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Effect of dissolved oxygen on nitrate removal using polycaprolactone as an organic carbon source and biofilm carrier in fixed-film denitrifying reactors

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ABSTRACT

Nitrate-nitrogen ($\text{NO}_3\text{-N}$) always accumulates in commercial recirculating aquaculture systems (RASs) with aerobic nitrification units. The ability to reduce $\text{NO}_3\text{-N}$ consistently and confidently could help RASs to become more sustainable. The rich dissolved oxygen (DO) content and sensitive organisms stocked in RASs increase the difficulty of denitrifying technology. A denitrifying process using biologically degradable polymers as an organic carbon source and biofilm carrier was proposed because of its space-efficient nature and strong ability to remove $\text{NO}_3\text{-N}$ from RASs. The effect of dissolved oxygen (DO) levels on heterotrophic denitrification in fixed-film reactors filled with polycaprolactone (PCL) was explored in the current experiment. DO conditions in the influent of the denitrifying reactors were set up as follows: the anoxic treatment group (Group A, average DO concentration of 0.28 ± 0.05 mg/L), the low-oxygen treatment DO group (Group B, average DO concentration of 2.50 ± 0.24 mg/L) and the aerated treatment group (Group C, average DO concentration of 5.63 ± 0.57 mg/L). Feeding with 200 mg/L of $\text{NO}_3\text{-N}$, the $\text{NO}_3\text{-N}$ removal rates were 1.53, 1.60 and 1.42 kg/m³ PCL/day in Groups A, B and C, respectively. No significant difference in $\text{NO}_3\text{-N}$ removal rates was observed among the three treatments. It was concluded that the inhibitory effects of DO concentrations lower than 6 mg/L on heterotrophic denitrification in the fixed-film reactors filled with PCL can be mitigated.

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Introduction

Aerobic nitrification is commonly used in recirculating aquaculture systems (RASs) to convert ammonium-nitrogen ($\text{NH}_4\text{-N}$) to nitrate-nitrogen ($\text{NO}_3\text{-N}$) through nitrite-nitrogen ($\text{NO}_2\text{-N}$) (US EPA, 1996; van Rijn et al., 2006; van Rijn, 2013). Without a nitrate removal unit in an RAS, $\text{NO}_3\text{-N}$ can accumulate as high as 400–500 mg/L (van Rijn et al., 2006). Although $\text{NO}_3\text{-N}$ is relatively non-toxic to aquatic organisms, in contrast to $\text{NH}_4\text{-N}$

and $\text{NO}_2\text{-N}$, there is increasing evidence that $\text{NO}_3\text{-N}$ accumulation can negatively impact aquaculture animals (Hamlin et al., 2008; Davidson et al., 2014). Additionally, $\text{NO}_3\text{-N}$ discharged from aquaculture systems is considered to be one of the most important factors related to eutrophication in aquatic environments (Passy et al., 2013). Therefore, increased efforts are now directed toward $\text{NO}_3\text{-N}$ control in RASs (Tsukudaa et al., 2015).

Heterotrophic denitrification has been demonstrated as one of the most feasible and cost-effective methods of $\text{NO}_3\text{-N}$

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removal (Chu and Wang, 2011). In the heterotrophic denitrifying process, denitrifying bacteria use organic carbon for growth and as an electron donor. The amount of biodegradable organic carbon in aquaculture water is much lower than that required for denitrifiers, thus organic sources are added intentionally (van Rijn et al., 2006). Several water-soluble carbon sources, such as ethanol, glucose, acetate and methanol, are readily available to heterotrophic bacteria, but require careful dosing and constant supervision to prevent overdosing and starvation periods (Lee et al., 2000), especially with the fluctuations of influent water quality and quantity in RASs (Singer et al., 2008). Recently, a new set of biological degradable polymers (BDPs), including polyhydroxyalkanoate (PHA), poly-3-hydroxybutyric acid (PHB), polycaprolactone (PCL), and polybutylene succinate (PBS), has been developed to serve as both an organic carbon source and a biofilm carrier for denitrification and is referred to as BDP-denitrification (Wu et al., 2012). BDP-denitrification is considered to require less supervision and management for its successful operation than previous methods (Boley et al., 2000).

