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Simultaneous nitrogen and carbon removal from swine digester liquor by the Canon process and denitrification

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ABSTRACT

A laboratory-scale completely autotrophic nitrogen removal over nitrite (Canon) biofilm reactor was applied to remove nitrogen and organic carbon from swine digester liquor for 150 d. A stable nitrogen removal rate was reached after a three-week adaptation period. The nitrogen loading rate of influent were 0.20 ± 0.04, 0.26 ± 0.04, 0.26 ± 0.07 kg N m^{-3} d^{-1} in period I, II and III, respectively. Nitrogen removal rates reached 0.096, 0.133 and 0.104 kg N m^{-3} d^{-1} when the C/N ratios of liquor were maintained at 0.81, 0.65, and 1.24, respectively. The Canon process was better than the denitrification process for nitrogen elimination when the C/N ratio of the influent was controlled below 0.8. PCR-DGGE analysis showed a decrease in microbial diversity of *Planctomycetes* during long-term operation. In contrast, the aerobic ammonium-oxidizing bacteria species were resistant to the changes due to replacement of synthetic wastewater with swine digester liquor.

1. Introduction

Agro-industrial pollution from swine wastewater has received considerable attention because it contains a high concentration of organic matter, nitrogen, phosphorus, hazardous heavy metals, and estrogenic compounds (Lee and Shoda, 2008). Nutrient-related eutrophication, resulting from untreated swine wastewater discharges, is becoming one of the most significant challenges to water bodies (Painting et al., 2007). Odor emissions from swine wastewater also have a great impact on air quality in the vicinity of swine farms (Hanajima et al., 2010). To reduce pollution due to livestock discharges, considerable treatment processes, both experimental and practical, have been developed to attain simultaneous carbon and nitrogen elimination. These processes include: (i) employment of conventional nitrification–denitrification biologic nitrogen removal (BNR) processes in the form of constructed wetlands (Dong and Reddy, 2010), and membrane bioreactors (MBR)-combined bioreactors (Kim et al., 2008), (ii) utilization of entrapped mixed microbial cell processes in a land-limited area (Yang et al., 2003), (iii) removal of nitrate and ammonia by methane and oxygen in the water phase via semi-partitioned reactors (Waki et al., 2008), and (iv) elimination of carbon, nitrogen, and phosphorus by microalgae under photosynthetic oxygenation (de Godos et al., 2009). Recently, a number of attempts have also been made to develop cost-effective and energy-saving BNR methods, among them, anaerobic ammonium oxidation (Anammox) (Kartal et al., 2010) is being investigated as an efficient process for autotrophic nitrogen removal. Compared with conventional nitrification–denitrification process, Anammox requires 90% less operational costs (Jetten et al., 2001). At the same time, a new technique coupling Anammox with partial nitrification in one single unit, namely completely autotrophic nitrogen removal over nitrite (Canon) (Sliekers et al., 2003) has been developed. In general, Anammox bacteria are capable of coexisting with heterotrophic denitrifiers in the same reactor (Pathak et al., 2007) and can also grow mixotrophically (Güven et al., 2005). Therefore, the use of Canon and denitrification processes in the degradation of real wastewater that contains high concentrations of organic carbon and nitrogen (i.e., livestock discharges, landfill leachate and sludge digester liquor) has been a promising technology of BNR processes (Kumar and Lin, 2010).

Yamamoto et al. (2008) reported a method to remove nitrogen from digester liquor of swine wastewater via partial nitrification and Anammox in two consecutive reactors. Results indicate that a long-term stable nitrogen removal rate (NRR) could be achieved through the inhibition of free ammonia and free nitric acid. However, little information is available on the coexistence of Anammox and denitrification. Wang et al. (2010) found that nitrogen and organic carbon from landfill leachate can be simultaneously removed by Canon and denitrification processes in a full-scale bioreactor. With limited aeration, Anammox is more effective than heterotrophic denitrification for total nitrogen (TN) removal.

Two factors may influence a successful Canon process in the presence of organic compounds. The competition of Anammox

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bacteria and heterotrophic denitrifiers (HD) could restrain the activity of Anammox bacteria due to the higher growth yield of HD (Dong and Tollner, 2003), and the nitrite produced from nitrate through dissimilatory nitrate reduction to ammonium (DNRA) process and denitrifiers may advance the Anammox process (Dalsgaard et al., 2005). In addition, organic carbon loading shocks can result in an unstable oxygen concentration in the bioreactor, preventing the growth of Anammox bacteria and inhibiting partial nitrification (Vázquez-Padín et al., 2010). Some operating conditions (i.e., C/N ratios, DO values, and reactor configuration) have definite impacts on activities of partial nitrification, Anammox and denitrification in a single reactor treating real wastewater (Kumar and Lin, 2010); however, these impacts have not been well documented. Therefore additional knowledge on these conditions is needed to further develop the application of the Canon process. The present research aimed to investigate the changes occurring when a biofilm Canon reactor fed synthetic wastewater is switched to a feed of raw swine digester liquor. The influence of C/N ratios of swine digester liquor on the performance of autotrophic nitrogen removal was also determined.

