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Influence of reflux ratio on two-stage anoxic/oxic with MBR for leachate treatment: Performance and microbial community structure

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ABSTRACT

A lab-scale two-stage Anoxic/Oxic with MBR (AO/AO-MBR) system was operated for 81 days for leachate treatment with different reflux ratio (R). The best system performances were observed with a R value of 150%, and the average removal efficiencies of chemical oxygen demand, ammonia and total nitrogen were 85.6%, 99.1%, and 77.6%, respectively. The microbial community were monitored and evaluated using high-throughput sequencing. *Proteobacteria* were dominant in all process. Phylogenetic trees were described at species level, genus *Thiopseudomonas*, *Amaricoccus*, *Nitrosomonas* and *Nitrobacter* played significant roles in nitrogen removal. Co-occurrence analyzing top 20 genera showed that *Nitrosomonas-Nitrobacter* presented perfect positive relationship, as well as *Paracoccus-Brevundimonas* and *Pusillimonas-Halobacteriovorax*.

1. Introduction

Landfill leachate is a kind of wastewater with high organic and nitrogen concentration (Chian and Dewalle, 1977). Moreover, the landfill leachate usually contains various polycyclic aromatic hydrocarbons, heterocyclic aromatic hydrocarbons, saturated aliphatic carboxyl compounds, aldehydes, phenols and other organic macromolecules, which are refractory to biodegradation (Zhang et al., 2015). The development of pertinent biological processes for landfill leachate treatment is challenging. Current studies showed that modified activated sludge processes (MASP) were satisfactory in performing the treatment of landfill leachate (Silva et al., 2013; Zhang et al., 2015). Chen et al. (2016) used a multi-stage process of anoxic/oxic/oxic/anoxic to realize nitrification and denitrification of landfill leachate, and the removal of ammonia (NH_4^+-N) and total nitrogen (TN) was stable at 95.0% and 66.4%, respectively. Anoxic/oxic (AO) process can effectively remove nitrogen via nitrification and denitrification. However, the landfill leachate usually presents a high chemical oxygen demand (COD) and nitrogen concentration, which have very great impact on sludge load, thus a new practical two-stage Anoxic/Oxic with MBR (AO/AO-MBR) was constructed to study the effectiveness and possible mechanisms of nitrogen removal from landfill leachate, as well as organic degradation (Liu et al., 2017). On the other hand, MBR can play a role of effective effluent separation from sludge, to ensure a high sludge concentration for enhancing pollutant removal (Boonnorat et al., 2014). Furthermore, successful implementation of this process is controlled by many factors, such as dissolved oxygen, hydraulic retention time (HRT), reflux ratio (R), temperature and so on. In general, the best biological treatment performance of landfill leachate in different regions were achieved with a dissolved oxygen (DO) of 2–4 mg/L, and a temperature of 25 °C. HRT and R was shown as the most important two parameters affecting the leachate treatment. According to our preliminary study on the treatment of Laohuchong landfill leachate (Shenyang, Liaoning Province, China), the suitable HTR of AO/AO-MBR was 7 d (Liu et al., 2018). This study will discuss the properties of landfill leachate treatment process with different R.

As the core of biological wastewater treatment processes, microorganisms play a key role in degrading and converting the pollutants (Bell et al., 2005; Chiu et al., 2015; Zhang et al., 2017). With high pollutant concentration of landfill leachate, the activity of microorganisms is usually inhibited, thus affecting the removal of organic matters and nitrogen. A thorough monitoring and analysis of microbial community composition and functions were necessary for in-depth understanding of this biological treatment process, which benefits the system manipulation and optimization (Yang et al., 2016). In previous studies, denaturing gradient gel electrophoresis (DGGE) was often used

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for only the detection of highly abundant bacterial population (Ma et al., 2013). However, rare species also have important contribution to the stability and function of biosystem (Isbell et al., 2011), more detail plotting of bacterial community structure and function is highly desirable. To date, few studies have been done on the functional microbial community in AO/AO-MBR process for landfill leachate treatment (Liu et al., 2018). Macro-genome Miseq sequencing platform has been adopted to detect the microbial community in different biosystems (Tang et al., 2017; Ye et al., 2018). The relationship between population community and environmental factors can be analyzed by means of bioinformatics methods, including phylogeny and significant difference of microbial population among different sludge samples (Widder et al., 2016; Qi et al., 2017).

In this study, the overall performance of pollutant removal of landfill leachate by AO/AO-MBR system was investigated with different R, and the composition of sludge microbes under the optimal operating conditions was analyzed. A series of bioinformatical analysis, such as phylogenetic trees and co-occurrence of five sludge samples in first-stage anoxic (A_1), first-stage oxic (O_1), second-stage anoxic (A_2), second-stage oxic (O_2) and MBR were performed to understand the denitrification mechanisms of AO/AO-MBR system.

