Effect of dissolved oxygen on biological denitrification using biodegradable plastic as the carbon source

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Abstract. Biological denitrification is currently a common approach to remove nitrate from wastewater. This study was conducted to evaluate the influence of dissolved oxygen on denitrification in wastewater treatment using biodegradable plastic as carbon source by designing the aerated, anoxic, and low-oxygen experimental treatment groups. The results showed that the removal rates of nitrate in anoxic and low-oxygen experimental treatment groups were 30.6 g NO\textsubscript{3}-N m\textsuperscript{-3} d\textsuperscript{-1} and 30.8 g NO\textsubscript{3}-N m\textsuperscript{-3} d\textsuperscript{-1} at 83 h, respectively, both of which were higher than that of the aerated group. There was no significant difference between the anoxic and low-oxygen treatment groups for the nitrate removal. Additionally, the nitrite accumulated during the experiments, and the nitrite concentrations in anoxic and aerated groups were lower than those in low-oxygen group. No nitrite was detected in all groups at the end of the experiments. These findings indicated that dissolved oxygen has important influence on denitrification, and anoxic and low-oxygen conditions can support completely denitrification when using BP as carbon source in nitrate-polluted wastewater treatment.

1. Introduction

Nitrate contamination of water resources has been reported worldwide. Potential sources of nitrate in water resources include agricultural fertilizers, atmospheric deposition, and irrigation with domestic wastewater [1-4]. A high concentration of nitrate in drinking water poses a risk to public health since it can lead directly to blue baby syndrome and may play a role in the development of some cancers [5, 6]. So it is important to develop efficient treatment processes for nitrate-polluted wastewater in order to avoid nitrate pollution in water resources. Biological denitrification is one of the most feasible processes for removing nitrate from wastewater because of its efficiency, moderate cost and environmentally sounded nature [7]. The denitrification efficiency is influenced by many factors including organic carbon, dissolved oxygen (DO), temperature, and phosphorus [8-10]. Denitrification produces less energy yield than oxygen respiration [11]. Therefore, denitrifying bacteria in aerobic conditions will choose to use oxygen as terminal electron acceptor. Consequently, denitrification mainly occurs under anaerobic conditions, and previous studies have found that completely denitrification can occur in a very low DO concentration [12,13]. Gómez et al. [14] has found that the presence of oxygen decreased the removal efficiency of inorganic nitrogen and caused an increase of nitrite concentration in the treated water. Shao et al. [15] also found that when the concentration of DO
in the influent was greater than 4.2 mg/L, the presence of oxygen decreased the removal efficiency of nitrate in a reactor using rice husk as the carbon source for water denitrification. Therefore, reducing DO by pretreatment always needs to be carried out before wastewater enters the denitrification systems [14]. However, all the pretreatment processes of wastewater increase the system running cost.

Denitrification occurs naturally when denitrifying bacteria use nitrate as a terminal electron acceptor in their respiration process. Many researchers have found that the presence of an organic carbon source is needed in denitrification system because the amount of organic matter in most wastewater is much lower than required for the denitrification process [9]. A great deal of attention has recently been focused on solid carbon sources, such as sawdust and synthesized biodegradable polymers [7,9]. Biodegradable plastic (BP), which are easily degraded by microorganisms, are commonly used worldwide. Studies have shown that reducing conditions can be created via biodegradation of BP that can develop and sustain an anaerobic-reductive environment conducive to denitrification [16, 17]. Thus, the negative effect of oxygen on the denitrification can be minimized when BP are used as carbon source. If the negative effect of oxygen on the denitrification process could be minimized with the use of BP as the carbon source, the cost of wastewater pretreatment would be significantly reduced. However, information regarding how different DO levels affect denitrification using a solid carbon source is lacking.

In this study, the influence of DO on biological denitrification was assessed by designing the aerated, anoxic, and low-oxygen groups when using BP as the carbon source.

2. Materials and methods

2.1. Chemicals
Chemicals used in this study were of analytical reagent grade. Biodegradable plastic (φ 2.0 mm x 5.0 mm) was selected as a potential carbon source for denitrifying bacteria. Biodegradable plastic was composed of 60% starch and 30% polypropylene. In this study, synthetic nitrate-polluted wastewater used in the experiment was prepared by spiking tap water with an appropriate mass of NaNO₃ to achieve a nitrate concentration of 110 mg NO₃⁻-N/L. Freshly prepared synthetic nitrate-polluted water had a pH of 6.5–7.5. Sludge was obtained from the Shiyang Wastewater Treatment Plant, Wuhan, China. The suspended solids (SS) and volatile suspended solids (VSS) were 1960 mg/L and 1375 mg/L, respectively.

