Bioresource Technology 169 (2014) 103–109

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microbial characteristics and nitrogen removal of simultaneous partial nitrification, anammox and denitrification (SNAD) process treating low C/N ratio sewage

Yuhai Liang [a], Dong Li [a,*], Xiaojing Zhang [b], Huiping Zeng [a], Zhuo Yang [a], Jie Zhang [a,b]

[a] Key Laboratory of Water Quality Science and Water Environment Recovery Engineering, Beijing University of Technology, Beijing 100124, China
[b] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

HIGHLIGHTS

- A start-up strategy for SNAD treating low C/N ratio sewage was proposed.
- The system achieved to simultaneous high-rate nitrogen and COD removal.
- *Nitrosomonas* and *Candidatus brocadia* were detected as predominant organisms.
- COD presence led to biodiversity increasing of AerAOB and decreasing of AnAOB.
- Dominant organisms in different phases were related to *Proteobacteria* and *Planctomycete*.

ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form 15 June 2014
Accepted 18 June 2014
Available online 27 June 2014

Keywords:
CANON
SNAD
AerAOB
AnAOB
Denitrifying

ABSTRACT

Simultaneous partial nitrification, anammox and denitrification (SNAD) process was successfully realized for treating low C/N ratio sewage, nitrogen and COD removal achieved to 3.26 kg m^{-3} d^{-1}, 81%, respectively. The nitrogen removal performance, microbial community and distribution of the functional microorganisms were investigated. Results suggested that the presence of COD performed activity inhibition on both aerobic ammonia-oxidizing bacteria (AerAOB) and anaerobic ammonia-oxidizing bacteria (AnAOB), and led to the number decreasing of both AerAOB and AnAOB. Even though COD presence resulted in the biodiversity increasing of AerAOB and decreasing of AnAOB, the dominant species were always *Nitrosomonas* and *Candidatus brocadia* during the whole experiment. Clone-sequencing of 16S rRNA results suggested the emergence of five different denitrifying species, which then led to a higher nitrogen removal. Results in this study demonstrated that the applied start-up strategy was feasible for SNAD process treating low C/N ratio sewage.

1. Introduction

In conventional nitrification–denitrification nitrogen removal processes, denitrifying bacteria utilize organic substrate as electron donor to reduce nitrate to nitrogen gas. However, 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 (Kartal et al., 2010), which leads to the lack of organic carbon available for denitrification and hence a large excess of nitrogen in effluent. Completely autotrophic nitrogen removal over nitrite (CANON) process which combines anammox with partial nitrification in one single unit could remove nitrogen with no organic carbon consumption and less operational costs (Bagchi et al., 2012; Sliekers et al., 2002; Third et al., 2001). Thus, the combination of anaerobic biogas production process with subsequent CANON process would be a better choice for wastewater treatment, which is feasible with the present state of the art (Zhang et al., 2013b).

CANON process has been successfully adopted to treat wastewater with no organic carbon in several systems as previously reported, which mainly focused on the nitrogen removal and microbial ecology (Liu et al., 2012; Sliekers et al., 2003; Vazquez-Padin et al., 2009; Zhang et al., 2013a,b). In CANON process, 89% of ammonia is converted to nitrogen gas by the cooperative work of aerobic ammonia-oxidizing bacteria (AerAOB) and anaerobic ammonia-oxidizing bacteria (AnAOB), leaving 11% of nitrogen as

* Corresponding author. Tel.: +86 18501276008.
E-mail addresses: lidong2006@bjut.edu.cn, bjut_lidong@163.com (D. Li).

http://dx.doi.org/10.1016/j.biortech.2014.06.064
0960-8524/

nitrate as shown in Eq. (1)(Strous et al., 1998). Thus, CANON process could not arrive a 100% removal of nitrogen. Additionally, organic compounds co-exist in most nitrogen wastewaters, and the biogas producing process could not remove the organic carbon totally, which thus result in a wastewater with low C/N ratio as influent for subsequent CANON process. The presence of organic carbon can destabilize the CANON process, which was regarded as the other challenge for CANON besides of the limiting nitrogen removal efficiency. Alternatively, the development of simultaneous partial nitrification, anammox and denitrification (SNAD) process, which combines CANON process and denitrification into one single reactor (Chen et al., 2009) can facilitate the simultaneous nitrogen and carbon removal, makes it possible to resolve the two challenges in CANON process simultaneously (Kumar and Lin, 2010).

$$1NH_3 + 0.85O_2 \rightarrow 0.11NO_3^- + 0.445N_2 + 0.14H^+ + 1.43H_2O \quad (1)$$