2. Materials and methods

2.1. Experimental set-up and its operation

A lab-scale AO/AO-MBR system was established (as shown in Fig. 1). The system contained two AO bioreactors in series, followed by a MBR. Each AO reactor contained an anoxic zone (15 L) and an aerobic zone (25 L), and the volume of MBR was 20 L. A flat membrane (Dayi Tech Co., Ltd, China, pore size $\leq 0.10\,\mu m$, effective area = $0.1\,m^2$) module were vertically centered in the bioreactor. The landfill leachate was fed via a peristaltic pump (Longer, China) to the anoxic zone of first AO bioreactor and successively flowed past the AO/AO-MBR system. The landfill leachate was continuously mixed in anoxic zones with overhead stirrers (Youlian Tech Co., Ltd, China) at a speed of 7.5 r/min. The air was continuously supplied to the oxic zones at a flow rate of 0.7 L/d to maintain a DO concentration of about 3 mg/L and to alleviate the membrane fouling. Physical cleaning of the flat membrane was carried out every two days with tap water. The system continuously operated for 81 d with a HRT of 7 d, a sludge retention time (SRT) of 14 d, a sludge return ratio of 100%, and the mixed liquor suspended solid (MLSS) remained at 6.0 ± 0.5 g/L throughout the operation.

The landfill leachate was collected monthly from Laohuchong landfill as the feed of AO/AO-MBR system. The main characteristics of landfill leachate are shown in Table 1. The leachate showed a high COD and nitrogen concentration with a carbon to nitrogen (C/N) ratio around 2.0–7.4.

Table 1
Quality of landfill leachate.

Parameter	Value	Parameter	Value
COD (mg/L)	3516–21,080	NO_2^--N (mg/L)	0.19–2.91
NH_4^+-N (mg/L)	862.18–2828.02	pH	7.2–8.5
TN (mg/L)	1308.57–2835.55	Color	Dark brown
NO_3^--N (mg/L)	5.46–68.95	Smell	Strong

2.2. Wastewater analysis

Wastewater samples were collected every alternate day from the influent, the effluent of A_1, O_1, A_2, O_2, and MBR. COD was determined by a Lian-hua COD quick-analysis apparatus (Lian-hua Tech Co., Ltd, China). NH_4^+-N, nitrite (NO_2^--N), nitrate (NO_3^--N) and TN was measured according to standard methods (APHA, 2005). DO were determined by a WTW Oxi 3310 m (WTW Company, Germany). The pH was monitored by a WTW Multi 3420 m (WTW Company, Germany). MLSS was measured via gravimetric method.

2.3. Microbial community analysis

2.3.1. DNA extraction and high-throughput sequencing analysis

To investigate the bacterial community in AO/AO-MBR system, sludge samples were collected during stable operation (Day 47) under a reflux ratio 150% from A_1, O_1, A_2, O_2 and the MBR, respectively. The genomic DNA was extracted using an E.Z.N.A. Soil DNA Kit D5625-01 (Omega, USA) according to the protocol provide by the manufacturer. The DNA concentration in extractives was determined using a Qubit 2.0 DNA Assay Kit Q10212 (Life, USA).

The 16S rRNA genes in V3-V4 region were amplified from the genomic DNA using primer pairs 341F/805R containing Miseq sequencing platform universal adaptors. The fusion primer pairs 341F and 805R were 5′-ccctacacgacgctcttccgatctgCCTACGGGN-GGCWGCAG-3′ and 5′-gactggagttccttggcacccgagaattccaGACTACHVGGGTATCTAA-TCC-3′ (the adaptors in lowercase letters), respectively. The nucleotide barcodes inserted between the adaptors and the primers allowed sample multiplexing during the sequencing. The PCR products were pooled and purified by a SanPrep DNA Gel Extraction Kit SK8192 (Sangon Biotech, China). The concentration of purified products was quantified by a Qubit2.0 DNA Assay Kit Q10212 (Life, USA), then the purified products were mixed in equal amounts based on DNA concentration, followed by sequencing on the Miseq sequencing platforms

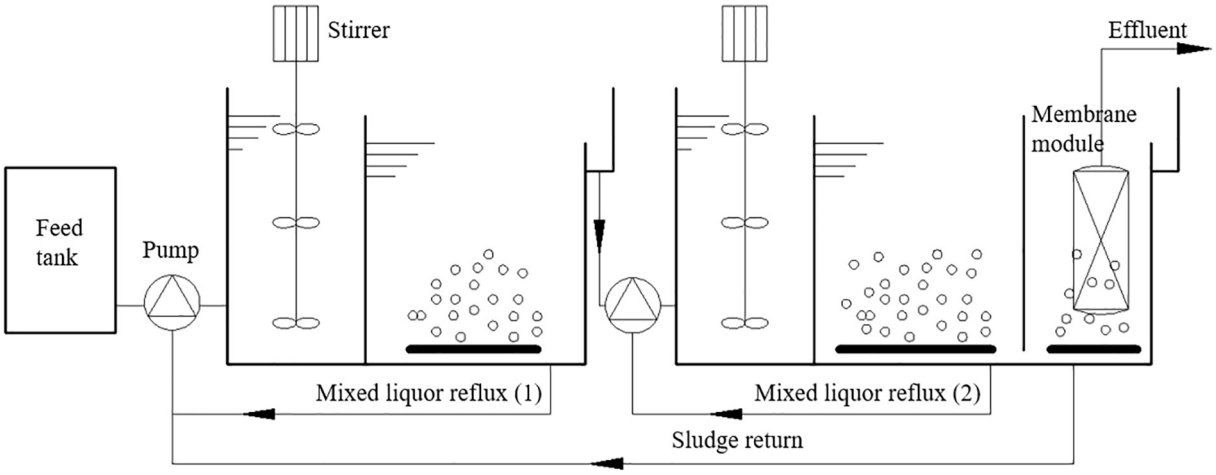

Fig. 1. Schematic diagram of AO/AO-MBR for landfill leachate treatment.