Microbial characteristics of landfill leachate disposed by aerobic moving bed biofilm reactor

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ABSTRACT

An aerobic moving bed biofilm reactor (MBBR) was applied to treat landfill leachate generated from a domestic waste incineration plant. Pollutant removal efficiency of this reactor under stable operating condition was studied. The biomass, bacteria species, and microbial metabolism in this reactor were investigated. These results showed that the average removal efficiency of chemical oxygen demand (COD) and ammonia nitrogen in the aerobic MBBR achieved 64% and 97% in the optimum conditions, respectively. The three-dimensional fluorescence spectrum revealed that the content of soluble microbial byproducts from extracellular polymeric substances extraction in suspended sludge was much higher than that on biofilm, and the types of pollutants were various in different regions of the reactor. It also indicated that the MBBR system had a stable, rich and regular microorganism community, including large amounts of nitrifying bacteria and denitrifying bacteria. Scanning electron microscopy suggested that biofilm attached to the packing provided a good anoxic–aerobic micro environment system to achieve a high metabolic activity, which favored COD and ammonia removal.

Key words | ammonia, denitrification, leachate treatment, MBBR, microorganism

INTRODUCTION

Incineration is one of the most common options for domestic solid waste treatment, which decreases weight and volume of municipal waste. One of the main problems during the stacking process is the production of leachate, which is known as a complex mixture of liquid effluent. The leachate contains high concentration of organic matter, heavy metals, ammonia nitrogen, and inorganic salts. The characteristics of leachate are influenced by many factors, including waste source, garbage collection time, and seasonal variation (Puig et al. 2014). Some organic matter, such as xenobiotic organic compounds and humic compounds, present a threat to human health and the environment if released to natural water (Mahmoud et al. 2016). Leachates need to be treated for the further discharge to sewer or disposal to the environment nearby. Several ways, such as physical, chemical and biological methods, are used together as a combined process to deal with landfill leachate. Physicochemical methods were usually used for the pretreatment of leachate (Deng & Ezyske 2011), while biological methods have been widely applied in pilot scale and practical engineering due to the advantages of low cost, effective management, and easy operation (Niu et al. 2016; Ye et al. 2016). Anaerobic treatment is usually applied to improve the biodegradability of raw leachate (Ağdag & Sponza 2005). However, after anaerobic treatment, the concentration of organic contaminants is still at a high level and ammonia increases slightly. Thus, aerobic treatment is used after the anaerobic process to further remove pollutants.

Based on the conventional activated sludge process, the aerobic process for leachate treatment still has several disadvantages, such as limited biological load, sensitivity to low temperatures and toxic compounds, and sludge loss (Chen et al. 2008). The moving bed biofilm reactor (MBBR), one of the biofilm systems, has been used in leachate treatment for recent years because of its good attached-growth and well mixing of wastewater. Suspended porous polymeric carriers in an MBBR always keep continuous movement in the aeration tank, and active biomass attaches to the carrier surface as a biofilm (Hajipour et al. 2011). On the basis of the conventional activated sludge process and fluidized-bed reactor, MBBR has the advantages of high biomass content, high chemical oxygen demand (COD) loading, small reactor...
Materials and Methods

Experimental reactor setup and operation

The experimental setup is shown in Figure 1. Anoxic, aerobic and precipitation zones were separated in an aerobic MBBR. Anoxic and aerobic zones were divided by a movable baffle. Sludge recirculation was set in the precipitation zone, and the nitrification liquid reflux was applied at the end of the aerobic zone. The size of anoxic zone and aerobic zone was 0.9 m × 0.3 m × 0.35 m (L × W × H), with the effective volume of 94.5 L. The size of precipitation zone was 0.245 m × 0.2 m × 0.25 m, with volume of 12.25 L. Polyvinylidene fluoride biofillers, with the average size of Φ12 mm × 12 mm, were added to the aerobic zone, the volume of which accounted for 20% of the reactor. In the aerobic zone, microporous aeration pipes were installed in the bottom of the tank for continuous aeration. The aeration strength was controlled by a rotor flow meter and fillers were kept suspended in water.