Most denitrifying bacteria are facultative anaerobes that reduce nitrate in the absence of oxygen (Gómez et al., 2002). Due to the intensive aeration of the grow-out tanks of RASs, high levels of oxygen in the influent process have the potential for inhibiting the denitrifying progress (Singer et al., 2008). Therefore, to create an anaerobic environment, water is pretreated to remove dissolved oxygen (DO) before entering denitrifying reactors. DO can be removed from water by adding sodium thiosulfate (Gómez et al., 2002), or through an ample residence time (Boley et al., 2000). Of course, pretreatment inevitably increases the cost and management needed.

During the BDP-denitrifying process, the biofilm forms on the surface of the polymers, diffusion of oxygen is impeded and large anoxic zones are created above the pores and cracks of the BDPs coupled with carbon storage in the depth of the granules, which should stimulate the heterotrophic denitrifying process (Gutierrez-Wing et al., 2012). Therefore, the negative effect of DO on the BDP-denitrifying process can be expected to be minimized. In our recent study, we observed the effects of DO on heterotrophic denitrification using PBS (Luo et al., 2014) and PCL (under review) as the carbon source and biofilm carrier, contained in beakers. It was determined that the removal of NO_3^- -N was highest with DO concentrations up to 6 mg/L achieved by aeration, compared with the anoxic treatment and low DO treatment groups. This finding led us to ask whether DO could also improve BDP-based denitrification in fixed-film denitrifying reactors. If the answer is yes, a convenient method could be supplied to remove NO_3^- -N from RASs. The current study was undertaken to investigate the effect of influent DO concentration on heterotrophic denitrification in fixed-bed reactors using polycaprolactone (PCL) as the carbon source and biofilm carrier. Three DO levels in the influent of the reactors, representing common situations encountered in the denitrifying process, were set up. The first group was the anoxic treatment group, in which gaseous nitrogen was added to the water and the water surface was sealed. The second group was the low DO treatment group, in which water was left exposed to air with no aeration and no wax sealing. The third group was the aerated treatment group, in which the water was aerated with a fine-bubble diffuser. The nitrogen removal performance and water quality in the

effluent were evaluated to assess the effect of DO in the influent on the PCL-denitrifying reactors.

1. Materials and methods

1.1. PCL

The physical characteristics of polycaprolactone ($[\text{C}_6\text{H}_{10}\text{O}_2]_n$) (Guanghua Weiye Co. Ltd., Shenzhen, China) are as follows: density, $1.08\text{--}1.12\text{ g cm}^{-3}$; melting point, 60°C . The PCL granules have an ellipsoid shape with dimensions of $2\text{ mm} \times 3\text{ mm} \times 4\text{ mm}$ (width \times length \times height). The granules were cleaned by the ultrasonic technique (0.1 kW, 40 kHz) and subsequently dried at 35°C to a constant mass of $\pm 0.0001\text{ g}$ in a vacuum oven.

1.2. Nitrate nitrogen removal in fixed-bed reactors with PCL as an organic carbon source and film carrier

A continuous experiment was conducted in three fixed-bed reactors (10.5 cm in diameter, 70.00 cm high and 3.5 L working volume; Fig. 1). Each reactor was filled with 50 vol.% fill of PCL granules (1.8 kg). The column surface of the reactor was not covered. The raw synthetic wastewater (SW) was kept in a tank at $22 \pm 1^\circ\text{C}$ by a temperature controller, then fed to the denitrifying reactors by a peristaltic pump (BT100-1F, Baoding Longer Precision Pump Co., Ltd., China). The SW was prepared by adding 78 mg K_2HPO_4 , 31 mg KH_2PO_4 , 95 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 37 mg KCl to 1 L of tap water and 0.2% (V/V) of a trace nutrient solution containing the following compounds: 640 mg EDTA, 550 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 230 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 340 mg $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 75 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 25 mg $(\text{NH}_4)_5\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$. The hydraulic retention time (HRT) was 5.5 hr according to our previous study.

The three DO groups in the tank that contained SW were the anoxic treatment group (group A), the low DO treatment group (group B), and the aeration treatment group (aerated with air; group C). The water surface of group A was sealed with liquid paraffin to create an anoxic environment in the water and maintain an average DO concentration ranging from 0.12 to 0.55 mg/L. Group B was left open to the air with no aeration and no wax seal, and contained DO in the range of 1.82 to 3.99 mg/L. The DO concentration ranged from 5.29 to 6.56 mg/L in group C and was controlled through an air flow adjuster.

