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Simultaneous nitrification and denitrification by EPSs in aerobic granular sludge enhanced nitrogen removal of ammonium-nitrogen-rich wastewater

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HIGHLIGHTS

- Nitrification and denitrification take place simultaneously.
- Nitrogen in different forms was present in EPSs and underwent dynamic changes.
- Biosorption affected the results of batch experiments.
- *Nitrosomonas eutropha* and heterotrophic denitrifiers were identified of the AGS.

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ABSTRACT

In this study, role of extracellular polymeric substances (EPSs) in enhancing nitrogen-removal from ammonium-nitrogen-rich wastewater using aerobic granular sludge (AGS) technology were analyzed. AGS enabled ammonium oxidation and denitrification to occur simultaneously. Air stripping and simultaneous nitrification–denitrification contributed to total-nitrogen removal. Clone-library analysis revealed that close relatives of *Nitrosomonas eutropha* and heterotrophic denitrifiers were dominant in the AGS, whereas anammox bacteria were not detected. EPSs adsorption of ammonium, nitrite, and nitrate nitrogen results in improved removal of nitrogen in batch experiments.

1. Introduction

Aerobic granular sludge (AGS) is a promising wastewater-treatment technology that has many advantages, including good sludge settleability, high processing efficiency, and powerful resistance to impact load. Long sludge age promotes growth and reproduction of microorganisms with long generation time, such as nitrifying bacteria, to improve the removal efficiency of ammonium nitrogen. AGS is utilized from the initial treatment of organic wastewater to the treatment of ammonium-nitrogen-rich wastewater and poisonous and harmful substances (Adav and Lee, 2008). The hierarchical structure of granular sludge allows the AGS to remove organic matter and ammonium nitrogen. Moreover, the dissolved oxygen diffusion restriction allows the granular sludge to form anoxic and anaerobic zones. The anoxic condition inside creates the conditions for simultaneous nitrification and denitrification process (Gao et al., 2011). Thus, with the coexistence of nitrifying and denitrifying bacteria, simultaneous nitrification and denitrification is possible (Yang et al., 2003). Large granular sludge particle size is favorable for simultaneous nitrification and denitrification (Wang et al., 2007; Shi et al., 2011). Although simultaneous nitrification–denitrification has been observed (Wang et al., 2007; Shi et al., 2011; Wei et al., 2014; Yan et al., 2014), further study is needed to investigate the elimination of nitrogen in the processing system.

Batch experiments are often used to analyze microbial substrate degradation ability (Wang et al., 2007; Shi et al., 2011), such as the denitrification of denitrifying bacteria. However, adsorption is often ignored in this process. Neglecting ammonium adsorption

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can lead to underestimations of at least 10–25% of ammonium available for nitrification (Lin et al., 2012). Experiments on ammonium nitrogen adsorption by active sludge, biological membrane, and granular sludge have been carried out. Temmink et al. (2001) studied the adsorption of ammonium nitrogen by biological membrane. Schwitalla et al. (2007) examined the adsorption ability of ammonium nitrogen by activated sludge floc and found that the adsorption capacity of ammonium nitrogen by activated sludge floc was between 0.07 and 0.20 mg of NH_4^+-N/(g/VSS). Bassin et al. (2011) studied the adsorption capacity of ammonium nitrogen and the desorbing ability by AGS based on the small and middle test. However, during the study of ammonium nitrogen as wastewater treatment, and rarely described ammonium nitrogen adsorption process in the operation of bioreactor. Bassin et al. (2011) found that the adsorbed ammonium nitrogen will dissolve back into liquid. As a microbial cell secretion, EPSs contain abundant charged groups (e.g., –COOH, –NH, –OH, –CO–). These groups can react with cations (metal and ammonium ions) and anions (phosphate radical) in wastewater by electrostatic reaction and ion exchange reaction. EPSs of activated sludge can adsorb metal ions in wastewater (D'Abzac et al., 2010; Sheng et al., 2013; Wang et al., 2014; Yan et al., 2015), and its function in phosphorus removal system was analyzed (Lin et al., 2012; Chen et al., 2015; Wang et al., 2015). However, knowledge about how EPSs affect the ammonium nitrogen content in biological treatment systems is very limited (Lin et al., 2012; Chen et al., 2015). The adsorption characteristics of ammonium nitrogen by AGS have been studied, and EPSs are believed to play an important role in ammonium nitrogen adsorption (Bassin et al., 2011). Extended aeration was used to eliminate ammonium nitrogen absorbed by granular sludge by desorption and oxidation. However, Bassin et al. (2012) did not conduct a comprehensive analysis of the ammonium nitrogen, especially nitrite nitrogen and nitrate nitrogen in EPSs.

