Bioresource Technology 244 (2017) 33–39

Contents lists available at ScienceDirect

Bioresource Technology

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

Effect of glucose on nitrogen removal and microbial community in anammox-denitrification system

Yujie Qin[a,b,c,d,*], Yan Cao[a], Junyi Ren[a], Tongyu Wang[a], Bin Han[a]

[a] School of Environment and Energy, South China University of Technology, Guangzhou Higher Education Mega Centre, Guangzhou 510006, PR China
[b] The Key Lab of Pollution Control and Ecosystem Restoration in Industry Clusters, Ministry of Education, South China University of Technology, Guangzhou Higher Education Mega Centre, Guangzhou 510006, PR China
[c] Guangdong Provincial Engineering and Technology Research Centre for Environmental Risk Prevention and Emergency Disposal, South China University of Technology, Guangzhou Higher Education Mega Centre, Guangzhou 510006, PR China
[d] Guangdong Provincial Key Laboratory of Atmospheric Environment and Pollution Control, South China University of Technology, Guangzhou Higher Education Mega Centre, Guangzhou 510006, PR China

ARTICLE INFO

Keywords:
Anammox
Glucose
Denitrification
High-throughput sequencing
Microbial communities

ABSTRACT

The effect of glucose on nitrogen removal and microbial communities in the anammox-denitrification systems was investigated. The optimal nitrogen removal could be achieved when the influent glucose concentration was 56.4 mg L^{-1}. The influent nitrite to ammonium ratio of 0.95–1.40 would not obviously affect the nitrogen removal due to the coexistence of anammox, denitrification and partial denitrification.

The anammox activity was deteriorated with increasing glucose concentration. When the influent glucose concentration was increased to 374.9 mg L^{-1}, the average ammonium removal efficiency decreased from 97% to around 10% and anammox activity was seriously inhibited. The anammox activity quickly recovered with decreasing influent glucose and increasing influent nitrite. High-throughput sequencing analysis suggested that the predominant genus changed from *Candidatus Kuenenia* to *Diaphorobacter* with the addition of glucose and then changed to *Hydrogenophaga* with the decrease of glucose. It indicated that organics concentration had an effect on the microbial communities.

1. Introduction

Anaerobic ammonium oxidation (anammox) has been regarded as a cost-effective and energy-saving way for nitrogen removal since discovered in a denitrifying bioreactor (Mulder et al., 1995). Compared with the traditional nitrification-denitrification, anammox can directly oxidize ammonium to N_2 using nitrite as an electron acceptor without the presence of organic carbon source and oxygen (Strous et al., 1998). Therefore, anammox process can save 60% aeration, 100% organic carbon and reduce 90% sludge production (Bagchi et al., 2012). However, concerning a stable anammox reactor, the ratio of nitrite to ammonium in influent is strictly required to maintain a high nitrogen removal efficiency (Strous et al., 1998). Theoretical maximum TN removal efficiency was 89% in an anammox process due to nitrate production (Xie et al., 2017). The stoichiometric ratios of nitrite consumption to ammonium consumption and nitrate production to ammonium consumption could indicate the nitrogen removal efficiency of an anammox process (Sun et al., 2011).

Combined with denitrification, anammox-denitrification process can firstly reduce nitrate produced in an anammox process to nitrite, and then to N_2. The nitrite produced from partial denitrification can be served as an electron acceptor for anammox under the lack of nitrite. Simultaneous anammox and denitrification process is relatively insensitive to the ratio of nitrite to ammonium in influent. Besides, this process can improve TN removal efficiency because denitrifiers can reduce nitrate or nitrite to N_2 using organic carbon as an electron donor (Ahn et al., 2004). However, anammox bacteria would be negatively affected by organic carbon because of the competition for nitrite between anammox bacteria and denitrifiers (Meolinuevo et al., 2009; Ni et al., 2012; Chen et al., 2016). For example, the anammox activity was completely inhibited and denitrification became the main nitrogen removal pathway when the influent COD concentration was increased to 290 mg L^{-1} (Meolinuevo et al., 2009). The denitrification prevailed over anammox when the ratio of COD/N was 4 (Ni et al., 2012). The ammonium removal efficiency dropped to 4% and denitrification became dominant at the influent COD concentration of 284 mg L^{-1} (Chen et al., 2016). It showed that high organic matter concentration had a negative influence on anammox (Cao et al., 2016). Therefore, it is