In SNAD process, most nitrogen is removed by CANON, and heterotrophic denitrification degrades the excess nitrate as well as the organic carbon (Lan et al., 2011). Therefore, the application of CANON and denitrification processes in the degradation of real wastewater that contains low organic carbon and high nitrogen would become a promising technology of biological nutrient removal processes (Kumar and Lin, 2010). Besides of application in the mainstream, adoption of SNAD for treating sidestream wastewater could also significantly improve the final effluent quality, since the sidestream sewage would bring 25% nitrogen loading to the mainstream (Ahn and Choi, 2006). Several studies about SNAD process have been carried out, all of which were focused on the start-up strategy or the nitrogen removal pathway (Chen et al., 2009; Daverey et al., 2013; Keluskar et al., 2013; Lan et al., 2011). Few study has been done about the microbial community and population dynamics about the functional organisms in SNAD system, including AerAOB, AnAOB and denitrifiers. Although anammox was first identified in a denitrification reactor (Mulder et al., 1995), the interaction of anammox organisms with denitrifiers and the role of organic compounds in CANON process are still unclear. Given that the presence of organic compounds would lead to either the competition of AnAOB and heterotrophic denitrifiers, or the fluctuation of DO due to the organic oxidation, certain species would be affected. Thus, the information related with microbial ecology in SNAD reactor is essential for a better understanding about the performance of the system, which, may further lead to the development of SNAD process.

In addition, previous study about SNAD process were all conducted in SBR system, the feasibility of biofilm reactor for SNAD was still unclear, let alone the corresponding microbial characteristics in the system. Therefore, the present study was aimed to (1) develop a SNAD process in a laboratory scale biofilm reactor using synthetic wastewater with low C/N ratio, and (2) investigate the microbiology of CANON and denitrification with and without addition of glucose, using DGGE, FISH and clone-sequencing techniques.

2. Methods

2.1. Experimental setup

The reactor used in this study was an up-flow biofilter packing with volcanic rock as biofilm carrier, which was made of polymethyl methacrylate. The particle size of the volcanic rock was 4–6 mm. The entire reactor with an effective volume of 2.65 L (diameter: 90 mm, height: 1000 mm, loading height: 850 mm) was placed in a water bath to ensure a constant reaction temperature (25 °C). The schematic diagram of the biofilter was shown in Fig. 1. The synthetic wastewater used in this study contained

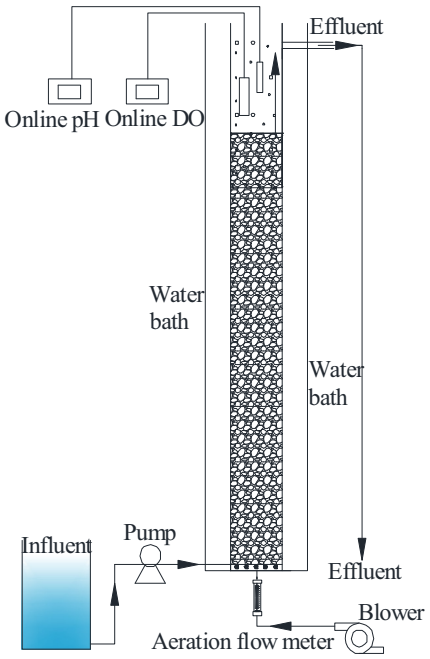

Fig. 1. Schematic diagram of the biofilter for SNAD process.

$(NH_4)_2SO_4$ and $NaHCO_3$ as main substrates, together with a small amount of KH_2PO_4, $CaCl_2$, $MgSO_4{\cdot}7H_2O$ and trace element solution (1 mL L^{-1}) (deGraaf et al., 1996). The influent ammonia was kept at 200 mg L^{-1} and the aeration rate was 4.5 L min^{-1} constantly throughout the whole experiment.

The whole experiment was conducted as two phases. In phase I, the reactor was operated as CANON process with no organic substrate feeding; In phase II from day 201, glucose was added to the synthetic wastewater with an influent C/N ratio of 0.2, to start-up SNAD process. Biofilm samples were obtained from the reactor on day 198 and 262, when the reactor got stable operation as CANON and SNAD process respectively, for DGGE, clone-sequencing and FISH analysis. The main operational conditions and the corresponding performance of the reactor, including ammonia removal rate (ARR), nitrogen removal rate (NRR), TN removal efficiency (TRE) and COD removal efficiency (CRE) were summarized in Table 1.

2.2. Analytical methods

Concentrations of NH_4^+, NO_2^- and NO_3^- in influent and effluent were daily measured according to Standard Methods (APHA, 1995). The temperature, DO and pH were detected using online instruments (WTW, Germany). COD was detected by 5B-1digestion instrument.

2.3. DNA extraction and PCR-DGGE

Some pieces of volcanic filter were collected and stored in 50 mL sterile plastic test tubes at −20 °C when the reactor was in stable operation as CANON and SNAD, respectively. Biofilm was removed from the volcanic filter with sterile brush and collected into 10 mL sterile plastic test tube. DNA was extracted using a bacterial genomic mini extraction kit (Sangon, China) and was detected by 0.8% (w/v) agarose gel electrophoresis.

All the primers and the corresponding PCR conditions used in this study were summarized in Table 2. To amplify 16S rDNA fragments of *β-Proteobacteria* AerAOB for DGGE analysis, primers