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Enhanced nitrogen removal from piggery wastewater with high NH_4^+ and low COD/TN ratio in a novel upflow microaerobic biofilm reactor

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ABSTRACT

To enhance nutrient removal more cost-efficiently in microaerobic process treating piggery wastewater characterized by high ammonium (NH_4^+-N) and low chemical oxygen demand (COD) to total nitrogen (TN) ratio, a novel upflow microaerobic biofilm reactor (UMBR) was constructed and the efficiency in nutrient removal was evaluated with various influent COD/TN ratios and reflux ratios. The results showed that the biofilm on the carriers had increased the biomass in the UMBR and enhanced the enrichment of slow-growth-rate bacteria such as nitrifiers, denitrifiers and anammox bacteria. The packed bed allowed the microaerobic biofilm process perform well at a low reflux ratio of 35 with a NH_4^+-N and TN removal as high as 93.1% and 89.9%, respectively. Compared with the previously developed upflow microaerobic sludge reactor, the UMBR had not changed the dominant anammox approach to nitrogen removal, but was more cost-efficiently in treating organic wastewater with high NH_4^+-N and low COD/TN ratio.

1. Introduction

With the rapid development of large-scale pig farms, the discharge of piggery wastewater rich in nutrients would result in serious health and environmental consequences (Wu et al., 2015a; Zhao et al., 2014). Characteristics of piggery wastewater vary significantly due to the difference of manure collection methods (Meng et al., 2015). Among the manure collection methods is the urine-free manure with resource recycling and widely used in developing countries like China. The piggery flushing wastewater is defined as manure-free piggery wastewater (MFPW) and characterized by high ammonium (NH_4^+-N) and low ratio of chemical oxygen demand (COD) to total nitrogen (TN) (Zhao et al., 2014). It is difficult to remove nutrient from the MFPW by traditional nitrification-denitrification processes due to the lack of carbon source (He et al., 2014; Zhang et al., 2016). Furthermore, the traditional nitrogen removal processes are complex in processing, high in engineering investment and management cost (Bernet et al., 2000; Canals et al., 2013). Thus, it is essential to develop novel processes for MFPW treatment more efficiently and economically.

Microaerobic biological treatment process with a dissolved oxygen (DO) ranged from 0.3 to 1.0 mg/L has been introduced to treat municipal wastewater (Chu et al., 2006; Zheng and Cui, 2012) because of its low yield of excess sludge, high COD removal, capacity of anti-impact load, and low treatment cost (Chu et al., 2006). Microaerobic condition could provide an appropriate growth environment for aerobe, anaerobe and facultative aerobe resulting in synchronous removal of organics and nutrients (Zheng and Cui, 2012). In the previous studies, a novel upflow microaerobic sludge reactor (UMSR) was constructed to treat the MFPW (Meng et al., 2015). Ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), anaerobic ammonium oxidation (anammox) bacteria and denitrifiers were found to coexist in the microaerobic process, resulting in a better synchronous removal of COD, NH_4^+-N and TN from the MFPW (Meng et al., 2015). To evaluate the performance of the UMSR, both effect of COD/TN ratio and reflux ratio on pollutant removal had been investigated at a constant temperature of 35 °C and a hydraulic retention time (HRT) of 8 h. With a reflux ratio of 45 and a COD/TN ratio averaged 0.84 in the MFPW, the COD, NH_4^+-N and TN removal in the microaerobic reactor reached 77.9%, 86.2% and 87.2%, respectively (Meng et al., 2015). Operated at a lower reflux ratio of 35, a COD, NH_4^+-N and TN removal of 86.9%, 77.4% and 80.0%, respectively, was also kept in the UMSR (Meng, 2016).

