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Effects of different carbon sources and C/N ratios on the simultaneous anammox and denitrification process

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ABSTRACT

The simultaneous anammox and denitrification (SAD) process is an emerging treatment for wastewaters containing nitrogen pollution and organics. However, there is little research on the effect of organic matter types on the SAD process. In this study, two reactors, one with glucose (R1) and the other with acetate (R2), were used to investigate the effects of different carbon sources and C/N ratios on the SAD process. The nitrogen removal performance, functional gene abundance, biomass activity and microbial community structures of the two reactors were analyzed and compared. Results showed that a desirable nitrogen removal efficiency (NRE) was obtained at the C/N ratio of 1.2 in R1, whereas a similar NRE could be achieved at a C/N ratio of 0.8 in R2, which was more susceptible to a high C/N ratio. Moreover, at high C/N ratios, the microbial species diversity of R1 was higher than that of R2. High-throughput sequencing analysis revealed that anammox bacteria predominated at low COD, but denitrifying bacteria grew rapidly at high C/N ratio. Removal of NO_3^--N in the glucose-driven reactor appeared to be more depended on other heterotrophic bacteria other than denitrification bacteria. In general, the glucose-driven reactor was more stable and resistant to high C/N ratios during the SAD process than the acetate-driven one. Results of this study provide information for practical treatment of wastewater with high nitrogen contents.

1. Introduction

Since anaerobic ammonium oxidation (anammox) was discovered in the 1990s, it has been regarded as a promising process for autotrophic nitrogen removal because no external carbon source is needed, there is a lower oxygen demand and decreased sludge production (Jetten et al., 2001; Meng et al., 2017). The anammox process involves the oxidation of ammonium (NH_4^+) to nitrogen gas (N_2), with nitrite (NO_2^-) as the electron acceptor. Compared to the conventional nitrification-denitrification process for nitrogen removal, anammox is a more sustainable and economical alternative, especially for the treatment of wastewater with a low C/N ratio (Jaroszynski et al., 2012).

In practice, wastewater from many sources, such as livestock operations, waste leachate, and hair product industries, have both high concentrations of nitrogen and chemical oxygen demand (COD), meaning that it is difficult for a single anammox process to effectively remediate the waste. Many studies have tried to combine the anammox process with other treatments to improve removal efficiency (Anjali and Sabumon, 2017; Chen et al., 2017). Currently there is growing interest in studying the simultaneous anammox and denitrification (SAD) process (Li et al., 2016; Takekawa et al., 2014). This combined process is highly efficient and cost effective because denitrifying bacteria utilize COD in wastewater as the electron donor and reduce the anammox byproduct (NO_3^-) to N_2. Previous studies have investigated the influence of various reaction conditions, such as salinity (Liu et al., 2009) and the C/N ratio (Bi et al., 2015), on the anammox/denitrification combined process.

It is known that high concentrations of organic carbon inhibit anammox activity, but the reason for this is complicated. There are two proposed mechanisms for the inhibition of anammox by organic carbon, namely “out-competition inhibition” and “metabolic pathway conversion inhibition” (Jin et al., 2012) In the first proposed mechanism, heterotrophic bacteria compete with anammox bacteria for space and nutrients under high COD concentrations; however, the heterotrophic bacteria grow faster than the autotrophic anammox bacteria, thus the anammox bacteria are quickly eliminated or decrease in proportion, thereby causing a reduction in anammox activity (Chamchoi et al., 2008; Güven et al., 2005). In the second proposed mechanism, the anammox bacteria, which are dominant in the given system, metabolize organic carbon rather than using ammonium and nitrite as a substrate, thus leading to lower anammox activity and decreased nitrogen removal (Güven et al., 2005; Kartal et al. 2007, 2008). Research

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demonstrates that adding glucose as the organic carbon source to an anammox reactor will rapidly stimulate growth of denitrifying bacteria (Chen et al., 2016); however, a low glucose concentration has no effect on the growth of anammox bacteria (Güven et al., 2005). Güven et al. (2005) reported that anammox bacteria exhibit different metabolic properties when using acetate as a substrate (Güven et al., 2005); thus, glucose can be used as the representative carbon source for "out-competition inhibition" and acetate can be used as the representative carbon source for "metabolic pathway conversion inhibition". Studies rarely mention the effect of carbon source type on the anammox/denitrification combined process. Jenni et al. (2014) reported that glucose and acetate have no significant influence on the nitritation/anammox process (Jenni et al., 2014); however, with respect to denitrification, different carbon sources corresponding to different absorption and bacterial growth rates could play an important role in the SAD system.

To our knowledge, the effect of carbon sources on the SAD process has not been reported. Thus, the objective of this study was to determine the most suitable carbon source for the anammox/denitrification process. The ultimate goal of this study was to test the influence of different carbon sources on the simultaneous anammox and denitrification (SAD) process and to achieve perfect coupling of anammox/denitrification using the selected carbon source in order to provide guidance for the remediation wastewater using the SAD process.

2. Materials and method

2.1. Synthetic wastewater

In this study, synthetic wastewater containing substrates (ammonium and nitrite), COD and trace elements were introduced into the two reactors as the influent. The reactors differed only in their carbon source, which was either glucose (reactor R1) or acetate (reactor R2). NH_4^+-N and NO_2^--N were provided by NH_4Cl (150 mg L^{-1}) and $NaNO_2$ (200 mg L^{-1}), respectively. Trace elements solution I and II were added according to a previous study (Du et al., 2014). Trace elements solution I contained (L^{-1}): 5 g EDTA, 5 g $FeSO_4$; and trace elements solution II contained (L^{-1}): 15 g EDTA, 0.014 g H_3BO_3, 0.43 g $ZnSO_4{\cdot}7H_2O$, 0.25 g $CuSO_4{\cdot}5H_2O$, 0.19 g $NiCl_2{\cdot}6H_2O$, 0.24 g $NaMoO_4{\cdot}2H2O$, 0.99 g $MnCl_2{\cdot}4H_2O$, 0.05 g $NaWO_4{\cdot}2H_2O$, 0.21 g $NaSeO_4{\cdot}10H_2O$, 0.24 g $CoCl_2{\cdot}6H_2O$.