Anaerobic effluent entered the anoxic zone for the denitrification process, then passed through the aerobic zone for a further nitrification process. Continuous operation was used in this aerobic MBBR. The flow rates of influent, sludge reflux and nitrifying liquid reflux were all controlled by peristaltic pumps.

Leachate influent

The leachate was collected from the stocking process in a waste incineration plant, located in Henan Province, China. The raw leachate contains high concentration of COD (50,000–60,000 mg/L) and ammonia nitrogen (2,000 mg/L). The value of pH varied from 6 to 6.5. The leachate used in this study was the effluent of an anaerobic process, with COD concentration of 4,000–7,000 mg/L, ammonia concentration of 2,100 mg/L, and pH from 7.8 to 8.2.

Analysis indexes and methods

Analysis methods

Chemical oxygen demand (COD$_{\text{C}}$), ammonia nitrogen (NH$_4^+$-N), mixed liquor suspended solid, and mixed liquor volatile suspended solid were respectively analyzed by closed reflux colorimetric method, Nessler's reagent spectrophotometry method and gravimetric method according to the standard methods (APHA 2005). Dissolved oxygen (DO) was measured using a digital DO meter (HQ30d, HACH, USA) and pH was measured by a pH meter (PHS-3C, INESA Scientific Instrument Co. Ltd, China). The biomass attached on the packing was first desquamated by ultrasonic vibration for 15 min, then the mixture was filtered by 0.45 um Millipore filter; after drying at 105 °C, the dry weight measured was attached biomass (Chen et al. 2008). The SOUR (specific oxygen uptake rate) test was carried out according to the US Environmental Protection Agency, using a DO probe to measure changes in the oxygen concentration of the mixture of suspended sludge and packing.

Figure 1 | Diagram of experimental device.
**EPS extraction**

At present, several methods can be applied for the extraction of extracellular polymeric substances (EPS), including ultrasonic method, high speed centrifugation, heating, NaOH method, sulfuric acid process, cation exchange resin, and formaldehyde–sodium hydroxide method (D’Abzac et al. 2010). A kind of optimization method was adopted in this study, and the extraction processes were as follows. The sludge samples in beakers were heated in a water bath at 80 °C and stirred under 600 rpm for 30 min. Then the samples were cooled to room temperature and stored in a refrigerator at 4 °C. Suspended sludge of 35 mL was sampled and centrifuged for 10 min at the speed of 400 rpm. The precipitates were kept and 0.85% NaCl solution was added to 50 mL. The mixed solution was centrifuged for 20 min at 3,000 rpm. The addition of NaCl and centrifugation were repeated three times. Then, 0.85% NaCl solution was added, and heated in a water bath at 80 °C. Finally, the samples were cooled to room temperature, and centrifuged for 10 min at the speed of 8,000 rpm. The supernatant was extracted by syringe then filtered with 0.45 um filter. The filtrate obtained was EPS extraction (Zheng et al. 2015). In this study, three-dimensional excitation emission matrix (EEM) fluorescence spectroscopy was applied to characterize the EPS in MBBR from anoxic zone, aerobic zone and biofilm on packing respectively.

**Scanning electron microscopy test**

Suspended sludge samples were first fixed for 2 hours in 2.5% glutaraldehyde solution, then they were stored in a refrigerator at 4 °C. After centrifugation for 5 mins at 8,000 rpm, the glutaraldehyde solution was discarded, PBS buffer (pH = 6.8) was added, and the samples were left to stand for 10 min and then centrifuged for 5 min at 8,000 rpm. The upper PBS solution was discarded and this step was repeated three times. For the dry-sectioning method, the sludge samples were dehydrated after fixation in an ethanol series (50%, 70%, 80%, 90%, 95%, 100%, 20 min per step), among which the 100% alcohol gradient dehydration was repeat for three times. At last, the supernatant was discarded, and the samples were put in a freezer at minus 80 °C (Alphenaar et al. 1994). The dry samples were fixed on a gold plate, and observed with scanning electron microscopy (SEM).

