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Effect of dissolved oxygen on denitrification using polycaprolactone as both the organic carbon source and the biofilm carrier

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ABSTRACT

Dissolved oxygen (DO) can inhibit the denitrifying process. Using polycaprolactone (PCL) as an organic carbon source and biofilm carrier (PCL-denitrifying process), an anoxic environment can be created to minimize the inhibition. This study investigates the effect of DO on the PCL-denitrifying process. Four DO conditions are established, ananoxic condition (group A, 0.44 ± 0.01 mg/L DO), a low DO condition (group B, 3.83 ± 0.22 mg/L DO), a moderate DO condition (group C, 6.12 ± 0.50 mg/L DO), and a high DO condition (group D, 10.45 ± 0.65 mg/L DO). This study demonstrates that the amount of released dissolved organic carbon is greater in the presence of DO compared to the anoxic condition. However, the amount of dissolved organic carbon released from PCL does not increase with an increase in DO. No significant differences in the nitrate nitrogen (NO_3^-–N) and total nitrogen (TN) removal efficiencies were between groups B and C. The NO_3^-–N and TN removal efficiencies in the group B and C were higher than those of group A and group D. The lowest NO_3^-–N and TN removal efficiencies were in the group D. These results indicate that the use of PCL as an organic carbon source and biofilm carrier can effectively minimize the inhibition of DO on the denitrifying process, although more than 10 mg/L DO in the water phase still inhibits this process to a certain degree.

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1. Introduction

Elevated NO_3^-–N concentrations in public water supplies have been causing eutrophication in many countries (Kim et al., 2015). High nitrate concentrations (up to 50 mg/L) can also affect the growth of commercially farmed fish (van Bussel et al., 2012). To alleviate this problem, researchers have been paying close attention to the process of efficient NO_3^-–N removal. Heterotrophic denitrification provides high selectivity for this process (Khardenavis et al., 2007).

Heterotrophic bacteria convert the NO_3^-–N supplied by the electron acceptor to N_2 during the heterotrophic denitrification process (Parka et al., 2015). Because oxygen is a more energy-favorable electron acceptor than nitrate, facultative heterotrophic denitrifiers use free oxygen as their electron acceptor when it is available (Feng et al., 2012). In addition to this competitive effect, oxygen inhibits nitrate reductase or nitrous oxide reductase, which can stop the enzymatic process, giving rise to a progressive reduction of molecular nitrogen production and the consequent accumulation of the different intermediates, principally nitrite, a highly toxic compound and nitrous oxide (Davies et al., 1989; Coyne and Tiedje, 1990; Fritsch and Saint-Blanquant, 1990). Many investigators had found that very low DO concentrations (0.02 mg/L) could cause a complete cessation of the denitrifying activity (Krul, 1976; Yang et al., 2012). Therefore, denitrification was believed to be a strictly anaerobic process (Tiedje, 1982; Hagedorn-Olsen et al., 1993; Chiu et al., 2007).

Oxygen is required for the survival of aerobic organisms. Generally, the dissolved oxygen should be maintained at above 6.0 mg/L, except for channel catfish, guppies, or eel, where 3.0–3.5 mg/L is acceptable (Timmons et al., 2002; Colt, 2006). To create an anaerobic environment for denitrification progress, in practice, aquacultural wastewater is pretreated to remove dissolved oxygen (DO) before it enters reactors. These pre-treatments inevitably increase the cost of wastewater treatment systems.

Heterotrophic denitrification processes require a sufficient organic carbon supply for nitrate removal (Her and Huang, 1995; Chiu and Chung, 2003). Methanol, ethanol, and acetic acid are

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commonly used to enhance the denitrification in carbon-poor effluents (Fajardo et al., 2012; Bill et al., 2009). The disadvantages of these soluble carbon sources are the need for constant supervision and the risks of insufficient doses or overdosing that can lead to the deterioration of the effluent quality (Müller et al., 1992). Recently, denitrification with biodegradable polymers (BDP-based denitrification) has been proposed to avoid the above-mentioned problems (Boley and Muller, 2005). BDPs were considered to be access only by enzymatic attacking, and the release of organic carbon from BDP_S was decided by the bacteria demand (Müller et al., 1992). It is likely that BDPs degradation in denitrification reactors is nearly equivalent to nitrate reduction under carbon-limited conditions, and the use of BDPs carries no potential risk of causing deterioration of the effluent quality by releasing dissolved organic carbon (Müller et al., 1992). Therefore, using BDPs can help to reduce the needed level of supervision and management (Boley et al., 2000; Chu and Wang, 2011; Shen et al., 2015).

Unlike a water-soluble carbon source, a solid carbon source must go through a degradation process to become water soluble so that it can supply the dissolved organic carbon that can be used by heterotrophic bacteria. The mechanism of PCL degradation has been described by Honda and Osawa (2002). The different degrees of the biodegradation are described as “Biodeterioration”, “Biodeterioration”, “Assimilation” and “Mineralisation” (Belal, 2003; Lucas et al., 2008). The term “biodegradation” indicates the predominance of biological activity in this phenomenon. DO is one of the most important factors that affect the efficiency of this degradation process. For instance, polyhydroxyalkanoates (PHB) have been demonstrated to be highly or completely degradable under anaerobic environments (Abou-Zeid et al., 2001)and aerobic conditions (Mergaert et al., 1994).