2.2. Reactor operation

Two EGSB reactors were operated in parallel to investigate the SAD process on synthetic wastewater (Fig. 1). The working volume of the EGSB reactors was 2.0 L and the internal diameter was 6 cm. The reactors has an inner diameter of 60 mm and a height of 90 cm. The effective volume of the reactor was 2 L. The two reactors were covered with black cloth to prevent the growth of phototrophic organisms (Wr et al., 2008) and light inhibition (Hulle et al., 2010). The reactor's temperature was maintained at 35 ± 1 °C by water recirculation (JingHong, CN) through the outer chamber. Synthetic wastewater was continuously introduced into the reactors with a peristaltic pump, and part of supernatant was recirculated to influent by a peristaltic pump (LongerPump, CN).

The seeding sludge for the two reactors was harvested from a successfully initiated laboratory-scale anammox EGSB reactor. The mixed liquor suspended solids (MLSS) of the sludge was 16 g/L and mixed liquor volatile suspended solids (MLVSS) was 10 g/L.

Two EGSB reactors were in operation for 91 days. The influent COD/N ratio was incrementally increased every 20 days by adjusting the glucose or acetate concentrations; therefore, the entire experimental course was divided into 5 phases according to the influent C/N ratios: phase 1 (C/N = 0.2), phase 2 (C/N = 0.4), phase 3 (C/N = 0.8), phase 4 (C/N = 1.2) and phase 5 (C/N = 1.6).

2.3. Sampling and DNA extraction

At the end of each phase, sludge from the two reactors was collected to characterize the microbial community structure of the SAD system via the Illumina high-throughput sequencing analysis. The sludge samples were mixed with an equal volume of anhydrous ethanol and stored at −20 °C. DNA was extracted using the FastDNA® Spin Kit Soil Kit (MP Biomedicals, USA) according to the manufacture's instruction. The isolated genomic DNA was stored at −80 °C for later use.

2.4. Polymerase chain reaction (PCR) and Illumina Miseq sequencing

The V3-V4 hypervariable region of the bacterial 16 S rRNA gene was targeted using the forward primer 515 F (5′-GTGCCAGCMGCCG CGG-3′) and reverse primer 806 R (5′-GGACTACHVGGGTWTCT AAT-3′); the nucleotide barcodes were inserted between the sequence adapter and the forward primer. After purification (TaKaRa MiniBEST DNA Fragment Purification Kit Ver. 4.0, TaKaRa, Japan), the amplicons for all samples were pooled in equimolar concentrations and sequenced at Shanghai Meiji Biological Analysis and Testing Co., Ltd. (Meiji, Shanghai).

Before analysis, raw reads were processed to remove low quality sequences according to the method previously described by Guo and Zhang (2012). After normalization to a uniform sequencing depth, DNA sequences for all samples were deposited into the Ribosomal Database Project (RDP; http://rdp.cme.msu.edu/). Sequences were systematically classified from the phylum level to the genus level, with an 80% confidence interval using the RDP bacterial functional model to complete the linkage cluster for all sample sequences. The sequences of the chained cluster analysis were then systematically clustered under the 3% confidence interval. The system cluster files were submitted to the relevant RDP models (Chao 1 & Shannon Index estimator and Rarefaction) to calculate the richness and diversity indices, as well as other data processing.

2.5. Additional analytic methods

Water samples were collected every 2 days from both reactors to determine the concentration of NH_4^+-N, NO_2^--N and NO_3^--N according to the standard methods (APHA, 1999). The pH of the samples was measured using a pH meter (METTLER TOLEDO, Switzerland), and the COD was analyzed using a COD quick-analysis apparatus (Jinghong Tech. Co., Ltd., JH-TC200, China).

3. Results and discussion

3.1. Performance of the glucose-driven SAD reactor

The SAD process in R1 was operated for 90 days with glucose as the organic carbon source and an influent NO_2^--N/NH_4^+-N ratio of approximately 1.2. The five experimental phases were based on different influent C/N ratios. Glucose was added stepwise to the reactor to increase the C/N ratio from 0.2 (phase 1) to 1.6 (phase 5) as shown in Fig. 2. Simultaneous consumption of NH_4^+-N and NO_2^--N was monitored following inoculation of anammox active sludge into the EGSB. In phase 1 (day 1–20), almost no NH_4^+-N and NO_2^--N was detected in the effluent and the removal rate of the two parameters reached over 98%, indicating good anammox activity; however, the NO_3^--N effluent concentration decreased sharply from 37.91 mg/L to 18.74 mg/L during phase 1. This could be caused by the rapid growth of denitrifying bacteria in the presence of COD and the reduction of NO_3^- produced by anammox (Du et al., 2017). During phase 2 (day 21–40), the NO_3^--N concentration in effluent continued to decline, but the rate of decrease slowed. The NH_4^+-N (0.91 mg/L) and NO_2^--N (0.52 mg/L) concentrations in the effluent remained low and corresponded to an average removal efficiency of 99.4% and 99.7%, respectively.