* Corresponding author at: School of Environment and Energy, South China University of Technology, Guangzhou Higher Education Mega Centre, Guangzhou 510006, PR China.
E-mail address: qinyujie@scut.edu.cn (Y. Qin).

http://dx.doi.org/10.1016/j.biortech.2017.07.124
Received 29 May 2017; Received in revised form 18 July 2017; Accepted 21 July 2017
Available online 23 July 2017
0960-8524/

necessary to investigate a range of organic matter concentration in favor of anammox bacteria and denitrifiers coexisting well. In this study, glucose was selected as organic carbon because glucose had little effect on anammox bacteria and could be easily decomposed by many bacteria (Zhong and Jia, 2013).

The microbial communities are related to nitrogen removal efficiency in an anammox reactor. Previous studies have reported that 16S rRNA gene sequences have been used to analyze microbial communities in various biological reactors, such as sequencing batch reactor (SBR), upflow anaerobic sludge blanket (UASB) and expand granular sludge bed (EGSB) (Qin and Zhou, 2009; Liao et al., 2013; liang et al., 2014). The high-throughput sequencing provides a chance of understanding the relationship between the microbial communities and nitrogen removal. In this study, the microbial communities in the upflow blanket filter (UBF) reactor was analyzed by Illumina Hisep sequencing. The UBF reactor was regarded as the suitable candidates to carry out anammox because it could promote the retention of slowly growing biomass (Jin et al., 2008).

The main objectives of this study are (1) to evaluate the nitrogen removal of the anammox-denitrification system under different glucose concentrations; (2) to achieve the simultaneous partial denitrification, anammox and denitrification in the anammox-denitrification system; (3) to gain a system overview of the microbial communities in the UBF reactor and a comprehensive insight of the relationship of the functional bacteria involved in nitrogen removal.

2. Materials and methods

2.1. Experimental setup

Two identical UBF reactors (R1 and R2) made of plexiglas were adopted in this study. The effective volume and inner diameter were 2.1 L and 0.08 m, respectively. The schematic diagram of the UBF reactor system was depicted in Fig. 1. Anammox sludge (1000 mL) was added to R1. Anammox sludge (800 mL) and denitrifying sludge (200 mL) were added to R2. The mixed liquor volatile suspended solids (MLVSS) of the anammox sludge was 15.2 g L^{-1}, and that of the denitrifying sludge was 17.1 g L^{-1}. The MLVSS ratio of anammox sludge to denitrifying sludge was approximately 0.89:1. The mixed liquor suspended solids (MLSS) of the anammox sludge was 27 g L^{-1}, and that of the denitrifying sludge was 29 g L^{-1}. The anammox sludge was taken from an anammox UBF reactor, which had been operated for more than 8 months. The denitrifying sludge was obtained from an anoxic pond of Lijiao Sewage Treatment Plant in Guangzhou.

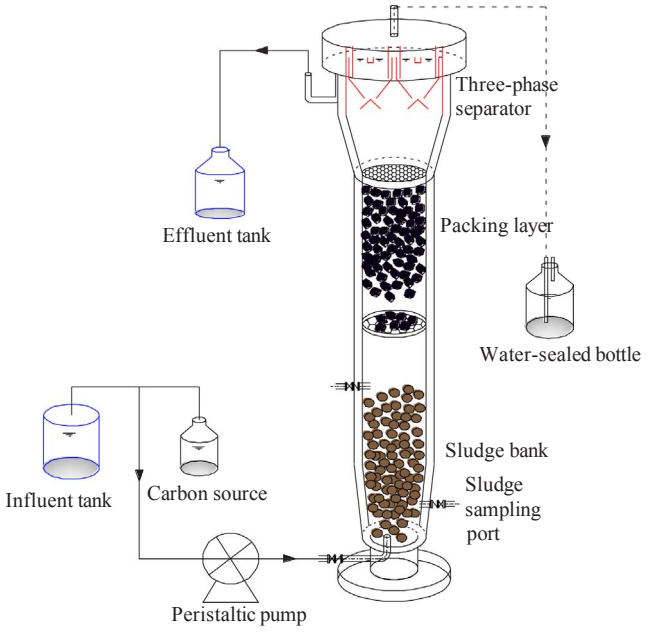

Fig. 1. Schematic diagram of the UBF reactor system.