**RESULTS AND DISCUSSION**

**The effect of aerobic MBBR in stable operation**

The results for COD and ammonia removal performance during 1 month operation are shown in Figures 2 and 3. The removal efficiency of COD in aerobic MBBR was about 60–70%, the maximum removal efficiency was close to 80%, and the average removal efficiency was 64.3%. As shown in Figure 3, the removal efficiency of ammonia nitrogen in aerobic MBBR system was above 90%, and the average removal efficiency was 97.4%. During the stable operation period, the COD removal efficiency of MBBR was above 60% when COD concentration in influent was 5,000 mg/L, the concentration of ammonia nitrogen was about 2,100 mg/L, and C/N ratio was nearly 2.5. After the concentration of COD in influent increased to 6,000 mg/L, the removal efficiency of COD
increased to over 65%. This was related to the increase of C/N ratio and the enhancement of microbial metabolic activity in the reactor. Previous studies have suggested that ammonia with high concentration has an inhibitory effect on the nitrification process, and it was known that free ammonia inhibited *Nitrobacter* and *Nitrosomonas* activities (Anthonisen et al. 1976). However, with the COD concentration increase in influent, C/N ratio also increased, the ability of microorganisms to remove pollutants was also enhanced, and the removal efficiency was relatively stable. The results indicated that aerobic MBBR system had a strong loading capacity and had a good performance on the pollutant removal for leachate in low C/N ratio.

**Analysis of biomass and biological activity**

SOUR, biomass of suspended sludge and biofilm on the fillers were measured, and the results are shown in Figure 4. The total biomass in aerobic zone was above 3,200 mg/L and the highest biomass was up to 4,300 mg/L. The biomass of biofilm attached to the packing was relatively stable, keeping from 1,000 mg/L to 1,500 mg/L, and it accounted for about 30% of the total biomass. The biomass of suspended sludge was from 2,000 mg/L to 3,300 mg/L, fluctuating very obviously. The results showed that SOUR was closely related to the biomass in the reactor, which changed with biomass. As shown in Figure 4, the value of SOUR varied from 17 mg·O₂/gSS·h to 22 mg·O₂/gSS·h with the change of biomass in aerobic zone. Comparing the first six results (No. 1 to No. 6) in Figure 4, SOUR value decreased when the biomass of the suspended sludge decreased, while the attached biomass kept steady. When the total biomass was unchanged (experiments from No. 4 to No. 9), the biomass of suspended sludge decreased and the attached biomass increased, while SOUR value kept steady. It was found from experiments No. 7 to No. 12 that the SOUR value increased, when the biomass of suspended sludge and packing all increased. By comparing the variation of biomass and SOUR in the reactor, it could be concluded that the total amount of microbes in MBBR aerobic zone was around 3,500 mg/L, which maintained at a high biological activity.

**Fluorescence analysis of EPS**

As the main components of bio-aggregates such as biofilm, sludge flocs and sludge mixtures, EPS consist of proteins, polysaccharides, nucleic acids, lipids, humic acids and other extracellular polymers (Duan et al. 2015). The amount of EPS in activated sludge affects the structure of activated sludge floc, sedimentation and adsorption properties of activated sludge.