2. Methods

2.1. Experimental set-up

A stable Canon process was successfully established in a laboratory-scale sequencing batch biofilm reactor (SBBR) fed with synthetic autotrophic medium before treatment with swine digester liquor. The performance of this biofilm Canon reactor is shown in Table 1. The working volume of the SBBR was 6.5 L, and the exchange volume of the reactor was kept at 50%. Non-woven porous polyester was selected as the biofilm carrier with a surface area of 645 $m^2\ m^{-3}$, and a packing rate of 40% (v/v). Fine bubble air diffusers provided oxygen at the bottom of the reactor. The temperature was regulated at 30 ± 2 °C during the whole experiment using a thermostated jacket. Swine digester liquor was collected as fermentation effluents from a pig farm of the Ying-xiang Food Group in Xia-men, China. The characteristic of the swine digester liquor are presented in Table 2.

The SBBR was operated for 150 days and divided into three periods according to the C/N values of the influent after a three-week adaptation period. Ammonium chloride and sodium acetate were selected to adjust the carbon and nitrogen content in the swine digester liquor. The SBBR was operated in a 12 h consecutive limited aeration cycle for the duration of the study. Setting, decanting and feeding phases were conducted during the last 25 min of the cycle, and the DO value was maintained at 1.60–2.00 mg $O_2\ L^{-1}$ during the limited aeration period.

2.2. Chemical analysis

At the start and end of each batch cycle, 50 mL of bulk solution was collected, filtered with 0.45 μm PVDF membrane before chemical analysis. The tests were performed once. The concentrations of NH_4^--N, NO_2^--N, NO_3^--N and total phosphorus were monitored by a flow injection analyzer (QuickChem 8500, USA). Total nitrogen (TN) was measured by a Shimadzu analyzer (TNM-1, Shimadzu, Japan). The pH and DO were determined by a HACH analyzer (HQ40d18, Hach, USA). COD was analyzed using a colorimetric method (Lian-hua, China), and the metals in the influent were measured using an inductively coupled plasma optical emission spectrometer (Optima 7000DV, USA) according to the manufacturer's instruction. Total suspended solids (TSS) content in the swine wastewater was analyzed by a standard method (Chinese NEPA, 2002).

2.3. Fluorescent in situ hybridization (FISH)

Ammonium-oxidizing bacteria (AOB) and Anammox bacteria populations at each operational period were identified by the FISH technique. Biofilm from the reactor was collected and fixed in 4% paraformaldehyde solution, and FISH was conducted according to Amann et al. (1990). The oligonucleotide probes were purchased as Cy3-, Cy5-, and fluorchromes fluorescein isothiocyanate (FITC)-labeled derivatives (Invitrogen, California, USA). Probes NEU653 and Amx820 were used to target most of the halophilic and halotolerant *Nitrosomonas* spp. and Anammox bacteria genera "*Candidatus* Brocardia" and "*Candidatus* Kuenenia", respectively (Vázquez-Padín et al., 2010). Fluorescence signals were recorded with a LSM 710 confocal laser scanning microscope (CLSM) (Carl Zeiss, Inc., Germany). The CLSM images were analyzed by the standard software for the LSM 710.

2.4. DNA extraction, PCR amplification, and DGGE analysis

Total DNA was periodically extracted from approximately 0.5 mL of the biofilm and purified with a Fast DNA spin kit (Omega Bio-tek Inc., USA) as described in the manufacturer's instruction. To investigate the AOB and *Planctomycetes* communities, a nested PCR was applied as described by Pynaert et al. (2003). For the first round, amplification of 16S rRNA genes of the β-proteobacterial ammonia-oxidizers was performed with primers CTO189fAB plus CTO189fC and CTO653r (Kowalchuk et al., 1998), and the primers Pla40f and P518r (Derakshani et al., 2001) were used for the amplification of *Planctomycetes*. The obtained fragments were used as templates for a second amplification with primers P338f (with a 40 bp GC-clamp) and P518r (Ovreas et al., 1997) that target all bacteria. Prior to DGGE analysis, the second round PCR products were purified with a cycle pure kit (Omega Bio-tek, Inc., USA). DGGE was performed with the Bio-Rad DGene™ system (Hercules, CA, USA). The products of the second round of PCR were loaded onto 8% (wt/vol) polyacrylamide gels in 1× TAE with a denaturing gradient ranging from 45% to 60%. The DGGE analysis was conducted at 60 °C and 37 V for 16 h. Gels were stained with SYBR Green I for 20 min and visualized using an imager (Ettan Dige, GE, USA). The DGGE gel images were analyzed in terms of Rang-weighted richness (Rr) and functional organization (Fo) according to Marzorati et al. (2008).

3. Results and discussion

3.1. Nitrogen and carbon removal performance

Simultaneous nitrogen and carbon removal from swine digester liquor in this laboratory-scale SBBR by Canon and denitrification processes was conducted for 150 d and consisted of three periods (Supplementary Fig. 1S, supporting information). Prior to the treatment periods, a three-week adaptation period was completed, and a new appropriate oxygen concentration (1.60–2.00 mg $O_2\ L^{-1}$) had been established due to the increase in organic carbon. The beginning of the adaptation period was considered as day 0. Raw swine digester liquor was diluted with tap water until on day 21, and the nitrogen loading rate (NLR) and COD loading rate (CLR) of the influent were increased gradually to 0.267 kg N $m^{-3}\ d^{-1}$ and 0.203 kg COD $m^{-3}\ d^{-1}$. SBBR showed a stable nitrogen and carbon removal efficiency under limited aeration at the end of the adaptation period (Supplementary Fig. 2S, supporting information and Supplementary Fig. 3S, supporting information). During treatment period I (days 22–65), no ammonium chloride or sodium acetate was added to the swine digester liquor, and the C/N ratios of the influent were kept at 0.81 ± 0.18, the NLR and CLR of the