The NO_3^- -N concentration in the SW tank was 200 mg/L. NO_3^- -N, NO_2^- -N, total nitrogen (TN), total ammonium-nitrogen

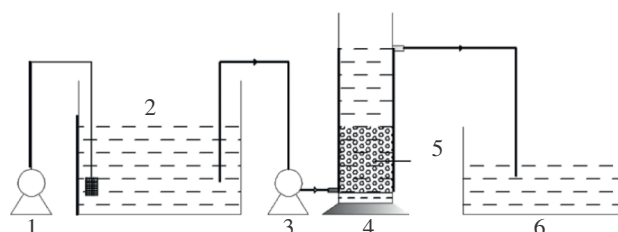


Fig. 1 – System diagram for the continuous experiment: (1) air compressor, (2) influent water reservoir, (3) influent peristaltic pump, (4) reactor column, (5) PCL media, and (6) effluent tank.

(TAN), DOC, alkalinity (ALK), pH and DO in the influent and the effluent were monitored daily. The weight loss of PCL granules was determined at the end of the test.

1.3. Analytical methods

The water temperature, pH, ALK and DO were measured with a YSI 556 m (YSI Incorporated 1725, Yellow Springs, OH, USA). The water samples were filtered through a 0.45- μm filter before determining the TAN, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, and DOC levels. TAN, $\text{NO}_2\text{-N}$, TN and $\text{NO}_3\text{-N}$ were analyzed according to Chinese SEPA Standard Methods (Chinese, 2004). The DOC sample was measured using a total organic carbon analyzer (TOC-V, CPH, Shimadzu Seisakusho, Japan).

1.4. Data analysis

All of the statistical analyses in the current study were performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). The parameters of water quality and weight loss of PCL were determined using one-way analysis of variance. The difference was considered significant at $p < 0.05$.

2. Results and discussions

2.1. DO Consumption

DO concentrations decreased from 0.5–6 mg/L in the influent to below 0.02 mg/L in the outlet for the three treatments (Fig. 2). A similar decrease in DO in denitrifying beds containing carbon substrates was also measured in Robertson (2010) and Warneke et al. (2011). The current experiment suggested that the biodegradation of PCL would consume DO under the aerobic condition. This perhaps was one of the main reasons that the BDP-denitrifying process could mitigate the inhibition of denitrification by DO.

2.2. $\text{NO}_3\text{-N}$ and TN removal performance

An acclimation period is required for the adaptation of denitrifying microorganisms. This period was defined as the point when the steepest negative concentration versus time occurred (Boley et al., 2000). 16 days and 8 days for acclimation were found in the denitrifying reactors with PCL and PHB as the organic carbon source, respectively (Boley et al., 2000). Gutierrez-Wing et al. (2012) allowed 2–7 days for the PHB-denitrifying reactor to acclimate. In the current experiment, $\text{NO}_3\text{-N}$ and TN removal efficiencies increased gradually and reached 90% on day 12 (Fig. 3). Thus, it was considered that the acclimation period of the reactors was 11 days.

After the start-up period, the $\text{NO}_3\text{-N}$ removal efficiency was maintained at approximately 90% in group A and group B. In group C, the $\text{NO}_3\text{-N}$ removal efficiency gradually decreased to 75% at the end of the experiment (Fig. 3a), which might be due to the blockage by the attached biofilm on the surface of the granules (Shen et al., 2013). This suggests that backwashing will be regularly required for reactors in practice. Although the $\text{NO}_3\text{-N}$ removal efficiency of group C was slightly lower than

