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Performance of autotrophic nitrogen removal from digested piggery wastewater

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HIGHLIGHTS

- An ANR process was successfully applied to remove nitrogen from DPW.
- Its nitrogen removal rate was higher than the maximum rate reported previously.
- Anaerobes, sulfur-oxidizing bacteria and methanogens were its specific microbes.
- *Nitrosomonas* at a high relative abundance played a key role.
- DPW reduced functional microbe abundances and activities and sludge concentration.

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ABSTRACT

The performance of an autotrophic nitrogen removal process to treat digested piggery wastewater (DPW) was investigated by gradually shortening the HRT and enhancing the DPW concentration during 390 days of operation. The results showed that the total nitrogen removal rate and efficiency reached 3.9 kg-N m^{-3} day^{-1} and 73%, which were significantly higher than the levels reported previously. A high relative abundance of *Nitrosomonas* (4.2%) and functional microbes (12.15%) resulted in a high aerobic ammonium oxidizing activity (1.25 ± 0.1 g-N g VSS^{-1} d^{-1}), and a good settling ability (SVI, 78.42 mL g^{-1} SS) resulted in a high sludge concentration (VSS, 11.01 g L^{-1}), which laid a solid foundation for the excellent performance. High-throughput pyrosequencing indicated that, compared with synthetic wastewater, the DPW decreased the relative abundances of every functional group of nitrogen removal microbes, and increased relative abundances of anaerobes (15.7%), sulfur-oxidizing bacteria (9.4%) and methanogens (40.8%).

1. Introduction

With the rapid development of an intensive piggery industry, increasing quantities of piggery wastewater are being discharged (Meng et al., 2016). It contains abundant nutrients, but also induces severe environmental crises and human health problems (Zhao et al., 2014). Anaerobic digestion is widely used all over the world to remove pollutants from piggery wastewater. One of its products, digested piggery wastewater (DPW), is characterized by a low ratio of chemical oxygen demand (COD) to ammonium (NH_4^+-N) (Meng et al., 2016). As traditional nitrification-denitrification requires COD as an electron donor, nitrogen removal is a serious challenge for the treatment of DPW because of its high NH_4^+-N content and low C/N ratio.

In general, biological processes, such as nitrification-denitrification and partial nitrification-anaerobic ammonium oxidation (PN-Anammox, including single stage PN-Anammox and two stage PN-Anammox), are the main processes used to remove nitrogen from wastewater (Wang et al., 2012). However, the traditional nitrification-denitrification process requires large amounts of oxygen and external carbon resources, which make the treatment of low C/N ratio wastewater including DPW costly (Soliman and Eldyasti, 2016). Compared to the traditional nitrification-denitrification process, PN-Anammox decreases the need for oxygen by about 60%, the organic carbon requirement by 100% and sludge production by about 90% (Lackner et al., 2014). It also

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has a higher nitrogen removal rate (NRR, 2.57 kg-N m^{-3} day^{-1}) (Wang et al., 2012), which is more suitable for the treatment of high NH_4^+-N and low C/N ratio wastewaters. In recent years, the full-scale PN-Anammox process has been successfully applied to treat some high NH_4^+-N and low C/N ratio wastewaters, such as the sludge-digestion liquid from municipal wastewater, reject water, landfill leachate and so on (Lackner et al., 2014).

Many studies have reported that DPW contains high concentrations of COD, heavy metals and antibiotics, which can inhibit the activity of ammonia oxidizing bacteria and Anammox bacteria (Lotti et al., 2012; Zhang et al., 2016; Zhao et al., 2010). Hence, few studies have successfully used a PN-Anammox process to treat DPW because of the many challenges, e.g. longer start-up period, low or unstable nitrogen removal rate and poor effluent water quality (Meng et al., 2016; Zhao et al., 2014). Furthermore, no information has been published about a full-scale PN-Anammox installation for treating DPW anywhere in the world.

In our previous research, an autotrophic nitrogen removal (ANR) process in a granular sludge bed reactor, a single stage PN-Anammox with a high nitrogen removal rate, was successfully developed to treat wastewater with a high NH_4^+-N concentration and low C/N ratio (Wang et al., 2012, 2014). Based on that work, this study investigated the feasibility and performance of an ANR process to treat DPW, as well as its microbiological mechanisms.

2. Methods

2.1. Experimental set-up and operation

Two 6-L internal loop air-lift reactors with effective volumes of 4.5 L were used for autotrophic nitrogen removal from synthetic wastewater (SYN reactor) and DPW (PIG reactor). The composition of synthetic wastewater was as described by Wang et al. (2012), and the concentration of $(NH_4)_2SO_4$ was regulated according to the ammonium concentration. The DPW contained 305–1000 mg L^{-1} COD (chemical oxygen demand), 277–661.3 mg L^{-1} NH_4^+-N, 0–3 mg L^{-1} NO_2^--N and NO_3^--N, 6.4–8.0 mg L^{-1} total phosphorous and 0.02 mg L^{-1} Total solids. The experimental system is presented in Fig. 1. The influent and air were continuously pumped into the bottom of the reactors. Driven by the air, the sludge and wastewater rose upwards in the riser part and flowed downwards in the downcomer part. The dissolved oxygen (DO) concentration was regulated in the range of 0.3–1.0 mg L^{-1} by a gas flow meter. The temperature was controlled at 30 ± 1 °C. A 0.5-L sample of sludge taken from our previous ANR reactor was used for inoculation of the reactors (Wang et al., 2012). The DPW was obtained from a pig farm in central Sichuan Province, China. The reactors were operated in continuous feeding mode, and the operation parameters were shown in Supplementary data Table S1.

