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Research article

The role of COD/N ratio on the start-up performance and microbial mechanism of an upflow microaerobic reactor treating piggery wastewater

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This study investigated the role of COD/N ratio on the start-up and performance of an upflow microaerobic sludge reactor (UMSR) treating piggery wastewater at 0.5 mgO_2/L. At high COD/N ratio (6.24 and 4.52), results showed that the competition for oxygen between ammonia-oxidizing bacteria, nitrite-oxidizing bacteria and heterotrophic bacteria limited the removal of nitrogen. Nitrogen removal efficiency was below 40% in both scenarios. Decreasing the influent COD/N ratio to 0.88 allowed achieving high removal efficiencies for COD (~75%) and nitrogen (~85%) due to the lower oxygen consumption for COD mineralization. Molecular biology techniques showed that nitrogen conversion at a COD/N ratio 0.88 was dominated by the anammox pathway and that *Candidatus Brocadia* sp. was the most important anammox bacteria in the reactor with a relative abundance of 58.5% among the anammox bacteria. Molecular techniques also showed that *Nitrosomonas* spp. was the major ammonia-oxidiser bacteria (relative abundance of 86.3%) and that denitrification via NO_3^- and NO_2^- also contributed to remove nitrogen from the system.

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1. Introduction

The rapid growth of human population and industrial production have resulted in an increasing discharge of domestic, industrial and livestock wastewater. One of the most important issues associated with the growth of the piggery industry is the discharge of untreated nutrient-rich wastewater into water bodies (Meng et al., 2018). Ammonium (NH_4^+) is a common contaminant of these wastewater steams, which can cause eutrophication of water bodies if discharged without proper treatment (Yamamoto et al., 2008; Zhang et al., 2016). In wastewater treatment plants, NH_4^+ is typically removed by an anoxic-aerobic (A/O) or by an anaerobic-anoxic-aerobic (A^2/O) process, where NH_4^+ is oxidised to nitrate (NO_3^-) under aerobic conditions (nitrification) and, subsequently, reduced to dinitrogen gas (N_2) under anoxic conditions (denitrification) (Obaja et al., 2003; Xie et al., 2017). Recently, anaerobic ammonium oxidation (anammox) has emerged as a cost-effective and space-saving alternative to conventional processes to treat wastewater with high NH_4^+ and low organic carbon (Fernández et al., 2016).

Anammox is an autotrophic process where NH_4^+ is oxidised to N_2 using nitrite (NO_2^-) as electron acceptor (Jetten et al., 1998; Kartal et al., 2010; Strous et al., 1999). Concomitantly, NO_2^- serves as electron donor for the formation of new biomass from bicarbonate (HCO_3^-), from which NO_3^- is stoichiometrically produced (Van de Graaf et al., 1996). The key advantages of the anammox process over conventional nitrification/denitrification processes are (i) the null requirement of organic carbon, (ii) the lower oxygen demand and (iii) the minimal sludge production (Fux et al., 2002; van Dongen et al., 2001; van Loosdrecht and Jetten, 1998). Nonetheless, anammox reactors are limited by the sensitivity of anammox bacteria towards oxygen and NO_2^- as well as the low biomass yield and growth rates (Fernández et al., 2012; Strous et al., 1999). The latter is the cause for the characteristic long start-up periods of anammox systems. The combination of aerobic NH_4^+ oxidation,

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anaerobic denitrification and anammox is essential to successfully remove nitrogen from the wastewater rich in NH_4^+ at a low treatment cost. However, achieving this goal in a single reactor is relatively complex since a co-culture of aerobic and anaerobic ammonium-oxidizing bacteria needs to be established and maintained (Vázquez-Padín et al., 2009). Therefore, there is a need to develop a cost-effective nitrogen removal process to treat wastewater with high NH_4^+ concentrations.

Microaerobic condition is a transitional state between aerobic and anaerobic condition, where dissolved oxygen (DO) levels range from 0.3 to 1.0 mg/L (Chu et al., 2006; Zheng and Cui, 2012). Wastewater treatment under microaerobic conditions is gaining attention due to its capacity to treat wastewater at a lower treatment cost than conventional technologies (e.g. nitrification-denitrification). For instance, Lu et al. (2018) revealed the co-existence of two metabolisms in a photosynthetic microaerobic reactor, i.e. degrading pollutants through light metabolic under light-anaerobic and oxygen metabolic pathways under dark-aerobic conditions. Jiang et al. (2018) supposed that the micro-aerobic environment would promote pyridine bio-mineralization in an electricity-assisted anaerobic reactor. Ramos et al. (2012) and Pokorna-Krayzelova et al. (2017) explored the feasibility of using microaeration conditions for biogas desulfurization. Regarding nitrogen removal, Zheng and Cui (2012) and Varas et al. (2015) reported that microaerobic condition provided an appropriate growth environment for ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), anammox bacteria and denitrifiers, resulting in a synchronous removal of organics and nutrients. In such systems, anammox bacteria are protected from oxygen exposure by growing in the aggregates inner layer (Vázquez-Padín et al., 2009; Winkler et al., 2012). To date, microaerobic biological conditions has been proved to remove COD and NH_4^+ from domestic wastewater, with removal efficiencies around 90% and 80%, respectively (Basu and Mino, 1993; Chu et al., 2006; Zheng and Cui, 2012). However, the removal of COD and NH_4^+ from livestock wastewater using microaerobic technology has received less attention.