2.2. Denitrification experiments
The experimental design included the aerated, anoxic, and low-oxygen groups. Prior to the experiments, BP was washed with distilled water and allowed to dry naturally. 10 g BP and 300 ml of synthetic nitrate-polluted wastewater were added to three 500 ml Erlenmeyer flasks, after which the flasks were sterilized in a pressure cooker for 15 minutes. Then, 10 ml of sludge was added to the flasks. One flask was prepared for the aerated group, which was aerated by an aerator with an air stone suspended in the upper layer of the liquid, with the DO concentration was 6.8 mg/L at the beginning of the experiment. The other flask were purged for 20 minutes with high purity nitrogen to lower DO concentration (final DO < 1.0 mg/L), and then were sealed with glass stopper. The low-oxygen group was left open to air with no aeration and no glass stopper, with the DO concentration was 4.8 mg/L at the beginning of the experiment. The experiments were conducted at room temperature (30±2℃).

Supernatants were taken from each flask at regular intervals. The supernatants were analyzed for NO₃⁻-N, NO₂⁻-N, and NH₄⁺-N. Triplicate analyses were carried out for each sample. The results of the parallel experiments were within the allowable range, and the arithmetic mean was used as the final result.

2.3. Analytical techniques
Electrodes were used to measure the pH (UB-7, Denver Instrument, USA) and DO (320D-02A, Thermo Electron Corporation). NO₃⁻-N, NO₂⁻-N, NH₄⁺-N were measured according to the Water and
Wastewater Monitoring Analysis Method [18]. Before analysis, all samples were filtered using 0.45μm membrane filters.

3. Results and discussion

3.1. Environmental parameters
The pH of all the flasks remained neutral over time, with values fluctuating between 6.5 and 7.8. Dissolved oxygen in the aerated and anoxic groups fluctuated from 6.5mg/L to 7.2 mg/L and below 1.0 mg/L during the experiments, respectively. At the beginning of the study, the DO concentration in the low-oxygen group was 4.8mg/L, and then it was rapidly reduced to almost the same level as in the anoxic group after 75h. This result might be due to the activity of the BP-degrading bacteria, and the DO in the low-oxygen group were used up by the respiration of these bacteria. The fast consumption of DO by the BP-degrading bacteria indicated the negative effect of oxygen on the denitrification process could be minimized when using BP as the carbon source.

3.2. Nitrate removal
The nitrate concentrations in the supernatants of the flasks are presented in Figure 1. The nitrate removal efficiency varied with the difference of the DO in the flasks. At the beginning of the experiments, the removal of nitrate was not obvious, and the maximum nitrate removal efficiency was only 9.3%. Afterwards, the nitrate removal efficiency was gradually increased in all flasks. For anoxic group, the nitrate concentrations decreased from the initial 110 mg NO$_3^-$-N/L to 4.2 mg NO$_3^-$-N/L within 83 hours. The removal rate of nitrate was 30.6 g NO$_3^-$-N m$^{-3}$ d$^{-1}$. Afterwards, the nitrate removal efficiency in the anoxic group was maintained at greater than 98% until the end of the experiment. For low-oxygen group, high nitrate removal efficiency was also observed. The nitrate concentrations decreased from the initial 110 mg NO$_3^-$-N/L to 3.4 mgNO$_3^-$-N/L at 83 h, and the nitrate removal efficiency was 97%. The removal rate of nitrate in low-oxygen group was 30.8 g NO$_3^-$-N m$^{-3}$ d$^{-1}$, which was almost the same level as in the anoxic group. In contrast, the denitrification rate proceed in aerated group was low, and the nitrate concentrations decreased to 38.8mg NO$_3^-$-N/L within 83 hours. The nitrate removal efficiency was only 65%, and the removal rate of nitrate was 20.6 g NO$_3^-$-N m$^{-3}$ d$^{-1}$, which were lower than those in the anoxic and low-oxygen groups. Thereafter, the nitrate concentrations were not completely removed until 120 h in aerated group.