The adsorbed materials react with the functional groups of EPSs, completing the process of adsorption. If the pollutant content of the EPSs in the reactor sludge can be measured, the adsorption process of the sludge would be understood. The contribution of adsorption to the pollutant removal helps to accurately reflect the microbial activity and kinetics process. Hence, this study was conducted to evaluate the possible improvement of nitrogen removal from ammonium-nitrogen-rich wastewater using AGS technology incorporating EPSs.

2. Methods

2.1. Reactor set-up and operation

A SBR reactor connected by two organic glass cylinders was used. The diameter and height of the bottom cylinder was 80 mm and 645 mm, respectively, whereas the diameter and height of the upper cylinder was 100 mm and 50 mm, respectively. The height of the middle connection was 100 mm, the effective volume was 5.6 L, exchange volume ratio was 50%, and the operating temperature was room temperature. Each stage was controlled by a time controller and operated six cycles every day, with each cycle involving 5 min of inflow, 180 min of aeration, 5 min of sedimentation, 2 min of drainage, and was left unused for 48 min. Air flow volume was 0.22 m^3/h through an aeration diffuser at the bottom of the reactor. The apparent air velocity was 1.22 cm/s (lower cylinder as the standard).

The sludge in the experiment was a shortcut nitrification granular sludge cultured in the laboratory, and the mass with particle size greater than 0.5 mm accounted for 81.37% of the total mass of AGS. Characteristics and particle size distribution of AGS are shown in Table 1.

Table 1
Characteristics and particle size distribution of AGS.

Sludge properties	Numerical value	Particle size (mm)	Percentage mass content (%)
MLSS (mg/L)	7865.08 ± 21.25	>1.7	14.21
VSS (mg/L)	6370.65 ± 9.32	1.4–1.7	21.21
SVI	30.57 ± 1.53	1.0–1.4	39.96
		0.85–1.0	0.24
		0.5–0.85	5.75
		<0.5	18.63

2.2. Experimental wastewater

Artificial wastewater was used as the experimental water, with glucose and sodium acetate as the carbon source and ammonium chloride as the nitrogen source. The specifics were as follows: COD, 200–350 mg/L; NH_4^+-N, 250–330 mg/L; pH, 7.4–8.3; $CaCl_2{\cdot}2H_2O$, 10 mg/L; $MgSO_4{\cdot}7H_2O$, 20 mg/L, 0.5 ml/L of trace elements were added to the wastewater as described by Yan et al. (2014).

2.3. Air stripping experiment

The air stripping process was conducted in the reactor. First, the reactor was emptied of mud mixture and washed with water to eliminate the influence of the biofilm adhering to the reactor wall on the test results. Then, the experimental sewage was pumped into the reactor as the gas flow was controlled at the normal operating conditions of the reactor. The inflow-water properties were as follows: pH 8.60, ammonium nitrogen concentration of 313.31 mg/L, and temperature of 20 °C.

2.4. Biological denitrifying batch experiments

Biological denitrification has two ways: heterotrophic denitrification and anammox. Before batch experiments, mixture samples were taken out of the reactor 2 min before the end of the aerobic reaction cycle. The supernatant was filtered through 0.45 μm cellulose and washed with deionized water once to eliminate the influence of sewage residue on the experimental results. Then it preserved for further use.

For the heterotrophic biological denitrification process, the granular sludge and the artificial wastewater (pH was 7.35, COD concentration was 447.60 mg/L, ammonium nitrogen concentration was 4.48 mg/L, nitrite nitrogen concentration was 103.97 mg/L, nitrate nitrogen concentration was 27.64 mg/L) were placed in a 1.0 L beaker to conduct a blocking test. The mixed liquid suspended solids (MLSS) was 6397 mg/L.

For the anammox procedure, the granular sludge and the artificial wastewater (pH was 7.60, ammonium nitrogen concentration was 137.20 mg/L, nitrite nitrogen concentration was 107.83 mg/L, nitrate nitrogen concentration was 27.46 mg/L) were placed in a 1.0 L beaker to conduct a blocking test. The MLSS was 6061 mg/L.

To prevent granular sludge settling and promote contact between sludge and sewage, a magnetic stirrer was placed in the beaker. The contents of ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen in wastewater and EPSs were sampled and measured.

2.5. Analytical procedure

The analysis of ammonium, nitrite, nitrate nitrogen, and MLSS was performed in accordance with the standard methods (APHA, 1998). UV absorbance was measured with a TU-1810 spectrophotometer (Puxitongyong Co., Beijing, China). The measurement of