COD/N ratio is an important parameter affecting the activity of denitrifiers and anammox bacteria. The presence of organic matter can improve nitrogen removal efficiency by promoting heterotrophic denitrification simultaneously with the anammox process (Du et al., 2015; Langone et al., 2014). However, an excess of organic carbon will hinder anammox activity due to competition between the anammox and the excessive growth of heterotrophic bacteria (Ibrahim et al., 2016); which hinders achieving the simultaneous removal of nitrogen and organic carbon (Kuba et al., 1996; Płaza et al., 2003). Lackner et al. (2014) and Ni et al. (2012) reported that anammox was the main nitrogen removal pathway at COD/N below 2. Additionally, the same authors suggested 1.7 and 3.1, respectively, as COD/N threshold value to have an anammox-driven nitrogen removal. Since COD/N controls the balance between heterotrophic bacteria, AOB, NOB and annamox bacteria, it is hypothesised that controlling the COD/N ratio would accelerate the start-up of a microaerobic system for synchronous removal of organic carbon and nitrogen.

In present study, an upflow microaerobic sludge reactor (UMSR) was constructed to synchronously remove nitrogen and organic carbon from piggery wastewater. The effect of influent COD/N ratio on reactor start-up performance, functional microbial populations and nitrogen removal pathways was evaluated. Molecular biology was investigated using real-time quantitative polymerase chain reaction (qPCR) and terminal restriction fragment length polymorphism (T-RFLP).

2. Materials and methods

2.1. Wastewater and inoculum origin

Raw piggery wastewater was collected from a local pig breeding farm in Harbin, China. Piggery wastewater is generated during the flush of the piggery floor after the removal of the solid manure. Piggery wastewater was collected once a month and stored at 4 °C. Beet molasses (Changjida, China) were used to adjust the wastewater COD/N ratio to the desired set point. The sludge used to inoculate the UMSR was waste activated sludge collected from the secondary clarifier of a wastewater treatment plant in Harbin, China. The initial sludge volatile solids (VS) concentration in the UMSR was 1.34 g/L.

2.2. Microaerobic treatment set-up and operation

A laboratory-scale UMSR of a working volume of 4.9 L (Fig. 1) was operated to remove nitrogen and organic carbon from piggery wastewater. Microaerobic conditions, DO level of about 0.5 mg/L, were maintained by aerating the effluent and recirculating it into the reactor. Influent and recirculation were continuous and carried out by peristaltic pumps. The temperature and hydraulic retention time (HRT) of the reactor were set at 35 ± 1 °C and 8 h, respectively. Reactor temperature was maintained by an electric jacket.

According to the influent COD/N ratio, the reactor operation was divided into 3 stages (Table 1). The influent COD/N ratio in Stage 1 and Stage 2 was controlled at 6.24 and 4.52, respectively, by adding molasses to the diluted piggery wastewater. In Stage 3, piggery wastewater was diluted but no molasses were added. The COD/N ratio in Stage 3 averaged 0.88. In all stages, piggery wastewater was diluted one third with tap water.

2.3. Analytical methods

Influent and effluent chemical analysis including COD, NH_4^+, NO_2^- and NO_3^- were measured according to the Standard Methods (APHA, 2005). pH was measured using a pH meter (Mettler-Toledo,

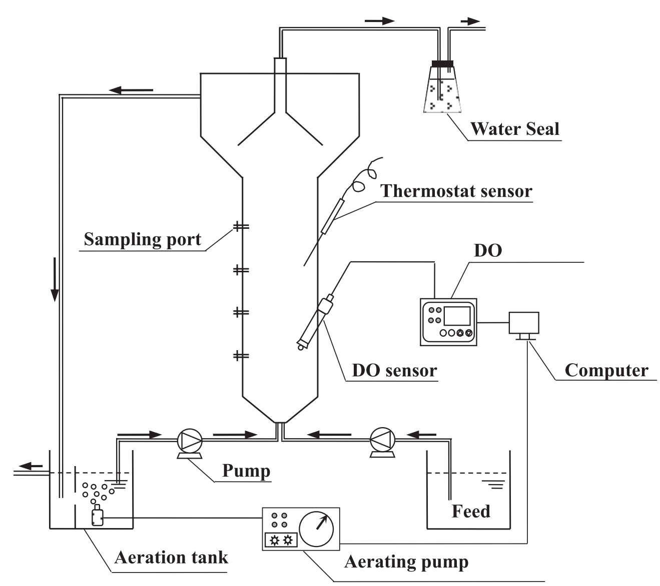

Fig. 1. Schematic representation of the lab-scale UMSR.

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