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Effect of dissolved oxygen on heterotrophic denitrification using poly(butylene succinate) as the carbon source and biofilm carrier

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HIGHLIGHTS

- Aeration with average DO of 5.2 mg L^{-1} could promote PBS-denitrification process.
- Pre-excluding DO in wastewater could be avoided using PBS as the carbon source.
- Aeration could accelerate the bio-degradation of PBS.

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ABSTRACT

The effect of dissolved oxygen (DO) on heterotrophic denitrification using poly(butylene succinate) as the carbon source and biofilm carrier was evaluated in a lab-scale experiment. Aerated, low-oxygen, and anoxic treatment groups were set up, which had average DO concentrations of 5.2 ± 1.0, 1.4 ± 1.2, and 0.5 ± 0.3 mg L^{-1}, respectively. The NO_3^--N and total nitrogen (TN) removal rates in the aerated group (37.44 ± 0.24 and 36.24 ± 0.48 g m^{-3} d^{-1}, respectively) were higher than those in the other two groups. There was no significant difference between the low-oxygen and anoxic groups for the NO_3^--N or TN removal rate. Accumulation of NO_2^--N reached 5.0 mg L^{-1} in the aerated group; no nitrite accumulation was found in the other two treatment groups. Bacterial communities of the low-oxygen and anoxic groups showed high similarity and were significantly different from those of the aerated group.

1. Introduction

Heterotrophic denitrification has been proven to be one of the most feasible processes for removing nitrate from wastewater and drinking water (Chu and Wang, 2011; Saliling et al., 2007). In this process, heterotrophic bacteria use organic carbon for both growth and as an electron source (Lee and Rittmann, 2003). In most wastewater, the amount of organic matter is much lower than required for the heterotrophic denitrification process; therefore, water-soluble organic sources, such as ethanol, glucose, acetate, and methanol, are added intentionally to support this process (Gómez et al., 2000). However, water-soluble organic sources often pose problems like overdosing, starvation periods, and increased dissolved organic carbon (DOC) in the effluent (Wu et al., 2013). An alternative is to use solid organic substrates such as inexpensive waste products (e.g., straw, wood, and bamboo fiber) from farms and forests (Lee and Wang, 2006) or biodegradable polymers (BDPs) (Boley et al., 2000; Wu et al., 2012).

BDPs can simultaneously serve as both an organic carbon source and biofilm carrier for denitrification and do not yield starvation periods or overdosing of the carbon source (Boley et al., 2000). 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; dissolved oxygen (DO) is one of the most important factors that affect the efficiency of this degradation process. For instance, polyhydroxyalkanoates have been demonstrated to be highly or completely degradable under anaerobic environments (Abou-Zeid et al., 2001) as well as under aerobic conditions (Mergaert et al., 1994).

Denitrification activity mainly occurs under anaerobic conditions. Intensive studies have found that even a very low DO concentration can cause complete cessation of heterotrophic denitrification (Oh and Silverstein, 1999; Yang et al., 2012). Moreover, a study by Mergaert et al. (2001) has shown that the denitrification process could not be initiated unless oxygen was depleted

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via continuous-upflow fixed-bed reactors when poly-β-hydroxybutyrate (PHB) was used as the carbon source for denitrification. Therefore, excluding DO by pretreatment always needs to be carried out before wastewater enters the denitrification reactor. This pretreatment can be achieved by the addition of a reagent such as Na_2SO_3 (Gómez et al., 2002) or by intentionally creating an anaerobic environment for denitrification through an ample residence time (Boley et al., 2000). Without a doubt, all of the above processes increase the system running cost.

Previous studies have demonstrated that an anaerobic environment can be created via biodegradation of a solid carbon source and respiration of microorganisms established on the solid granules (Boley et al., 2000). As the biofilm forms on the BDPs, diffusion of oxygen is impeded and large anoxic zones are created above the pores and cracks of the BDPs. The presence of an anoxic zone coupled with carbon storage in the depth of the granules stimulates the heterotrophic denitrification process (Gutierrez-Wing et al., 2012). Thus, with BDPs as the organic carbon source, the negative effect of oxygen on the denitrification process can be minimized. Nevertheless, information regarding how different DO levels affect denitrification using a solid carbon source is lacking and extremely needed. If the negative effect of oxygen on the denitrification process could be minimized with the use of BDPs as the organic carbon source, the cost of wastewater pretreatment would be significantly reduced.

The current study was carried out to evaluate how different DO levels affect heterotrophic denitrification in wastewater using poly(butylene succinate) (PBS), a novel biodegradable aliphatic polyester and an inexpensive BDP, as the carbon source and biofilm carrier. Three treatment groups were set up: aerated group, anoxic group, and low-oxygen group to determine whether DO is a factor that should be considered when BDPs are used as a carbon source for denitrification.

