



Achievement, performance and characteristics of microbial products in a partial nitrification sequencing batch reactor as a pretreatment for anaerobic ammonium oxidation



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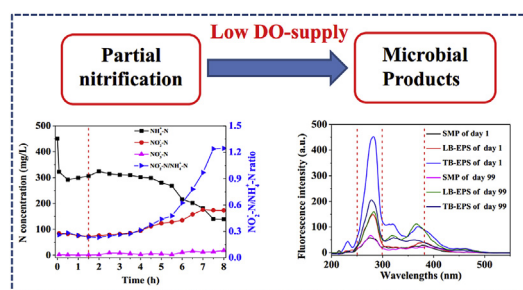
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HIGHLIGHTS

- Partial nitrification SBR was successfully achieved under low dissolved oxygen condition.
- PN and PS contents of EPS and SMP were reduced during the achievement of partial nitrification.
- Chemical components of EPS and SMP were assessed by 3D-EEM and synchronous fluorescence spectra.
- FA was responsible for inhibiting the activity of NOB.

GRAPHICAL ABSTRACT



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ABSTRACT

This study was carried out to evaluate achievement, performance and characteristics of microbial products in a partial nitrification sequencing batch reactor as a pretreatment for anaerobic ammonium oxidation (anammox). After 100 days long-term operation, the effluent NO₂⁻-N/NH₄⁺-N ratio of the reactor was average at 1.3 and NO₃⁻-N concentration was low by controlling low dissolved oxygen (DO) concentration, which was considered as the ideal influent for anammox. Specific oxygen uptake rate (SOUR) implied that (SOUR)_{NH₄} and (SOUR)_{NO₂} of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in sludge changed from 21.73 ± 0.52 and 27.39 ± 0.50 O₂/g SS/h to 36.37 ± 0.85 and 12.04 ± 0.17 O₂/g SS/h, respectively. The main compositions of extracellular polymeric substances (EPS) and soluble microbial products (SMP), including proteins (PN) and polysaccharides (PS), were both reduced during the achievement of partial nitrification. Three-dimensional excitation-emission matrix (3D-EEM) and synchronous fluorescence spectra revealed that PN-like, fulvic acid-like and humic acid-like substances were identified in both EPS and SMP, and their fluorescence intensities changed significantly after partial nitrification achievement. It was found from typical cycle that free ammonia (FA) may play a significant role on inhibiting the activity of NOB. The obtained results could provide more information on the performance of partial nitrification through the characteristics of microbial products when treating high ammonium wastewater.

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1. Introduction

Nitrogen-rich wastewater is generated from many different pathways, including pharmaceutical effluent, fertilizer waste, urban solid waste, pig farms waste, sludge dewatering and landfill leachate etc. (Buss et al., 2004). Traditional biological nitrogen removal (BNR) process applied in wastewater treatment plants (WWTP) mainly includes two phases: the conversion of NH_4^+-N to NO_3^--N by aerobic nitrification bacteria and subsequent NO_3^--N to nitrogen gas by anoxic denitrification bacteria (Carrera et al., 2003). In recent years, many novel BNR processes have been developed, such as simultaneous nitrification and denitrification (SND), membrane bioreactor (MBR) and anaerobic ammonium oxidation (anammox) etc. (Ni et al., 2009; Wang et al., 2010; Lee et al., 2013; Kong et al., 2014). Among them, anammox has been considered as one of the most promising technologies due to its significant advantages, including no oxygen demand, low sludge production and no organic carbon demand (Wu et al., 2015).

Partial nitrification, as an important preliminary process for anammox, plays an important role in supplying a suitable influent (50% NH_4^+-N to NO_2^--N) to feed an anammox reactor (Zhang et al., 2011a). The main influencing factors to achieve partial nitrification include dissolved oxygen (DO), solid retention time (SRT), temperature, free ammonia (FA) and free nitrous acid (FNA) etc. (Wang et al., 2014c). The key strategy to achieve partial nitrification is inhibition of nitrite oxidizing bacteria (NOB) and enrichment of ammonia oxidizing bacteria (AOB). Since the oxygen saturation coefficients of AOB and NOB are 0.3 and 1.1 mg/L, respectively, and therefore NOB could be washed out under low DO concentration (Ciudad et al., 2005). Till now, many literature have been well reported by using the strategy of low DO condition (Wiesmann, 1994). Sinha and Annachhatre (2007) successfully achieved the effluent ratio of nitrite to ammonium around 1.0 through adjusting DO in the range of 0.3–0.5 mg/L. Guo et al. (2013) reported nitrite accumulation in the limited filamentous bulking (LFB) process at low DO supply (0.5–1.0 mg/L) in a lab-scale anoxic/oxic (A/O) system.