Though the developed UMSR performed well in synchronous removal of organics and nitrogen, pollutant removal efficiency of the microaerobic process is expected to be further improved to reduce capital investment and management cost. Compared with suspended activated sludge processes, biofilm processes have advantages in biomass retention, specific pollutant removal rate and resistance to shock loading, and has been widely used in wastewater treatment (Liu et al.,

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in press; Ning et al., 2014). Furthermore, biofilm also plays an important role in nitrogen removal because of the heterogeneous commensalism of aerobic nitrifiers and anaerobic denitrifiers (Tsushima et al., 2007; Zhang et al., 2014). Therefore, a microaerobic biofilm process was suggested to enhance the pollutant removal, especially the nitrogen removal, from the MFPW.

In the present research, an upflow microaerobic biofilm reactor (UMBR) was constructed and employed to treat MFPW that was collected from the same pig farm as that in the previous reports (Li et al., 2016; Meng et al., 2015, 2016b). Its performance in pollutant removal was evaluated under various reflux ratios and COD/TN ratios, compared with the previous UMSR. To get a comprehensive insight into the biological mechanism for the simultaneous removal of COD, NH_4^+-N, TN and total phosphorus (TP) in the UMBR, functional microbial populations in the biofilm of the microaerobic process were further investigated by 16S rRNA gene amplicon on the Illumina Miseq platform.

2. Materials and methods

2.1. Microaerobic treatment systems and seed sludge

Fig. 1A illustrated the UMSR used in the previous studies (Li et al., 2016; Meng et al., 2015). To enhance the pollutant removal efficiency by biofilm, a UMBR (Fig. 1B) was constructed with the same configuration and size of the UMSR (Fig. 1A). PVC carriers with the specifications of Φ16 × 10 mm were loaded into the UMBR to construct a 200 mm high packed bed with a natural accumulation porosity of about 95%. As shown as Fig. 1, both of the microaerobic reactors were composed by a 0.5-meter-high plexiglass column with a working volume of 4.9 L, respectively. The piggery wastewater was introduced into the reactor by a peristaltic pump. Part of the effluent was aerated and then recirculated into the reactor to maintain an internal microaerobic condition with a DO of about 0.5 mg/L all through the operation process.

The seed sludge used for UMBR inoculation was collected from the UMSR (Li et al., 2016). When the activated sludge was collected, the UMSR was being operated at HRT 8 h, with COD, NH_4^+-N and TN in influent of about 114, 294.1 and 328.1 mg/L, respectively. The removal of COD, NH_4^+-N, TN and TP in the reactor averaged 66.2%, 92.4%, 91.4% and 57.5%, respectively. The initial biomass in the UMBR was 1.19 and 0.65 g/L in terms of mixed liquor suspended solids (MLSS) and in terms of mixed liquor volatile suspended solids (MLVSS), respectively.

2.2. Feed and operation of the UMBR

The raw manure-free piggery wastewater was collected from a local pig breeding farm in Harbin, China. The quality of the wastewater fluctuated following the breeding seasonality. COD, NH_4^+-N, NO_2^--N, NO_3^--N, TN, TP and pH ranged from 99 to 561 mg/L, 170.3 to 367.9 mg/L, 0.0 to 0.6 mg/L, 0.0 to 21.3 mg/L, 170.6 to 368.5 mg/L, 8.0 to 22.7 mg/L and 7.0 to 8.4, respectively, without deliberate control.

Based on reflux ratio and the wastewater quality of MFPW, the UMBR was operated in a 4-stage procedure including startup stage with a constant HRT of 8 h and temperature of 35 ± 1 °C. Wastewater quality including COD/TN ratios and the key controlling conditions including reflux ratios in the 4 stages are illustrated in Table 1.

2.3. Analytical methods

COD, NH_4^+-N, NO_2^--N, NO_3^--N and TP in influent and effluent of the UMBR were daily measured according to the Standard Methods (APHA, 2005). MLSS and MLVSS in the reactor were also analyzed according to the Standard Methods (APHA, 2005). DO, pH and TN were daily measured with a dissolved oxygen meter (Taiwan Hengxin, AZ 8403), a pH meter (Switzerland Mettler Toledo, DELTA320) and a total nitrogen analyzer (Germany Analytikjena Multi, N/C 2100S), respectively.