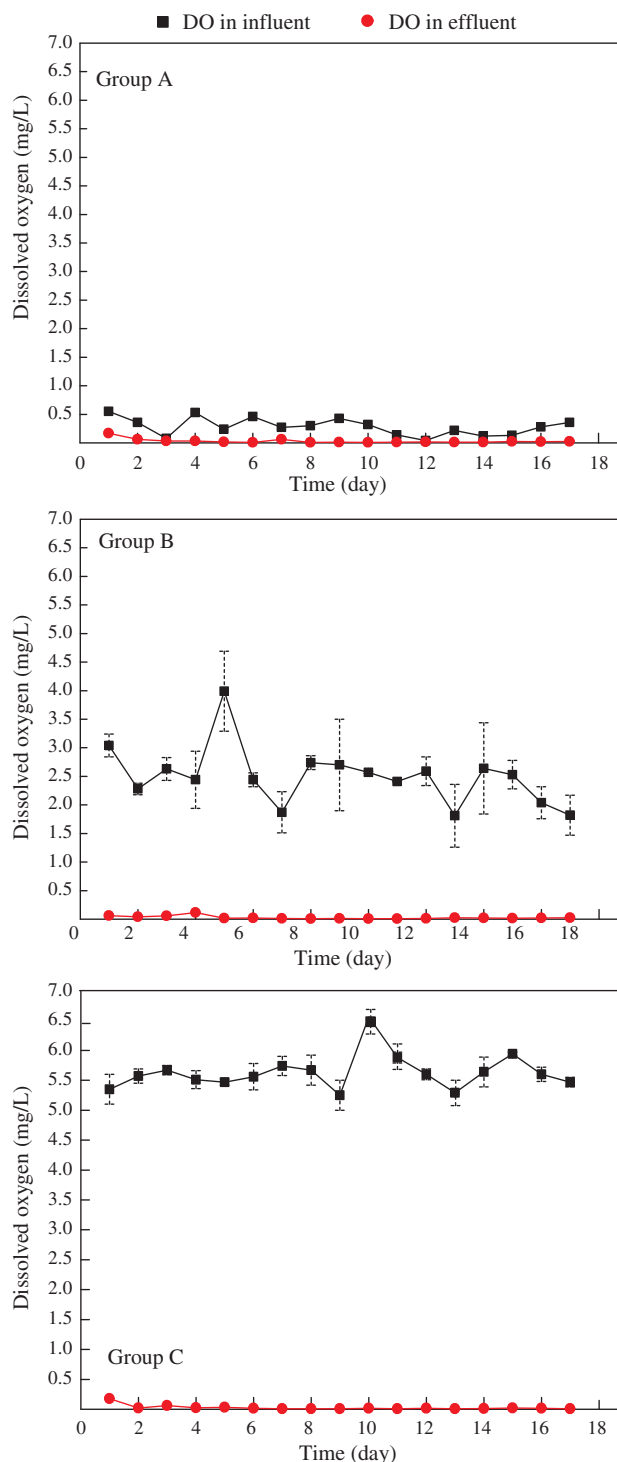


Fig. 2 – The dissolved oxygen (DO) concentrations in the influents and effluents of the polycaprolactone (PCL)-denitrification reactors under different DO conditions. Group A: anoxic treatment group, group B: low DO treatment group, group C: aeration treatment group.

that of groups A and B, the TN removal efficiency in group C was the highest among the three treatments (Fig. 3b). This may be because the amount of nitrate, which was transformed to nitrogen gas and completely removed from the system in group

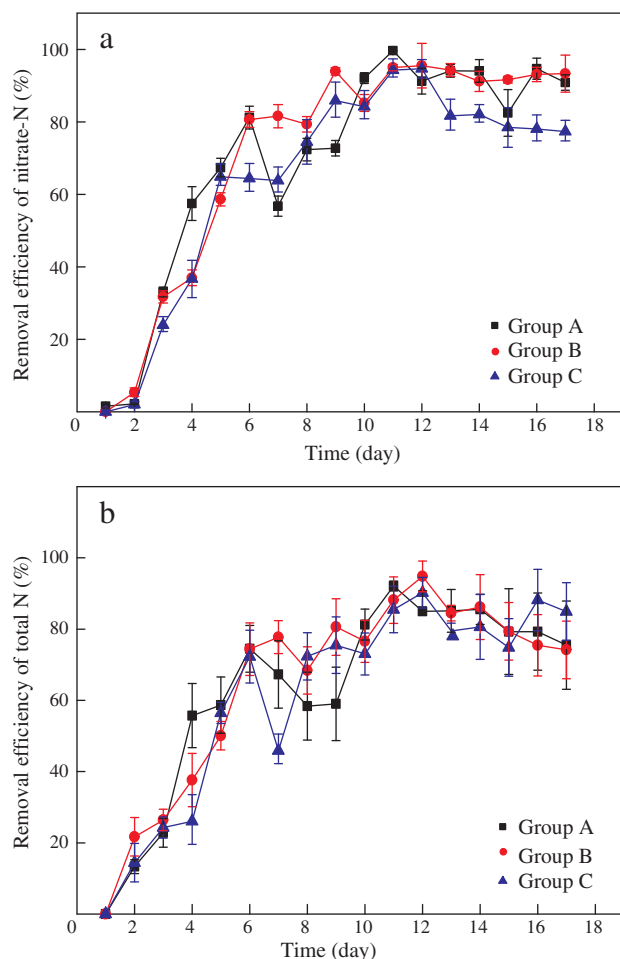


Fig. 3 – Performance of nitrate-nitrogen ($\text{NO}_3\text{-N}$) (a) and total nitrogen (TN) (b) removal of the PCL-denitrification reactors under different DO conditions.

