a marine anoxic denitrification system, as the denitrifiers deplete nitrate and nitrite, sulfate as the next best terminal electron acceptor can be used to produce toxic sulfide with sufficient organic electron donors as the redox potential decreases (Lee et al., 2000; Zhu et al., 2015). Besides, high carbon to NO_3^- ratios, high levels of sulfide and salinity provide favorable conditions to support dissimilatory nitrate reduction to ammonium (DNRA) over denitrification (Giblin et al., 2013), and this phenomenon as a competition to the denitrification process was widely detected in anoxic BDPs denitrification systems, especially in marine environment conditions (Chu and Wang, 2013; Shen and Wang, 2011; Zhu et al., 2015). Though those two problems can be solved by controlling residual DOC concentration in the reactor, effective methods were not reported, which inhibited the application of solid-phase anoxic denitrification in marine RAS practice.

In general, a biological reaction is a respiration process in which electron transport is accomplished for energy supplement. In a marine denitrification reactor, carbon source, the only electron donor, is competed for among several substances which play the roles of electron acceptors, such as oxygen, nitrate and sulfate (Gutierrez-Wing et al., 2012). The priority is established mainly by the energy yields, and oxygen provides the most energetic efficiency then followed by nitrate and then sulfate (Lee et al., 2000). Therefore, to solve the two problems using an anoxic PBS denitrification reactor mentioned above, a potential solution might be to apply oxygen to cut off the route of electron transport through DNRA and SRB under aerobic/anoxic operation. Traditionally, though oxygen can inhibit anoxic denitrification process either by direct competition or by enzyme inhibition (Gómez, 2002), we hypothesize it is still feasible for denitrification due to the characteristic of the packed media acting both as carbon source and biofilm carrier. Our hypothesis is based upon the fact that the anoxic micro-zones exists in the deeper layer of the biofilm below the aerobic outer layer and are developed with the gradual degradation of packing carriers, which could create a sufficient zone suitable for anoxic denitrification. Besides, several denitrifying groups such as *Comamonadaceae*, *Brevundimonas* and *Acidovorax* have facultative respiration processes to survive under aerobic condition, as well as many genuses such as *Rhodococcus* and *Acinetobacter* prefer to function as aerobic denitrifiers (Chen et al., 2012; Huang et al., 2013). If this process can be demonstrated, then there are additional benefit of the process providing ammonia removal being achieved during aeration period in one reactor meanwhile.

In this study, an airlift inner-loop sequencing batch reactor (SBR) using PBS as carbon source and biofilm carrier was studied for simultaneous ammonia and nitrate removal of marine RAS wastewater through alternant aeration and non-aeration strategy. The feasibility and nitrogen removal rates were evaluated through long-term reactor performance and cycle profiles. Meanwhile, to have a better insight of nitrogen (ammonia and nitrate) metabolism pathway, batch tests as well as isobaric tags for relative and absolute quantitation (iTRAQ) proteomic analysis were also carried out. Moreover, the bacteria communities over the course of the study were investigated through Illumina high throughput sequencing to provide microbial evidence of nitrogen removal. Overall, our results might contribute to elucidate the mechanisms of inorganic-N removal process and provide a potential alternative for nitrogen removal treatment in marine RAS practice.

2. Materials and methods

2.1. Biodegradable materials and airlift inner-loop SBR

The biodegradable PBS granules used in this experiment were identical with our previous study of a marine anoxic fixed bed denitrification reactor (Zhu et al., 2015). The schematic representation of the airlift inner-loop SBR is shown in Fig. 1. The main reactor vessel has a working volume of 5.5 L, the outer cylindrical tube has the height of 300 mm and diameter of 150 mm, while the inner tube has the height of 150 mm and diameter of 30 mm. The 2.3 L of PBS carriers filled the reactor up to a height of 150 mm, resulting in 50% fill ratio. Air was diffused into the bottom of the inner tube periodically to form an inner-loop driving force for water circulation. The feeding influent of RAS wastewater (salinity, 25‰) was slightly adjusted by additions of NH_4Cl and KNO_3 to obtain approximately 10–15 mg/L TAN and 100–150 mg/L $NO_3{}^-$–N, which were similar to the conditions of our previous study (Zhu et al., 2015).

In this study, the reactor was operated on sequencing mode with 4 h cycle procedure including an initial feeding for 6 min, followed by 25 minor cycles of aeration reaction for 1 min and intermittent settling for 8 min, and a final effluent discharge for 9 min. The operating procedure was automatically controlled for 6 cycles each day by a programmable logic controller (PLC). Meanwhile, the volumetric exchange ratio was determined as 80%, resulting in a hydraulic retention time (HRT) of 5 h. The overall apparatus was placed in a dark artificial climate room to maintain the temperature at 25 ± 1 °C. The reactor was pre-cultured for four weeks before the experiment started to accelerate to an acclimated performance condition.

2.2. Reactor operation performance

2.2.1. Long-term performance and cyclic profile

In order to assess the performance and stability of the reactor, long-term water qualities (inorganic-N and DOC) of the influents and effluents were monitored. The apparent inorganic-N removal rates were calculated based on the following equations:

$$TAN_r = 0.024Q(C_{TAN-in} - C_{TAN-eff})/(V * HRT)$$

$$DEN_r = 0.024Q(C_{NO3-in} - C_{NO3-eff})/(V * HRT)$$

where TAN_r (kgTAN m^{-3} d^{-1}) and DEN_r (kg$NO_3{}^-$–N m^{-3} d^{-1}) were the TAN and nitrate removal rate, respectively. C_{TAN-in} (mg/L), $C_{TAN-eff}$ (mg/L) and C_{NO3-in} (mg/L), $C_{NO3-eff}$ (mg/L) were the concentrations of TAN and nitrate in the influent and effluent. Q (L) and V (L) were the bulk volume of influent solution and concrete PBS carriers, respectively.

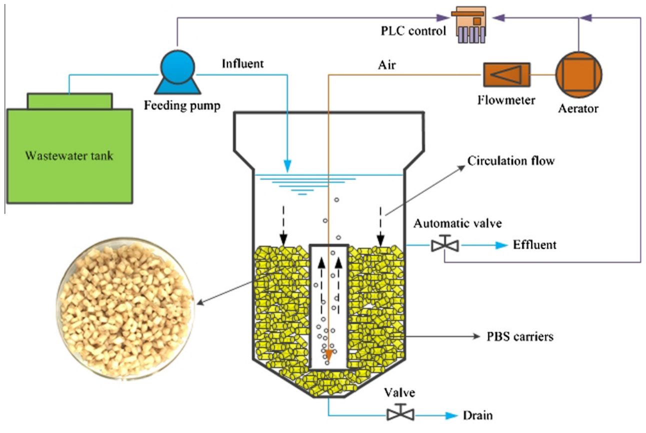

Fig. 1. Schematic representation of the PBS airlift inter-loop sequencing batch reactor.