2.2. Synthetic wastewater and operation

Synthetic wastewater was composed of NH_4Cl, $NaNO_2$ and glucose as the main source of ammonium, nitrite and organic carbon, respectively. The concentrations of ammonium, nitrite and organic carbon were changed according to the requirement of experiment. The other components included $NaHCO_3$ (1000 mg L^{-1}), $MgSO4{\cdot}7H_2O$ (473 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (180 mg L^{-1}), KH_2PO_4 (27 mg L^{-1}), trace element solution I (1 mL L^{-1}) and trace elements solution II (1 mL L^{-1}). The trace element solution I contained EDTA (5000 mg L^{-1}) and $FeSO_4{\cdot}7H_2O$ (5000 mg L^{-1}). The trace elements solution II contained $ZnSO_4{\cdot}7H_2O$ (430 mg L^{-1}), $GuSO_5{\cdot}5H_2O$ (240 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (990 mg L^{-1}), $NiCl_2{\cdot}6H_2O$ (190 mg L^{-1}) and $CoCl_2{\cdot}6H_2O$ (24 mg L^{-1}) (Qin et al., 2017).

The reactor was operated at room temperature (16–27 °C). The hydraulic retention time (HRT) was adjusted at approximately 12 h and pH was maintained in the range of 7.5–8.0. The operation was divided into seven phases. During the first six phases, the ammonium and nitrite concentrations in influent were 82.7–107.5 mg L^{-1} and 90.8–120.6 mg L^{-1}, respectively. The glucose concentration was increased from 0 in phase 1 to 18.8, 56.4, 112.8, 224.6 and 374.3 mg L^{-1} in phases 2–6, respectively. In phase 7, the glucose concentration was reduced to 56.4 mg L^{-1}. The ammonium and nitrite concentrations were 96.0–105.8 mg L^{-1} and 129.2–141.3 mg L^{-1}, respectively.

2.3. Analytic methods

2.3.1. Wastewater quality analysis

The influent and effluent water samples were analyzed for ammonium, nitrite and nitrate according to the standard methods (APHA, 2012). MLVSS was determined by weight method. TN concentration was the sum of ammonium, nitrite and nitrate concentration.

2.3.2. DNA extraction, PCR and high-throughput sequencing

The sludge samples were collected on days 7, 68 and 79 in R1 and R2, respectively, which were named A1, B1 and C1 in R1 and A2, B2 and C2 in R2, respectively. DNA was extracted using the Powersoil DNA isolation kit (MiBio Laboratories, Inc., USA) according to the manufacturer's instruction. 16S rRNA genes segments were PCR-amplified using forward primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) for the V4 region. The PCR reaction mixture contained 10 × Ex Taq Buffer (6 μL), dNTP (6 μL), BSA (0.6 μL), Ex Tap (0.3 μL), Primer F (1.2 μL), Primer R (1.2 μL), DNA (1 μL) and ddH_2O (43.7 μL). The PCR thermal program was performed at 94 °C for 5 min; 31 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min. The PCR products were examined on agarose gels. Sequences of amplification were carried out on Illumina Hisep PE250. Sequence data have been deposited in the NCBI Sequence Read Archive (SRA) database with accession number SRP110646.

3. Results and discussion

3.1. Performance of nitrogen removal

Fig. 2 shows the nitrogen removal performance of R1. In phase 1 (days 1–7, without organic matter), the average removal efficiencies of ammonium, nitrite and TN were 99.3%, 99.6% and 92.3%, respectively. In phases 2–3 (days 8–34), when the glucose concentration was increased from 18.8 mg L^{-1} to 56.4 mg L^{-1}, the TN removal efficiency increased slightly from 95.3% to 96.7% with the effluent nitrate decreasing from 8.7 mg L^{-1} to 4.5 mg L^{-1}. It indicated that the nitrate