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Ammonia removal and microbial characteristics of partial nitrification in biofilm and activated sludge treating low strength sewage at low temperature

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ABSTRACT

In this study, the ammonia removal and stability of partial nitrification (PN) were investigated in an activated sludge system (R1) and a biofilm system (R2). The two systems were operated with identical setup and seeded with same sludge, to treat the sewage with ammonia of 60–65 mg L^{-1} at 14–16 °C. R1 performed a higher ammonia removal ability but a lower stability, while R2 showed a contrary result. In R1, both ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) showed higher bioactivity, AOB and *Nitrospira* presented lower biodiversity while *Nitrobacter* showed higher result than those of R2. FISH results indicated the lower proportion of AOB in R2, and relative low proportion of NOB in both the two systems. Activated sludge system should be operated as half PN or treat high ammonia sewage under low DO, while biofilm system could run as totally PN or treat low ammonia sewage with high DO.

1. Introduction

Nowadays, many wastewaters do not contain sufficient amounts of biodegradable carbon, making them less suitable for nitrogen removal via the nitrification–denitrification process. Moreover, with the development of anaerobic treatment process, most organic compounds in wastewater are converted to biogas, which is feasible with the present state of the art (Kartal et al., 2010). The autotrophic nitrogen removal process which based on partial nitrification (PN) and anaerobic ammonia oxidation (Anammox) could remove nitrogen from wastewater with no organic carbon consumption, which has attracted increasing attention because of its ability to achieve high nitrogen removal rate with less energy consumption (Ni and Zhang, 2013). So it could be an alternative technology for treating the sewage with low ratio of COD to nitrogen (C/N) (Bagchi et al., 2012; Li et al., 2015).

The autotrophic nitrogen removal technology include two process types, PN—Anammox (two stages, as shown in Eqs. (1) and (2)) (Strous et al., 1998) and completely autotrophic nitrogen removal over nitrite (CANON) (single stage, as shown in Eq. (3)) (Sliekers et al., 2002). Since the occurrence of Anammox requires a certain proportion of nitrite nitrogen and ammonia nitrogen of 1.32 in the feed solution, part of ammonia in the wastewater should be oxidized to nitrite for the uptake by Anammox bacteria. Thus, the PN process which undertakes the oxidation of ammonia to nitrite is critical and indispensable for the autotrophic nitrogen removal from low C/N sewage.

$$1NH_4^+ + 1.5O_2 \rightarrow 1NO_2^- + H^+ + 1.0H_2O \quad (1)$$

$$1NH_4^+ + 1.32NO_2^- + 0.13H^+ \rightarrow 0.26NO_3^- + 1.02N_2 + 2.03H_2O \quad (2)$$

$$1NH_3 + 0.85O_2 \rightarrow 0.11NO_3^- + 0.44N_2 + 0.14H^+ + 1.43H_2O \quad (3)$$

In PN process, the oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB) should be enhanced while the oxidation of nitrite to nitrate by nitrite-oxidizing bacteria (NOB) should be inhibited since Anammox bacteria consumes only ammonia and nitrite. In consequence, a prominent and stable PN requires enrich-

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ment of AOB and inhibition of NOB (Ganigue et al., 2012; Kartal et al., 2010), to achieve high ammonia removal efficiency (ARE) and nitrite accumulation rate (NAR). It didn't seem quite so difficult to achieve high-rate and stable PN when treating sewage with high temperature or high ammonia in side-stream such as sludge digestion and landfill leachate with (Gabarro et al., 2012; López-Palau et al., 2011; Van Hulle et al., 2007). However, there were still some challenges in PN process for treating the mainstream sewage with low ammonia or low temperature such as the influent of municipal plant. On one hand, the oxidation rate of ammonia by AOB is severely affected by temperature (Kim et al., 2008), the lower temperature would lead to lower ARE. Moreover, the higher activation energy of AOB than NOB resulted in the difficulty of nitrite accumulation under low temperature, and many studies have reported failure during winter temperatures (Hendrickx et al., 2012; Hu et al., 2013; Vazquez-Padin et al., 2011). On the other hand, the lower ammonia concentration would also impose restrictions on PN process, since the lower free ammonia (FA) could not perform effective suppression on NOB (Vadivelu et al., 2007). In conclusion, the low temperature and ammonia concentration would not only limit the efficiency, but also affect the stability of PN, so the low ammonia sewage treated system could not reach the effluent demand in winter temperature (Gilbert et al., 2014; Hu et al., 2013). The operation of municipal plant in winter was one problem that could not be ignored during the application of PN, thus it was essential to gain more information about the performance and microbial characteristics of this process under low temperature.

Generally, there are two kinds of wastewater treated systems, including activated sludge system and biofilm system, in which the organisms respectively survived in suspended and fixed condition. Activated sludge system could achieve high removal loading since its better mass transfer, which could be flexibly operated and controlled. For biofilm system, it enables a larger biodiversity of the microorganisms due to its long SRT, and has shown great potentials for PN as a result of sufficient oxygen usage and well stratified distributions of AOB and NOB within the biofilm. In previous studies, PN reactors have been conventionally operated as activated sludge system, whereas it has been confirmed that biofilm PN processes with attached biomass also have advantages (Rodriguez-Sanchez et al., 2014). Both the two systems could be effectively used for PN process, but it was doubtless that the suitable operational condition could be different between each other. However, no study has been done simultaneously in both the two systems and the specific suitable strategies for each system were still not clear, let alone for treating sewage in main-stream.