C, was higher than that of group A and group B. In these two groups, some of the removed $\text{NO}_3\text{-N}$ might be transformed into ammonium or nitrite and still remain in the system.

The $\text{NO}_3\text{-N}$ removal rates of the three groups were 1.53 ± 0.24 , 1.60 ± 0.20 , and 1.42 ± 0.18 kg/m^3 PCL-day, respectively. No significant difference was found among them. This suggests that 2.50–5.43 mg/L DO in the influent did not inhibit the denitrifying process significantly. The $\text{NO}_3\text{-N}$ removal rates obtained in the current experiment are within the range or higher than those reported in related BDP-denitrifying studies. For example, 0.50–3.98 $\text{kg NO}_3\text{-N}/\text{m}^3$ media/day was found using PCL as a carbon source in RASs (Boley et al., 2000), and 0.64 $\text{kg NO}_3\text{-N}/\text{m}^3$ media/day was found using a cross-linked starch/PCL blend as a substrate in the treating of ground water (Shen et al., 2013).

2.3. TAN, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ in the effluent

TAN accumulation during steady-state was observed in all three groups, averaging 1.93 ± 0.37 , 1.37 ± 0.15 , and 1.72 ± 0.46 mg/L, respectively (Fig. 4a). This indicates that the process of dissimilatory nitrate reduction to ammonia (DNRA) was occurring in the systems. Several studies also suggested that DNRA is a

common minor process involved in $\text{NO}_3\text{-N}$ removal (Greenan et al., 2006; Shen and Wang, 2011). Therefore, a method to inhibit or minimize the DNRA process in BDP-denitrifying systems needs to be studied in the future.

During the start-up period, $\text{NO}_2\text{-N}$ accumulation ranged from 0.52 to 1.25 mg/L in group A, and 0.5 to 7.88 mg/L in group B. Less than 0.5 mg/L of $\text{NO}_2\text{-N}$ was observed in group C. After the start-up period, less than 0.20 mg $\text{NO}_2\text{-N}/\text{L}$ of the outlet concentrations was observed in groups B and C. The average outlet concentration of $\text{NO}_2\text{-N}$ in group A was 0.62 ± 0.07 mg/L during the steady-state period (Fig. 4b). The $\text{NO}_2\text{-N}$ and TAN