2.4. High-throughput pyrosequencing

Biofilm on the carriers in the steady phase of Stage 2, Stage 3 and Stage 4 (Table 2) were sampled from the UMBR and named S_{S2}, S_{S3} and S_{S4}, respectively. The total DNA of the samples were extracted using the Bacteria DNA Isolation Kit (Power Soil DNA Isolation Kit-MOBIO Laboratories, CA) as that reported in the previous work (Meng et al., 2016a). The genomic DNA samples were amplified with the primers 341f (5′-CCCTACACGACGCTCTTCCGATCTG (barcode) CCTACGG GNGGCWGCAG-3′) and 805r (5′-GACTGGAGTTCCTTGGCACCCGA-GAATTCCAGACTACHVGGG TATCTAATCC-3′) for the V3-V4 regions (Antwi et al., 2017). Each of the PCR was performed in a 50 μL system loaded with 10 ng template DNA, 0.5 μL forward and 0.5 μL reverse primers (with the same 50 μM), 0.5 μL Taq (5 U/μL), 0.5 μL dNTP (10 mM each), 5 μL 10 × PCR buffer, PCR-grade sterile water added to 50 μL. The PCR program was as follows: 3 min at 94 °C followed by 5 cycles each including 30 s at 94 °C, 20 s at 45 °C and 30 s at 65 °C; and

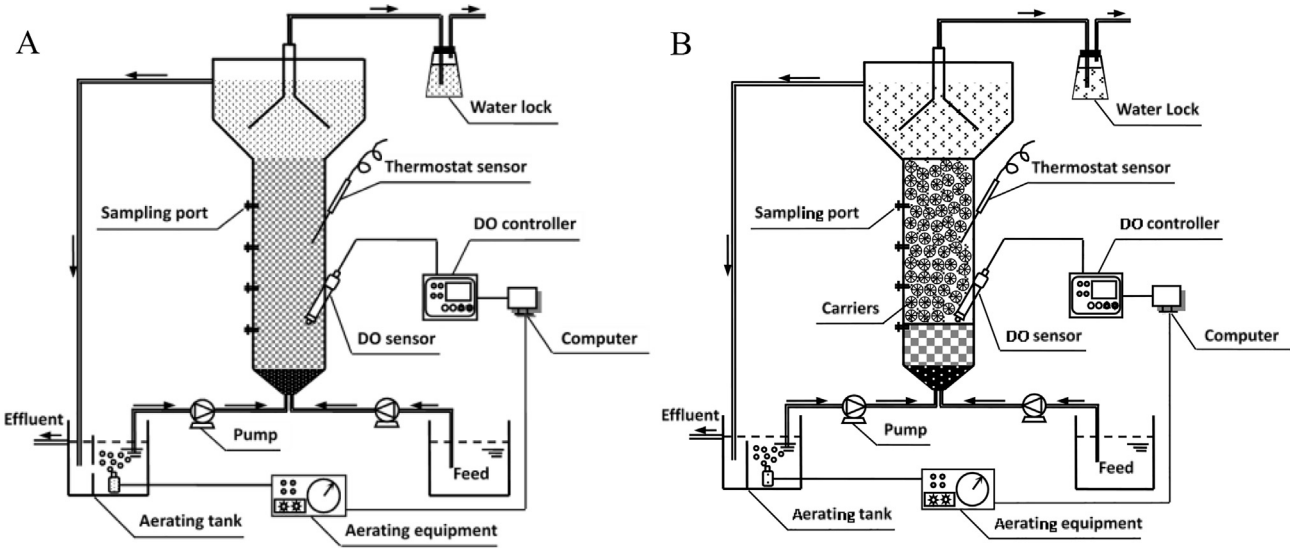

Fig. 1. Schematic representation of the lab-scale UMSR (A) and UMBR (B) processes.