The EEM spectra in the different EPS fractions are presented in Figure 5. Figure 5(a)–5(c) were from suspended sludge in anoxic zone, aerobic zone and biofilm attached to the packing from aerobic zone respectively. Three main peaks could be identified from Figure 5(a): peak A was located at the excitation/emission wavelengths (Ex/Em) of 280/350 nm and reported as soluble microbial byproduct-like material, and the second peak (peak B) was observed at the Ex/Em of 230/340 nm and described as tryptophan protein-like material (Wen & Westerhoff 2003). The third peak (peak C) occurred at the Ex/Em of 230/306 nm and characterized as tyrosine protein-like material (Ahmad & Reynolds 1999). There were two main peaks in Figure 5(b), corresponding to peak A and peak B, and another peak (peak D) occurred at the Ex/Em of 365/416 nm and was associated with humic acid-like material (Wen & Westerhoff 2003). Three main peaks were also observed from Figure 5(c): they were peak A and peak D and peak E. Peak E was located at Ex/Em of 245/400 nm and it represented fulvic acid-like materials (Mobed 1996).

Some additional information about the chemical nature of the biological material could be provided through the peak intensity ratios (Sheng & Yu 2006). Generally speaking, the concentration of organic matter was proportional to the intensity of the corresponding fluorescence. Different peak intensity ratios and fluorescence values are shown in Table 1. Soluble microbial byproduct-like material existed in all parts of the reaction, and constituted 69% and 65% in EPS from anoxic zone and aerobic zone respectively much higher than that of 33% on biofilm. Tryptophan protein-like material existed in anoxic and aerobic zone, while
tyrosine protein-like material was only observed in anoxic zone and humic acid-like material only existed in aerobic zone. Compared with the EPS from suspended sludge, EPS from biofilm on the packing in aerobic zone did not contain protein but contained fulvic acids.

According to Table 1, compared with Figure 5(b) and 5(c), it could be found that the location of peak A in this study was blue-shifted. Blue shift was mainly related to the splitting decomposition of complex aromatic ring system and the break-up of large molecules into smaller fragments. It mainly involved the following two aspects: one is a reduction in the degree of the π-electron system, such as the reduction of the aromatic ring, a reduction of conjugated bonds in a chain structure, or a conversion of a linear ring system to a non-linear system; the other one is the reduction of particular functional groups including amine, hydroxyl, carboxyl, carbonyl and other functional groups in the organic structure (Coble 1996). Thus, the fluorescence characteristics could be well correlated with the decomposition of organic matter, and it is obvious that the complex organic matter in the reactor could be degraded by the microorganisms through the metabolic activity. The fluorescence intensity also indicates that there was much humic acid and fulvic acid on the biofilm, but the content was little in suspended sludge. The reason may be the adsorption of humic acid by biofilm, while the suspended sludge did not have this function. The results of EPS analysis showed that the packing used in this study was a good carrier of microorganisms, which could promote the removal of pollutants.

### Study on microbial species and morphological

### Study on microbial species

In this study, the phylogenetic classification of effective bacterial sequences were conducted at phylum and genus...
taxonomic levels. Proteobacteria, Bacteroidetes, Firmicutes, Deinococcus-Thermus and Actinobacteria were dominant in the community from anoxic and aerobic zone at the stable operation period (Figure 6). The relative abundance of the total amount was more than 90%.

The most abundant phylum was Proteobacteria, with more than 50% of the sequences on average. Previous research indicated that it had been found as the dominant phylum in various municipal wastewater treatment plants (Ma et al. 2015). The following major phyla were Bacteroidetes, Firmicutes, Deinococcus-Thermus and Actinobacteria, which were also widespread in various bio-treatment systems. Previous studies have shown that Proteobacteria was mainly separated from fecal and anaerobic sludge, and has the function of degrading organic matter. Bacteroidetes, which could exist in high salinity environment, were reported to possess the capability of degrading macromolecular carbohydrates, and can realize the effective removal of COD and ammonia nitrogen. Some refractory substances, such as cellulose, can be degraded into small molecular substances by Firmicutes, which could produce endophytic spores; thus, it had a strong resistance to the extreme environment of leachate, and is meaningful for the stable operation of MBBR.