The research purpose of this study was to find the suitable operational strategies for different PN systems treating low ammonia sewage at low temperature. Two identical reactors were simultaneously adopted, in which one was operated as activated sludge system and another as biofilm system. The ammonia removal and nitrite accumulation performance were comparative studied between the two reactors, and the microbial communities and distributions of functional organisms in the two systems were determined using DGGE, clone-sequencing and FISH techniques.

2. Materials and methods

2.1. Experimental setup

Two reactors (R1 and R2) with identical setup were used to investigate the PN performance and microbial community in this study. The two reactors were both made of polymethyl methacrylate, with effective volume of 6 L (diameter: 15 cm, height: 50 cm), as shown in Fig. 1. R1 was operated as completely mixed activated sludge system, and R2 was run as biofilm system packing with polyurethane stuffing as biofilm carrier. The strips of carrier were hung along the reactor, with the two ends respectively fixed to the top and bottom of the reactor. The seeded sludge used in this study was conventional activated sludge taken from a municipal plant, with the mixed liquor suspended solids of 7.1 g L^{-1} and mixed liquor volatile suspended solids of 5 g L^{-1}. The sludge was firstly pre-acclimatized with the ammonia of 200 mg L^{-1} in a SBR system for one week. Then 3 L of this pretreated sludge was simultaneously seeded to R1 and R2 after cleaning by tap water and distilled water for three times, to eliminate the dissolved impurities. The whole experiment was conducted without heat supply, and the temperature in the two reactors was both around 14–16 °C. R1 and R2 were both daily operated for four cycles and each cycle contained: feeding, 5 min; reacting with aeration, 4 h; settling, 0.5 h; draining, 5 min. The operational conditions of the two reactors were totally same: DO in the initial of each cycle was 0.3 mg L^{-1}, pH was 7.8, and the ratio of the draining to feeding was 83.3%.

The synthetic wastewater used in this study contained $(NH_4)_2SO_4$ and $NaHCO_3$ as main substrate, together with small amount of KH_2PO_4, $CaCl_2$, $MgSO_4{\cdot}7H_2O$ and trace element solution. The ammonia nitrogen concentration during the whole experiment was 60–65 mg L^{-1}, to stimulate the influent of municipal plant.

2.2. Analytical methods

According to the standard methods (APHA, 1995), the concentrations of NH_4^+ and NO_2^- in influent and effluent were daily measured using colorimetric methods in a visible spectrophotometer, and the concentrations of NO_3^- was daily analyzed using ultraviolet spectrophotometric method in an ultraviolet spectrophotometer. The temperature, pH and DO were detected using online instruments (WTW, Germany). ARE and NAR were calculated as Eqs. (4) and (5), respectively.

$$\mathrm{ARE} = \frac{[\mathrm{NH_4^+}]_{Inf.} - [\mathrm{NH_4^+}]_{Eff.}}{[\mathrm{NH_4^+}]_{Inf.}} \times 100\% \quad (4)$$

$$\mathrm{NAR} = \frac{[\mathrm{NO_2^-} - \mathrm{N}]}{[\mathrm{NO_2^-} - \mathrm{N}] + [\mathrm{NO_3^-} - \mathrm{N}]} \times 100\% \quad (5)$$

2.3. DNA extraction, PCR-DGGE, cloning and sequencing

Some mixed liquor was collected from R1, while biofilm was taken from R2 at the end of the experiment on day 60, and then stored in 10 mL sterile plastic test tubes for DNA extraction. DNA was extracted using a bacterial genomic mini extraction kit (Sangon, China) and was detected by 0.8% (w/v) agarose gel electrophoresis.

To amplify 16S rDNA fragments of *β-Proteobacteria* AOB for DGGE, primers CTO189fA/B and CTO189fC of a 2:1 ratio, together with the reverse primer CTO654r were used for the first round PCR, following by 1 s round using universal primer set F338 (with GC-clamp)/R518 (Kowalchuk et al., 1997; Muyzer et al., 1993). In addition, primers F1nxrA (with GC-clamp)/R2nxrA were used to amplify 16S rDNA fragments of *Nitrobacter*-like NOB (Attard et al., 2010), while Primers 27F/705R (with GC-clamp) were used for the PCR of *Nitrospira*-like NOB (Freitag et al., 2005). Thermocycling was performed in PCR Thermal Cycler Dice (TakaRa, Japan), the conditions were summarized in Table 1. Then the qualified PCR production were separated on 8% polyacrylamide gels with a 30%–60% linear gradient of denaturant (100% denaturant = 7 M urea plus 40% formamide). The gel was conducted at 60 °C, 120 V for 5 h (AOB) or 7 h (NOB) on Dcode Universal Mutation Detection System (Bio-Rad). After electrophoresis, the gel was stained using silver-staining method followed by taking photos on Gel Doc XR 192 system (Bio-