Although DO is a key operation parameter for the achievement and maintenance of partial nitrification system, especially treating high ammonium wastewater, it is evident that low DO concentration largely affects the amount and activity of microorganisms. As two kinds of important microbial products relating to microbial activity, extracellular polymeric substances (EPS) and soluble microbial products (SMP) are mainly composed by proteins (PN), polysaccharides (PS), humic acid, nucleic acids and lipids etc. (Shaw et al., 2002). Wang et al. (2014a) reported that PS was the main component of EPS in the partial nitrification system and EPS were beneficial to improve the process of partial nitrification. Moreover, EPS have a dynamic multilayered structure consisting of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) (Liang et al., 2010; Ye et al., 2011). It has been well reported that two types of EPS fractions played different roles in the partial nitrification process, LB-EPS fraction were more significant than TB-EPS fraction in the process of initiating a partial nitrification process under salinity stress (Zhang et al., 2011). SMP are usually regarded as the group of soluble organic compounds that are originated from cell lysis and hydrolysis, substrate metabolism and biomass decay in wastewater treatment process (Kunacheva and Stuckey, 2014). SMP have a significant effect on formation of disinfection by-products in WWTP and degrade the quality of effluents (Urrea et al., 2016). Ng et al. (2006) found that the release of EPS into the solution that would be further hydrolyzed into SMP. During the achievement of partial nitrification process, component and structural characteristics of EPS and SMP may influence the performance and effluent of reactor. However, little information about the characteristics of

EPS and SMP in the partial nitrification formation process could be found.

Based on the above discussion, the aim of this study was to: (1) achieve partial nitrification in a sequencing batch reactor (SBR) through low DO supply treating high ammonium wastewater; (2) quantitative analysis the contents of EPS and SMP during the achievement of partial nitrification; (3) evaluate the variations of component of EPS and SMP by using fluorescence spectra. The obtained results could provide further information in the development of partial nitrification process through the changes of microbial products.

2. Materials and methods

2.1. SBR operation

Fig. S1 shows the experimental column-type SBR, which is conducted with the internal diameter and height of 12 and 30 cm, respectively. The SBR was operated in a cycle of 8 h, consisting of influent (5 min), anoxic phase (85 min), aeration (360 min), settling (20 min), idling and effluent (10 min). Effluent was drawn from the middle port of the column-type reactor with a volumetric exchange ratio of 50%, causing the hydraulic retention time (HRT) was 16 h. The air was supported by using an aeration pump with a constant airflow of 1 L/min, resulting in the DO was about 1 mg/L. The SBR was performed at room temperature (25 °C).

2.2. Synthetic wastewater and seed sludge

The synthetic wastewater was used as influent in the reactor with the following compositions: 400 mg/L of COD (chemical oxygen demand, as CH_3COONa); 400–500 mg/L of NH_4^+-N (as NH_4Cl); 30 mg/L of P (as K_2HPO_4); 40 mg/L of CaCl_2 ; 20 mg/L $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$ of; 20 mg/L of $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ and 1.0 mg/L of trace element solution. The composition of the trace element solution derived from the previous literature (Tay et al., 2002) was as follows: H_3BO_3 0.05 g/L, ZnCl_2 0.05 g/L, CuCl_2 0.03 g/L, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.05 g/L, $(\text{NH}_4)_6\text{MoO}_{24} \cdot 4\text{H}_2\text{O}$ 0.05 g/L, AlCl_3 0.05 g/L, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.05 g/L, NiCl_2 0.05 g/L.

The pH value of influent wastewater was adjusted to 8.0 by using NaHCO_3 and HCl. Seed sludge was obtained from a laboratory SBR which has an effective volume of 17 L that running for over 1 year. The initial mixed liquor suspended solids (MLSS) of SBR was set as about 3.0 g/L.

2.3. EPS and SMP extractions

EPS and SMP were collected by using a modified extraction method as described previously (Xu et al., 2016). Briefly, 50 mL of sludge suspension was firstly centrifuged at 6000 rpm for 5 min. The supernatant was regarded as SMP. Then, the sludge pellet left in the centrifuge tube was resuspended into 50 mL of 0.05% NaCl solution (pre-heated to 70 °C). The sludge suspension was centrifuged at 6000 rpm for 10 min, and the organic matter in the supernatant was LB-EPS. The remaining sludge pellet in the centrifuge tube was resuspended into 50 mL of 0.05% NaCl solution and heated (60 °C) in a water bath for 30 min. Lastly, the suspension was centrifuged at 6000 rpm for 15 min to obtain TB-EPS.

2.4. Specific oxygen uptake rate

The measurement of specific oxygen uptake rate (SOUR) for AOB and NOB was determined as described by Shi et al. (2009). Briefly, 100 mL sludge sample (3 g/L) was first taken from the partial nitrification SBR at the end of aeration process, and washed three times by using deionized water. The substrate solution concentrations of

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