The phylogenetic classification at genus taxonomic levels from different zones is shown in Figure 7. It is obvious that microorganisms from anoxic zone, aerobic zone, and biofilm were similar but their relative abundances were slightly different. The genus Thauera belonging to Proteobacteria phylum was dominant both in anoxic and aerobic zones, but little on biofilm. Thauera genus was reported to be capable of denitrification and biodegradation of organic compound, which had been identified in wastewater bioreactors (Liu et al. 2006). The content of Thauera, Pseudomonas, Hydrogenophaga, Luteimonas, Serpens and Tissierella was higher in suspended sludge from anoxic and aerobic zone compared to that from biofilm, which indicated that the denitrification process was mainly completed in the suspended sludge from anoxic and aerobic zone. However, the contents of Nitrosomonas and Nitrobacter on the packing were higher than that in the aerobic suspended sludge, which indicated that the nitrification reaction was mainly completed in the biofilm. Thauera, Pseudomonas and Paracoccus belong to aerobic denitrifying bacteria. In aerobic environment, their main function is to convert nitrate nitrogen into nitrogen, which can be released into the atmosphere, and organic matter can also be degraded by these bacteria, which play an important role in the process of biological nitrogen removal (Liu et al. 2006). The main function of dominant species in the samples from different areas are shown in Table 2.

Results of high-throughput sequencing analysis indicated that organic matter in leachate could be removed effectively by microorganisms. Ammonia nitrogen was mainly removed through the nitrification–denitrification processes; aerobic denitrifying bacteria had a high abundance in this system, and also had a high growth rate and high denitrification rate, and they could adapt to the environment well.

Study on microbial morphology

SEM analysis of sludge and biofilm is shown in Figure 8. The samples were observed at two different magnifications with zoom of 3,000 and 10,000. Under the zoom of 3,000, a rough appearance and irregular shape of suspended sludge samples could be seen, with many gaps in the sludge, showing a disordered state. However, a smooth appearance and three-dimensional structure were presented for biofilm, which could form a good anoxic-aerobic
microenvironment. There were many pores on the surface of the biofilm, which could connect the inside and outside of the biofilm through material exchange channels.

Additionally, a high magnification with zoom of 10,000 is also presented in Figure 8. Cocci and bacilli were dominant in the suspended system. The number of microbes in
sludge was not very large, but they were evenly distributed around the sludge particles. However, rich microorganisms were observed on the biofilm; the main microorganisms were bacillus, arranged neatly on the sample.

High-throughput sequencing results indicated that there were abundant nitrifying bacteria and denitrifying bacteria in the reactor. Nitrobacter and Nitrosomonas were the two main nitrifying bacteria, while denitrifying bacteria included Bacillus, Alcaligenes (bacilli), Paracoccus and Hyphomicrobium (coci). Most of the bacteria observed by SEM were cocci and bacilli, which were consistent with the results of high-throughput sequencing. And the operating mechanism of MBBR was better explained from the microscopic point of view.

CONCLUSIONS

Aerobic MBBR operated under the optimum conditions achieved 64% COD removal and 97% ammonia nitrogen removal, with initial COD concentration of 4,000 mg/L–7,000 mg/L and initial NH₄⁺-N from 1,800 mg/L to 2,500 mg/L.

The total microbial biomass in MBBR was abundant and stable, with a high metabolic activity. In particular, the microorganisms attached to the packing had a high metabolic activity and stable biomass. The amount of biomass was improved after the usage of packing in the reactor. The type of organic matter degraded by microorganisms from different zones of MBBR was different.

The results of high-throughput sequencing and SEM analysis showed that suspended sludge from anoxic zone, aerobic zone and biofilm on the packing were beneficial for microorganisms to grow. The predominant bacteria of bacillus and coccus belonged to Proteobacteria, Bacteroides and Firmicutes. Proteobacteria contained a variety of nitrifying bacteria and denitrifying bacteria, which favored COD and ammonia removal in the aerobic MBBR.

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