2. Methods

2.1. Biodegradable carriers

The physical characteristics of PBS (Anqing Hexing Chemical industry Co., Ltd., China) are as follows: density, 1.26 kg L^{-1}; melting point, 115 °C; crystallinity, 30–40%; tensile elongation, 600%; tensile breaking strength, 28 Mpa; and tensile yield strength, 35 Mpa. PBS has an ellipsoid shape with dimensions of 2 mm × 3 mm × 4 mm (width × length × height). The PBS granules were cleaned by the ultrasonic technique (0.1 kW, 40 kHz) and subsequently dried at 40 °C to a constant mass of ±0.0001 g in a vacuum oven.

2.2. Acclimation

The batch experiment was carried out in 500-mL Erlenmeyer flasks, each filled with 30 ± 0.01 g of PBS pellets and 400 mL of synthetic wastewater. The synthetic wastewater was prepared by adding 720 mg of KNO_3 (approximately 100 mg of NO_3^--N), 45 mg of Na_2HPO_4, 45 mg of KH_2PO_4, and 120 mg of NaCl to 1 L of tap water and 0.1% (v/v) of a trace nutrient solution containing the following compounds: 2.50 g L^{-1} $MgSO_4{\cdot}7H_2O$, 1.50 g L^{-1} $CaCl_2$, 0.28 g L^{-1} $FeSO_4{\cdot}7H_2O$, 0.13 g L^{-1} $MnCl_2{\cdot}4H_2O$, 0.12 g L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.004 g L^{-1} $CoCl_2{\cdot}6H_2O$, 0.003 g L^{-1} H_3BO_3, and 0.003 g L^{-1} $Na_2SeO_3{\cdot}5H_2O$. The flasks were kept in the dark at room temperature (30 ± 2 °C). Water in the flasks was changed weekly, and nitrate concentrations in the flasks were measured every 3 days. After 40 days of culture, the nitrate removal rate in the flask was stable and the biofilm was considered mature.

2.3. DO supplying strategy

After acclimation, 400 mL of synthetic wastewater was added into each flask. In order to maintain a relatively stable DO concentration in the flasks, static batch tests were set up. The experimental design included triplicate replications of the aerated, anoxic, and low-oxygen groups. The aerated group was aerated by an aerator (electromagnetic air pump, 135 W) with an air stone suspended in the upper layer of the liquid to avoid disturbance of the biofilm on the PBS. The water surface of the anoxic group was sealed with liquid paraffin to make an anoxic environment in the water. The low-oxygen group was left open to air with no aeration and no seal with wax. The supernatant was collected in triplicate from the flasks every 4 h to determine the oxidation–reduction potential (ORP), pH, and concentrations of DO, NO_3^--N, NO_2^--N, total nitrogen (TN), total ammonia nitrogen (TAN), and dissolved organic carbon (DOC). The flasks used for sampling were not used for the remainder of the experiment to avoid DO contamination in the flasks. At the end of the study, PBS granules in the last nine replicates were gathered for electron scanning experiments. The biofilm on the PBS surface was stripped for denaturing gradient gel electrophoresis (DGGE).

2.4. Analytical methods

2.4.1. Water samples

The DOC was measured using a Total Organic Carbon Analyzer (TOC-V.CPH, Shimadzu Seisakusho, Japan). The parameters DO, ORP, and pH were measured using a YSI 556 meter (YSI Incorporated 1725, Yellow Springs, OH, USA). TN, TAN, NO_2^--N, and NO_3^--N were analyzed spectrophotometrically according to standard methods (Eaton et al., 2005).

2.4.2. The NO_3^--N and TN removal rates

The NO_3^--N and TN removal rates of the three treatments during the experimental period were calculated based on the following equation:

$$E = 24\ (C_{in} - C_{en})/T$$

where E (g m^{-3} d^{-1}) was the removal rate of NO_3^--N or TN in the flask during the experimental period, and C_{in} (mg L^{-1}) and C_{en} (mg L^{-1}) were the concentrations of NO_3^--N or TN in the flasks at the beginning and end of the study, respectively. T was the duration of the study in hours. If the NO_3^--N or TN was eliminated before the study finished, then C_{en} would be zero and T would be the time when NO_3^--N or TN was completely removed.

2.4.3. Scanning electron microscopy (SEM)

Fresh PBS and the PBS granules removed from the last sampling were immediately fixed in 9 mL of 2.5% glutaraldehyde for subsequent SEM analysis. Fixed PBS granules were dehydrated by equilibration for 10 min each in a sequence of aqueous acetone solutions (30%, 50%, 70%, 90%, and 100% acetone; two equilibrations were performed in 100% acetone solutions. The PBS beads were critical-point dried, fixed onto aluminum stubs, sputter-coated with gold–palladium, and examined using a scanning electron microscope (JSM-5600LV, JEOL Ltd., Japan).

2.5. DNA extraction and polymerase chain reaction (PCR) amplification

2.5.1. DNA extraction and purification

The biofilm attached to the PBS was removed by ultrasonic treatment (0.1 kW, Ultrasonic cleaner KQ2200E, Kunshan Ultrasonic Instrument Co., Ltd., China) for 30 min at a frequency of 50 kHz. The biofilm samples were filtered through 0.22-μm