From these results, it is reasonable to conclude that anoxic and low-oxygen conditions were all favorable for denitrification when using BP as carbon source, and the presence of DO in low-oxygen didn’t limited the denitrification process. Many investigators had found that only very low DO concentrations could cause complete cessation of denitrifying activity [14,19]. In this study, low-oxygen conditions can also support completely denitrification, so pretreatment of wastewater with a DO concentration of about 4.8 mg/L could possibly be avoided when BP is used as the carbon source. And when the DO concentration in wastewater is higher, DO concentrations only need to be reduced to below 4.8mg/L by pretreatment, thus the cost of pretreatment will be greatly reduced. However, Luo et al. [20] found the NO$_3^-$-N and total nitrogen (TN) removal rates in the aerated group were higher than those in the low-oxygen and anoxic groups. They thought denitrifying bacteria, the heterogeneity of the microenvironments on the poly(butylene succinate) (PBS) carrier, and the PBS biodegradation process may cause this difference.
3.3. The accumulation of nitrite and ammonium

Nitrite, the intermediate product of nitrate reduction, is undesirable because it is more toxic to human health and ecosystem functioning than nitrate. From Figure 2, it is clear that the nitrite accumulated significantly during the experiments. The nitrite accumulation in the flasks was lower than 0.05 mgNO$_2$-$N$/L in 16 hours; afterwards, there was an abrupt increase in nitrite accumulation, from the initial low levels to 1.47 mg NO$_3$-$N$/L for anoxic group, and 1.49mgNO$_2$-$N$/L for the aerated group. In addition, the nitrite concentrations in low-oxygen group were higher than those in anoxic and aerated groups, with the value of 4.36mgNO$_2$-$N$/L at 40 h. The concentrations of nitrite then rapidly decreased, with the concentrations in all flasks falling below 0.10 mg NO$_2$-$N$/L by day 83, after which they continued to decrease gradually, with no nitrite being detected at the end of the experiments.

Previous studies found that many factors can influence the accumulation of nitrite [21-23]. DO is one of the most important factors affecting the accumulation of nitrite during the denitrification process. By inhibiting the enzymatic processes of nitrate reductase and nitrous oxide reductase, oxygen can cause accumulation of different intermediates such as nitrite [14]. In this study, although significant accumulation of nitrite was observed during the experiments, no nitrite was detected at the end of the experiments. So nitrate can be reduced completely in all flasks, and dissolved oxygen did not cause nitrite accumulation when using BP as carbon source.

Likewise, some amounts of accumulated ammonium were observed in this experiment, with the maximum ammonium concentration being 0.64 mgNH$_4$-$N$/L in the aerated group, 0.99 mgNH$_4$-$N$/L in the low-oxygen group, and 0.99 mgNH$_4$-$N$/L in the anoxic group (Figure 3). The formation of ammonium suggested that dissimilatory nitrate reduction to ammonium (DNRA) was occurring.
DNRA is a further anaerobic reduction reaction that competes with denitrification and converts nitrate to ammonium rather than N₂ [24]. As shown in Figure 3, the concentration of ammonium in all flasks were very low relative to the initial nitrate concentration, indicating that the principal mechanism of nitrate removal in the flasks might be denitrification, with DNRA only contributing a minor role. From hour 34 onwards, the ammonium concentration in the aerated group were lower than those in the low-oxygen and anoxic groups. This primarily occurred probably because DO is adverse to DNRA, which resulted in decreased ammonium accumulation [25, 26].

![Figure 3. Concentrations of NH₄⁺-N of supernatants during the three experiments (aerated, low-oxygen, and anoxic treatment groups).](image)

### 4. Conclusions

In this study, the nitrate concentrations in anoxic and low-oxygen groups decreased from the initial 110 mg NO₃⁻-N/L to 4.2 mg NO₃⁻-N/L and 3.4 mg NO₃⁻-N/L within 83 hours, with the removal rate of nitrate was 30.6 g NO₃⁻-N m⁻³ d⁻¹ and 30.8 g NO₃⁻-N m⁻³ d⁻¹, respectively, both of which were higher than that of the aerated group. It can be concluded that anoxic and low-oxygen conditions were all favorable for denitrification when using BP as carbon source, and the presence of DO in low-oxygen didn’t limited the denitrification process. So pretreatment of wastewater with a DO concentration of about 4.8 mg/L could possibly be avoided when BP is used as the carbon source; thus the cost of pretreatment will be greatly reduced. Additional, although the nitrite accumulated during the experiments, no nitrite was detected at the end of the experiments. So nitrate can be reduced completely in all flasks, and dissolved oxygen did not cause nitrite accumulation when using BP as carbon source. Similarly, some amounts of accumulated ammonium were observed in this experiment, which suggested that DNRA was occurring in this study.

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