Bioresource Technology 192 (2015) 603–610

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biological denitrification using poly(butylene succinate) as carbon source and biofilm carrier for recirculating aquaculture system effluent treatment

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HIGHLIGHTS

- PBS polymer showed well performance for real RAS wastewater denitrification.
- High nitrate loading was favor to inhibit sulfate reduction and DNRA activity.
- Variation in microbial population was responsible for changed reactor performance.
- Precise carbon release was crucial for RAS denitrification process in practice.

ARTICLE INFO

Article history:
Received 9 April 2015
Received in revised form 2 June 2015
Accepted 4 June 2015
Available online 10 June 2015

Keywords:
Poly(butylene succinate)
Denitrification
Recirculating aquaculture system
Wastewater treatment
Salinity

ABSTRACT

Nitrate removal is essential for the sustainable operation of recirculating aquaculture system (RAS). This study evaluated the heterotrophic denitrification using poly(butylene succinate) as carbon source and biofilm carrier for RAS wastewater treatment. The effect of varied operational conditions (influent type, salinity and nitrate loading) on reactor performance and microbial community was investigated. The high denitrification rates of 0.53 ± 0.19 kg NO_3^--N m^{-3} d^{-1} (salinity, 0‰) and 0.66 ± 0.12 kg NO_3^--N m^{-3} d^{-1} (salinity, 25‰) were achieved, and nitrite concentration was maintained below 1 mg/L. In addition, the existence of salinity exhibited more stable nitrate removal efficiency, but caused adverse effects such as excessive effluent dissolved organic carbon (DOC) and dissimilation nitrate reduce to ammonia (DNRA) activity. The degradation of PBS was further confirmed by SEM and FTIR analysis. Illumina sequencing revealed the abundance and species changes of functional denitrification and degradation microflora which might be the primary cause of varied reactor performance.

1. Introduction

The indoor recirculating aquaculture system (RAS) is a potential sustainable alternative to traditional aquaculture systems (Midilli et al., 2012). It provides opportunities to reduce water consumption and to improve waste treatment and nutrient recycling, which makes intensive fish production compatible with environmental sustainability (Martins et al., 2010). During the process, RASs employ biological filters to oxidize ammonia to nitrate with nitrite as intermediate product, since ammonia and nitrite are toxic to cultured species (Gutierrez-Wing and Malone, 2006). Thus, high concentration of nitrate can be accumulated in intensive RASs due to the continuous nitrification and less effluent discharge (van Rijn et al., 2006). Compared with ammonia and nitrite, though nitrate has relatively low stress effect to aquatic animals temporarily, long-time threat was detected to cultured species (van Bussel et al., 2012). Meanwhile, nitrate was considered as one of the main reasons for terrestrial ecosystems eutrophication (McIsaac et al., 2001). Therefore, nitrate removal has become an inevitable potential problem to be solved in RAS practice, considering the aquatic animal welfare, environment pollution and production sustainability (van Rijn et al., 2006).

Biological heterotrophic denitrification was proved to be an efficient way for nitrate removal in wastewater treatment (Pan et al., 2015). In this process, heterotrophic microorganisms use organic carbon as electron donor, while nitrate as electron acceptor, and ultimately change nitrite or nitrate into nitrogen (N_2). Therefore, sufficient organic carbon concentration or suitable C/N ratio was demonstrated as a crucial factor to ensure the desired nitrate

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http://dx.doi.org/10.1016/j.biortech.2015.06.021
0960-8524/

removal efficiency (Chen et al., 2015). For RAS, daily feed contained relative high protein and low carbohydrate, which resulted in low C/N ratio of water quality characteristics. Thus, added liquid organic carbons such as methanol or ethanol were needed for RAS effluent denitrification (Müller-Belecke et al., 2013). However, the supplied organic carbon might cause water quality fluctuation as the continuous water recirculation. Because pulses addition organic carbon would result in organic carbon loss and water dissolved oxygen (DO) drop, which was a stress to fish and might cause increase mortality. Meanwhile, less amount of liquid organic cannot achieve acceptable denitrification performance while more liquid organic would add organic loading to the bio-filter, which would have a negative effect on the system stability. On the other hand, the fluctuation of nitrate concentration in RAS caused by different species and cultured objectives also increase the difficulty for precise dosage of liquid carbon. Though bypass system can solve the above concerns, it lead to more footprint, tanks and equipment, which increases the complexity and cost of control process.

An interesting alternative is to use insoluble solid carbon for denitrification, which is mainly divided into two methods. One is using natural materials (e.g., straw, and wood) which were demonstrated to have acceptable nitrate removal efficiency (Saliling et al., 2007), but the excessive effluent dissolved organic carbon (DOC) concentration and color problems limit its universality (Boley et al., 2000). The other one is using biodegradable polymers as solid carbon source and biofilm carrier for denitrification. It can release organic carbon slowly and automatically, which means it can be used directly in the system, and provides an alternative to sustainable RAS. For instance, polycaprolactone (PCL) (Chu and Wang, 2013) or starch/polycaprolactone (SPCL) (Shen et al., 2015), polyhydroxybutyrate (PHB) (Gutierrez-Wing et al., 2012) and poly(butylene succinate) (PBS) (Luo et al., 2014; Wu et al., 2013) were all proved to have high nitrate removal efficiency. Because of the specific material component and reaction product, denitrification based on biodegradable polymers seemed to be more convenient and competitive when the treatment objects have high water quality requirement (e.g., drinking water, groundwater, and RAS water) (Lucas et al., 2008). However, previous researches of solid-phase denitrification mostly focused on synthetic wastewater (Luo et al., 2014; Shen and Wang, 2011; Shen et al., 2013, 2015; Wu et al., 2013), whose composition is relatively unitary, while real RAS wastewater were seldom studied. Though these target wastewaters share the common water quality characteristic of low C/N ratio, RAS effluent contained more complex substrate component due to the interaction of cultured animals and feeding (van Rijn, 2013). Moreover, the increase in production of saltwater species appeal to more suitable denitrification systems, because salinity was demonstrated to be detrimental as its effect to bacteria osmotic pressure and enzyme inhibition (Lefebvre and Moletta, 2006). Therefore, further researches of real wastewater treatment were necessary for the sustainable and practical application of RAS.