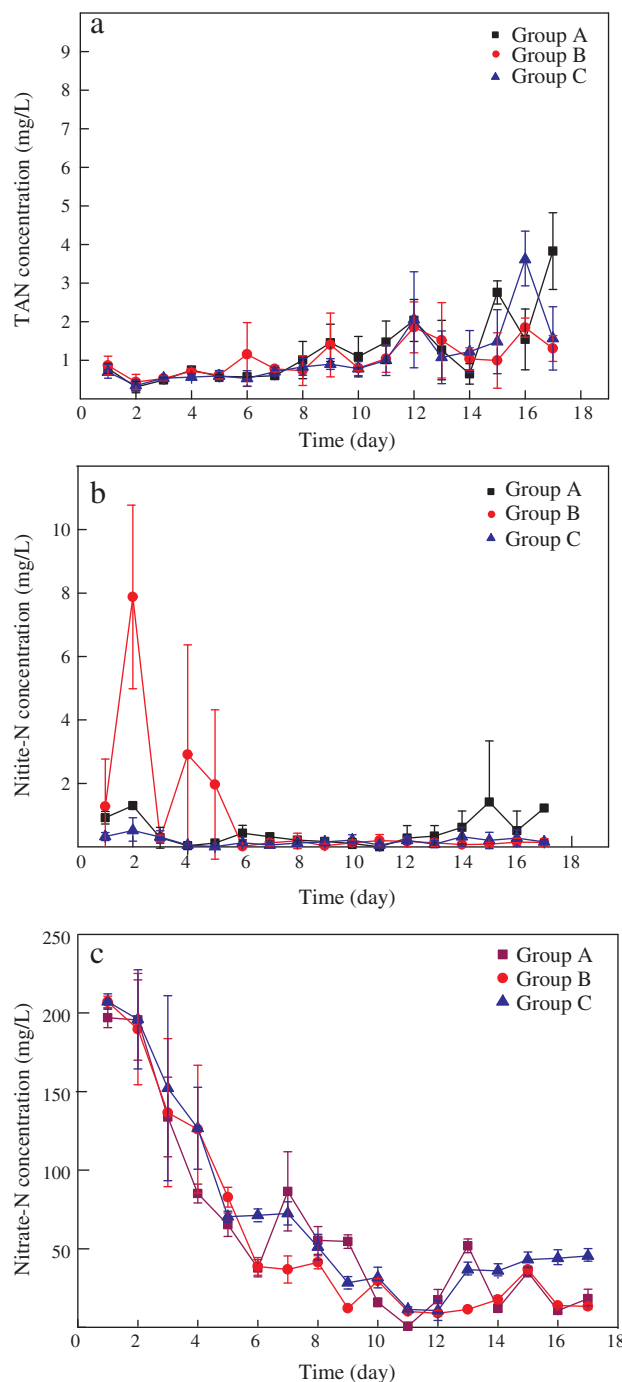


Fig. 4 – TAN-N (a), $\text{NO}_2\text{-N}$ (b) and $\text{NO}_3\text{-N}$ (c) in the effluents of the PCL-denitrification reactors under different DO conditions.

in the effluent from the denitrifying reactors can input into the nitrifying reactor for further treatment (Tal et al., 2009).

The NO_3^- -N concentrations in the effluent for the three groups were 24.21 ± 0.55 , 17.00 ± 0.28 and 35.94 ± 1.37 mg/L during the steady-state period, respectively (Fig. 4c). These values are higher than those in related studies (e.g., Boley et al., 2000; Wu et al., 2013; Shen et al., 2013; Chu and Wang, 2013). This is perhaps because the NO_3^- -N concentration in the influent was as high as 200 mg/L in the current experiment, which was more concentrated than the water in the RASs. Noting the originality of our experiment for NO_3^- -N removal in RASs, approximately 30 mg/L of NO_3^- -N is considered within the allowable range for most cultured organisms (Kincheloe et al., 1979; McGurk et al., 2006).

2.4. Residual DOC in the effluent and the weight loss of PCL

The residual DOC in the effluent of group A (53.06 ± 5.93 mg/L) was higher than that of group B (35.50 ± 6.26 mg/L) and group C (26.12 ± 3.12 mg/L) in the steady state (Fig. 5a). DOC accumulation in the effluent was also found in related studies (e.g., Wu et al., 2012; Shen and Wang, 2011). It has been noted that bio-deterioration of polymer substrates can rarely reach 100% because a small portion of the polymer will be incorporated

into microbial biomass, humus and other natural products (Atlas and Bartha, 1997; Narayan, 1993). This may be one of the reasons that the residual DOC accumulated in the effluents.

At the end of the experiment, the weight loss of the PCL in group C was highest among the three treatments (Fig. 5b). However, the NO_3^- -N removal rate and DOC in the effluents were lower than those of group A and group B. This may be because the released CO_2 from group C was greater than in the other two treatments because of the aeration activity.

2.5. pH and ALK in the effluent

The pH of the effluent of groups A and B was slightly higher than that of the influent. The pH of the effluent of group C was slightly lower than that of the influent (Fig. 6a). No significant difference was observed among the three treatments. However, the ALK of the effluents of the three treatments was significantly higher than those of the influent. The ALK in group A was lower than that of group B and group C (Fig. 6b).

Heterotrophic denitrification positively influenced the pH of the water (Lee and Rittmann, 2003). The pH decreased during the process of PCL degradation (Shah et al., 2008). The current experiment suggested that the PCL-denitrifying process can increase pH and ALK, especially the latter.