DO levels have a significant influence on the microbial community of biofilm in biological treatment systems (Feng et al., 2012). In BDP-based denitrifying processes, denitrifying microorganisms simultaneously use a bio-polymer as a biofilm carrier and a water-insoluble organic carbon source (Müller et al., 1992; Shen et al., 2013). The biofilm forms on the surface of the polymers, impeding the diffusion of oxygen and readily creating large anoxic zones above the pores or cracks of the BDPs (Gutierrez-Wing et al., 2012). Substantial biodegradation can be expected, primarily in an aerobic aquatic environment, and oxygen is consumed during this process (Marusincová et al., 2013). This lack of oxygen coupled with carbon storage deep within the granules should stimulate the heterotrophic denitrifying process (Gómez et al., 2002; Gutierrez-Wing et al., 2012). Therefore, the negative effect of DO on the BDP-based denitrifying process is expected to decrease.

In our recent study, we observed that the presence of DO with an average concentration of 5.2 mg/L in the influent did not inhibit the denitrifying performance (Luo et al., 2014). This finding led us to investigate the effect of a greater presence of DO. We added a treatment supplied with pure oxygen to the aqueous phase during the current experiment. The three other DO conditions in the aqueous phase were as follows: one treatment removed DO to simulate anoxic conditions that are encountered in the wastewater treatment process, one treatment did not apply any pretreatment process, and one treatment involved aeration to simulate a DO-rich situation, such as aquaculture water and drinking water. Polycaprolactone (PCL), a solid, non-water -soluble biopolymer can be used as an alternative self-regulating carbon source (Boley et al., 2000). We used PCL as the organic carbon source for denitrification to verify the universality of the results. Additionally, we investigated the amount of dissolved organic carbon (DOC) released from PCL and the microbial community structure under the four DO conditions to explain the primary mechanism.

2. Materials and methods

2.1. PCL

The PCL ($[C_4H_6O_2]_n$) used in the current study was supplied by Yisheng Material Ltd. Company (Shenzhen, China) and had an ellipsoid shape with dimensions of 2 mm × 3 mm × 4 mm (width × length × height). The main characteristics of the PCL are as follows: melting point, 60 °C; elongation at break, 800%; and heat-deflection temperature, 45 °C. Before use in this study, PCL granules were cleaned via an ultrasonic technique (0.1 kW, 40 kHz) and subsequently dried at 35 °C in a vacuum oven to a constant weight within ±0.0001 g.

2.2. DO supplying strategy

The batch experiments were conducted in triplicate in 1000-ml Erlenmeyer flasks at 25 ± 1 °C and 90 rpm in a shaking incubator (Tuhua HY-5, Shanghai, China). Four DO conditions were designed, including anoxic (group A), low DO (group B), moderate DO via aeration with air (group C), and high DO via the supply of pure oxygen (group D). For group A, gaseous nitrogen was added to the water, and the water surface was sealed to create an anoxic environment in the water and maintain the DO concentration at 0.44 ± 0.01 mg/L. For group B, the flasks were left open to air with no aeration and no sealing with wax, and the DO concentration was 3.83 ± 0.22 mg/L. For groups C and D, the DO concentrations were controlled via an air flow adjuster. For group C, the average DO concentration was maintained at 6.12 ± 0.50 mg/L with a fine-bubble diffuser at the bottom of the flask. Group D flasks were supplied with pure oxygen to maintain an average DO concentration of 10.45 ± 0.65 mg/L. The DO concentration in the every flask was measured before and after sampling to confirm the consistency of the DO concentration over the complete experimental period.

2.3. Determination of the DOC release under different DO conditions

First, each flask was filled with 60 ± 0.01 g of PCL pellets and 900 mL of synthetic wastewater (SW) to evaluate the amount of DOC released in the four groups from day 1 to day 22. The SW was prepared by adding 78 mg of K_2HPO_4, 31 mg of KH_2PO_4, 95 mg of $MgSO_4 \cdot 7H_2O$, and 37 mg of KCl to 1 L of degassed tap water and subsequently adding 0.2% (v/v) of a trace nutrient solution containing the following compounds: 640 mg of EDTA, 550 mg of $FeSO_4 \cdot 7H_2O$, 230 mg of $ZnSO_4 \cdot 7H_2O$, 340 mg of $MnSO_4 \cdot H_2O$, 75 mg of $CuSO_4 \cdot 5H_2O$, and 25 mg of $(NH_4)_5Mo_7O_{24} \cdot 4H_2O$. The SW in the flasks was replaced completely with fresh SW every 2 days, and the operation was completed within 30 s to avoid an error caused by the action of water sampling. The PCL pellets were not removed from the flasks for weighing to avoid disturbance of the DO conditions between the tests. The alkalinity (ALK), pH value, total ammonium nitrogen (TAN), nitrite nitrogen (NO_2^-–N), NO_3^- N, and DOC in the influent and effluent were monitored. On day 4 of the DOC release test, the PCL granules were sampled from the flasks for bacteria community analysis.

2.4. NO_3^-–N removal efficiency

At the end of the DOC release test, 100 mg/L NO_3^-–N (KNO_3:720 mg/L) was added to the SW to evaluate the NO_3^-–N removal rate over a period of 10 days. Next, the concentration of NO_3^-–N in the SW was increased to 200 mg/L (KNO_3: 1440 mg/L) for another 10 days to confirm this effect further. The water in the flasks was replaced with SW containing NO_3^-–N every 2 days. The

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