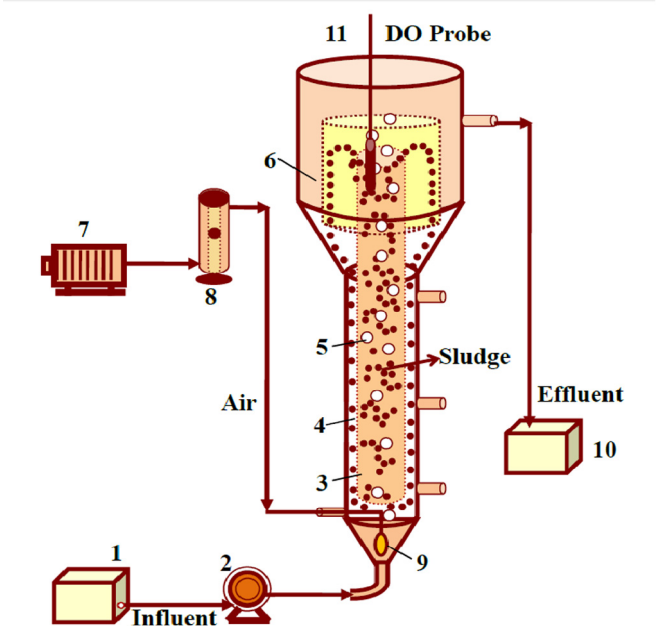

Fig. 1. Schematic diagram of the internal loop air-lift reactor. (1 Influent tank; 2 feeding pump; 3 riser part; 4 downcomer part; 5 air bubble; 6 settling zone; 7 air pump; 8 gas flow meter; 9 air distributor; 10 effluent tank; 11 DO probe.)

2.2. Analytical methods

The volatile suspended solids (VSS) and the concentrations of ammonium, nitrite and nitrate were determined according to standard procedures (Sliekers et al., 2002). The air flow rate and the DO concentration were determined using an LZB-4 glass tube rotameter (Zhejiang Yuyao Instrument Factory, China) and SevenGo pro SG9 meter (METLER TOLEDO, Switzerland). The pH was measured by a PHS-3C pH meter (Shanghai Leici Equipment Factory, China).

2.3. Batch activity tests

The batch tests were conducted to determine the aerobic ammonium oxidizing activity of the aerobic ammonia oxidizing microbes (AOM), the anaerobic nitrite oxidizing activity of the Anammox bacteria, the aerobic nitrite oxidizing activity of the nitrite-oxidizing bacteria (NOB) and the anaerobic denitrifying activity of the denitrifying bacteria (DB). Sludge samples were taken from the SYN and PIG reactors in periods II–IV and washed three times with 2 g L^{-1} $KHCO_3$. Then, 0.1 g of VSS from the washed sludge was used for the batch tests. Each test was conducted on a shaking table at 150 rpm and 30 °C. And the sampling times were 0.25 h for the AOM test, 1 h for the NOB and Anammox bacteria tests and 5 h for the DB test. The detailed method has been reported by Wang et al. (2014).

2.4. High-throughput pyrosequencing

Triplicate sludge samples were taken from the inoculum, the SYN reactor at period IV and the PIG reactor at period IV, and labeled, respectively, seed.1, seed.2, seed.3, SYN1.1, SYN1.2, SYN1.3, PIG2.1, PIG2.2 and PIG2.3. The total genomic DNA from the samples was extracted using the CTAB/SDS method. 16S rRNA genes of the 16SV3-V4 regions were amplified using specific primer sets (bacteria: 341F-806R (Kuppusamy et al., 2016; Trivedi et al., 2016) and archaea: Parch519F-Arch915R (Lin et al., 2011)). The PCR conditions were as described by Kuppusamy et al. (2016). PCR products were detected on 2% agarose gels. Samples with a bright main strip between 400 and 450 bp were chosen for further experiments. PCR products were purified, pooled and sequenced by an Illumina HiSeq 2500 platform (Illumina, USA) according to the manufacturer's instructions. The detailed methodology and following bioinformatics analysis were followed by Yan et al. (2015). The raw sequences were deposited into the NCBI SRA database (accession number: SRX2641391).

Alpha diversity was used to analyze the complexity of species diversity for a sample using six indices, including Observed-species, Chao1, Shannon, Simpson, ACE and Good's coverage. All indices for our samples were calculated with QIIME software (Version1.7.0) and displayed with R software (Version 2.15.3). Cluster analysis was preceded by principal component analysis (PCA), which was applied to reduce the dimensions of the original variables using the FactoMineR package and ggplot2 package in R software (Version 2.15.3). Rarefaction curve analysis reflected the