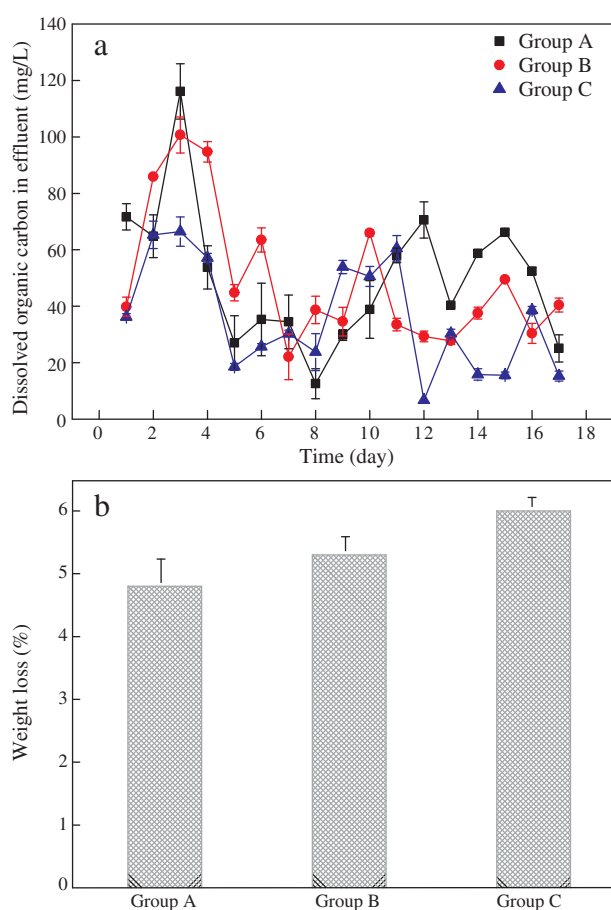


Fig. 5 – Dissolved organic carbon (DOC) in effluents of the PCL-denitrification reactors (a) and PCL weight loss (b) of the PCL granules in the denitrification reactors under different DO conditions.

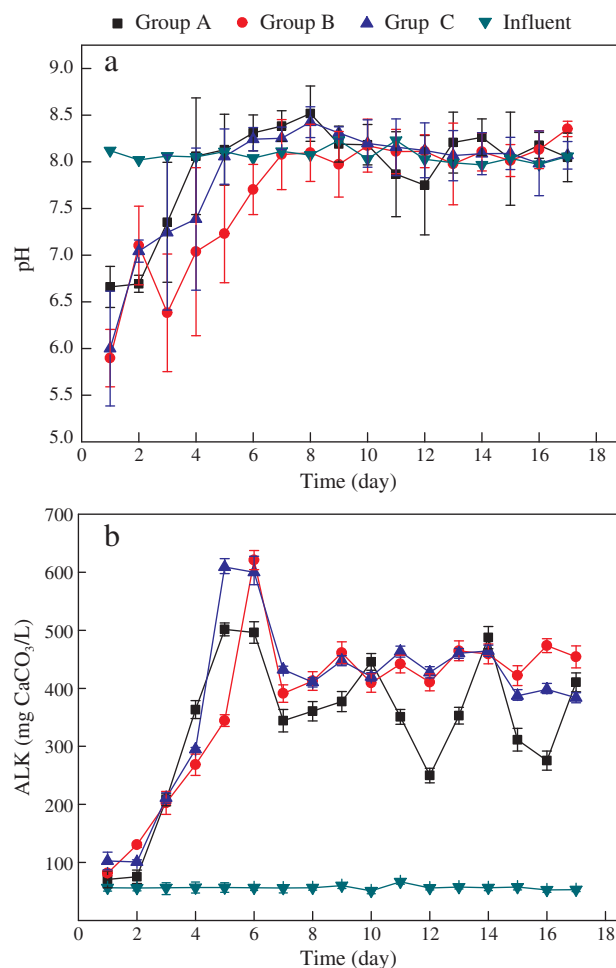


Fig. 6 – pH (a) and alkalinity (ALK) (b) of effluent during the batch experiment under different DO conditions.

3. Conclusions

The effect of dissolved oxygen (DO) levels on heterotrophic denitrification using polycaprolactone (PCL) as a carbon source and biofilm carrier was investigated. DO conditions in the influent of reactors were set up using an anoxic treatment group, a low-oxygen treatment DO group and an aerated treatment group. The levels of NO_3^- -N, NO_2^- -N, NH_4^+ -N, pH and alkalinity in the effluents of the three treatment reactors could meet the requirements of nitrifying reactors. There was no significant difference for both NO_3^- -N and TN removal efficiency among the three treatments. It was concluded that the inhibitory effects of DO concentrations lower than 6 mg/L can be mitigated in the PCL-denitrifying process.

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