In this study, we evaluated the performance of two up-flow fixed bed reactors (salinities, 0‰ and 25‰) packed with PBS granules under different operational conditions. The feasibility and efficiency of nitrate removal were studied in terms of denitrification efficiency and effluent characteristics using synthetic and real RAS wastewater as influent, respectively. To have a better understanding of the PBS biodegradation process, the granules before and after experiment were characterized by FTIR and SEM observation. Furthermore, the microbial diversities during different operational conditions were also analyzed to elucidate the variation in microbial population and reactor performance.

2. Methods

2.1. Reactors and biodegradable PBS materials

The schematic representation of the two up flow fixed-bed PBS denitrification reactors is shown in Fig. 1. The two identical reactors were 60 mm inner diameter and 1000 mm height, with the PBS granules packed the column up to the height of 900 mm. The varied types of influents were fed into the two reactors by peristaltic pumps (BT100-2J, Baoding Longer Precision Pump Co., Ltd., China) and the effluents were collected for further analysis. The overall apparatus was placed in a dark artificial climate room to keep the temperature at 19 ± 1 °C and 24 ± 1 °C (Table 1), which were thought to be the suitable temperatures for the cold-water fish and warm-water fish, respectively.

The biodegradable injection level PBS granules were purchased from a commercial company, and the main physical characteristics are as follows according to the manufacturer description: density (25 °C), 1.26 kg/L; melt flow index, 21 g/10 min; tensile strength, 39%; and flexural strength, 27 MPa. The PBS granule has cylindrical size of 3 mm × 5 mm (diameter × height), and the porosity is 34.6%.

2.2. Experimental procedure

The different operational conditions during whole stage were shown in Table 1. For inoculation, 20 mL deep deposit-mixtures from local fresh lake and intertidal zone (Luchao port, Shanghai, China) were used to seed Reactor I and Reactor II, respectively. During stage I, synthetic RAS wastewater used as influent for both reactors was according to (mg/L): 360 KNO_3 (around 50 mg/L NO_3^--N), 78 K_2HPO_4, 31 KH_2PO_4, 95 $MgSO_4{\cdot}7H_2O$, 37 KCl, and 0.2% (v/v) trace element (mg/L): 640 EDTA, 550 $FeSO_4{\cdot}7H_2O$, 230 $ZnSO_4{\cdot}7H_2O$, 340 $MnSO_4{\cdot}H_2O$, 75 $CuSO_4{\cdot}5H_2O$, 47 $Co(NO_3)_2{\cdot}6H_2O$, 25 $(NH_4)_5Mo_7O_{24}{\cdot}4H_2O$ (Ruan et al., 2011). For other stages (II–VI), the real RAS wastewater was used to feed the reactors with only moderate added KNO_3 (around 50–150 mg/L NO_3^--N). The tilapia RAS contained with four tanks (4 m^3) and one MBBR bio-filter (0.5 m^3) was relative stable (survival rate, 95.8%) operated in our laboratory for nearly 1 year. Besides, the artificial sea salt (Blue starfish salt product Co., Ltd., Zhejiang, China) was used to keep the salinity at 25‰ for Reactor II in the whole stage, while the Reactor I was continuously operated at 0‰. In addition, DNA samples were also collected from the two reactors in different stages to evaluate the bacterial community variation.

The nitrate removal efficiency and denitrification rate of the reactors were calculated based on the following equation:

$$R_e = (C_{in} - C_{ef})/C_{in} \times 100\%$$

$$DEN_r = 0.024Q(C_{in} - C_{ef})/(\eta V)$$

where R_e (%) and DEN_r (kg NO_3^--N m^{-3} d^{-1}) were the nitrate removal efficiency and denitrification rate, respectively. C_{inf} (mg/L) and C_{eff} (mg/L) were the concentrations of NO_3^--N in the influent and effluent, respectively. Q (L/h) was the flow rate, V (L) was the bulk volume of the PBS, and η was the porosity of the PBS.

2.3. Analytical methods

The water samples were filtered through 0.45 μm filter membrane before analysis. Total ammonia nitrogen (TAN), NO_2^--N and NO_3^--N concentration were analyzed according to standard methods (SEPA, 2002). DOC was measured using a TOC analyzer (Multi N/C 2100, Analytik Jena, Germany). The morphology of PBS granule and attached biofilm was observed by scanning electron microscope (SEM) (TM-1000 and SU8010, Hitachi High-Technologies Corporation, Japan) on days 0, 25 and 205.