Journal of Bioscience and Bioengineering
VOL. xx No. xx, 1–5, 2017

www.elsevier.com/locate/jbiosc

Simultaneous chemical oxygen demand removal, methane production and heavy metal precipitation in the biological treatment of landfill leachate using acid mine drainage as sulfate resource

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Received 19 July 2016; accepted 11 February 2017
Available online xxx

Biological treatment played an important role in the treatment of landfill leachate. In the current study, acid mine drainage (AMD) was used as a source of sulfate to strengthen the anaerobic treatment of landfill leachate. Effects of chemical oxygen demand (COD) and SO_4^{2-} mass concentration ratio on the decomposition of organic matter, methane production and sulfate reduction were investigated and the microbial community was analyzed using the high throughout methods. Results showed that high removal efficiency of COD, methane production and heavy metal removal was achieved when the initial COD/SO_4^{2-} ratio (based on mass) was set at 3.0. The relative abundance of anaerobic hydrogen-producing bacteria (*Candidatus Cloacamonas*) in the experimental group with the addition of AMD was significantly increased compared to the control. Abundance of hydrogenotrophic methanogens of *Methanosarcina* and *Methanomassiliicoccus* was increased. Results confirmed that AMD could be used as sulfate resource to strengthen the biological treatment of landfill leachate.

[**Key words:** Acid mine drainage; Anaerobic digestion; Chemical oxygen demand/sulfate ratio; High-throughput sequencing; Landfill leachate]

The sanitary landfill method for the ultimate disposal of municipal solid waste continues to be widely used due to its economic advantages. Leachates are defined as the aqueous effluent generated as a consequence of rainwater percolation through wastes, biochemical processes and the inherent water content of wastes themselves. Leachates may contain large amounts of organic matter (biodegradable, but also refractory to biodegradation), as well as ammonia-nitrogen, heavy metals, chlorinated organic and inorganic salts (1). Currently, landfill leachate is treated commonly by a combination of anaerobic/aerobic biological reactions, physical and chemical reactions (2,3).

Organic matter and heavy metals were two key issues in the treatment of landfill leachate. Biological process plays an important role in the decomposition and degradation of organic matters in the landfill leachate. As reported previously complicated organic compounds in the industrial wastewater had great removal efficiency under the sulfigenic conditions (4,5). According to the principle of thermodynamics sulfate reducing bacteria (SRB) would compete with the methanogenic microorganisms and methane production was inhibited. On the other hand, in the identical reactor with a high initial organic loading would provide efficient carbon source for the sulfate reduction and methane production process. The generated sulfide would precipitate the heavy metals. In such reactors recovery of methane gas could be achieved as well as the precipitation of heavy metals by the generated sulfide. This would be helpful to resolve the two key problems in the treatment of landfill leachate.

Acid mine drainage (AMD), a major environmental concern currently facing active and inactive mining industries throughout the world, results from chemical and biological oxidation of residual sulphide minerals. The seepage of such acidic discharges into the water system constitutes a potential risk to natural ecosystems. AMD potentially can be used as a sulfate source and results in a passive sulfidogenic system treatment process to increase the treatment efficiency of landfill leachate. Furthermore, it is crucial to comprehensively understand the microbial mechanisms for the fundamental improvement of the anaerobic digestion. Development of the next generation high throughput sequencing technologies such as Illumina Miseq enables us to determine a much larger number of sequences in a shorter analytical time and has been developed to fully explore the microbial community in various environments (6,7). However, few studies have been conducted on anaerobic treatment of landfill leachate using AMD as the sulfate sources.

Therefore, the objectives of the study were: (i) to test the feasibility of simultaneous COD removal, methane production and heavy metal precipitation with the addition of AMD; (ii) to find out the optimal initial ratios of COD/SO_4^{2-} (based on mass); (iii) under the optimum conditions, the microbial community was analyzed using a high through testing method.

MATERIALS AND METHODS

Experimental design Landfill leachate was collected from the Long Quan Shan landfill located in Hefei, Anhui province, China. AMD was taken from an

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http://dx.doi.org/10.1016/j.jbiosc.2017.02.009

Please cite this article in press as: Li, Y.-L., et al., Simultaneous chemical oxygen demand removal, methane production and heavy metal precipitation in the biological treatment of landfill leachate using..., J. Biosci. Bioeng., (2017), http://dx.doi.org/10.1016/j.jbiosc.2017.02.009

TABLE 1. Characteristic of landfill leachate and AMD.

	COD/mg/L	SO_4^{2-}/mg/L	TN/mg/L	NH_3-N/mg/L	NO_3-N/mg/L	pH
Land fill leachate	20078 ± 300	272 ± 18	2665 ± 65	2017 ± 11	26 ± 2	7.40 ± 0.30
AMD	400 ± 50	10013 ± 97	71 ± 5	45 ± 2	14 ± 1	2.75 ± 0.48

abandoned mine in Ma'anshan, China. The major composition of leachate and AMD are listed in Table 1. Anaerobic digestion tests were performed in serum bottles with a working volume of 500 mL. The volume of landfill leachate added into all reactors was 180 mL. AMD and distilled water were mixed with the landfill leachate to make three COD/SO_4^{2-} ratios (1.0, 3.0 and 5.0) based on mass. The reactors were labeled as reactor 1.0, reactor 3.0 and reactor 5.0, respectively. Leachate without added AMD was set as the control group. All treatments were carried out in three replicates. The initial pH was adjusted to 7.0 ± 0.1, and the reactors were purged with argon gas for 5 min and sealed with aluminum caps to maintain anaerobic conditions. The reactors were placed an in incubator maintained at 35 ± 1°C.

Analytical methods Methane was determined by gas chromatography (GC-2010, Shimadzu Co., Ltd.) equipped with a RTX-1 column (30 m × 0.25 mm × 0.25 μm). The supernatant liquid was filtered through 0.45 μm membranes. Elemental HS^-/S^{2-} and H_2S were determined by the Gas-phase molecular absorption spectrometry method (8). SO_4^{2-} was measured using ion chromatography (Dionex ICS-3000) COD was determined based on the standard methods. Metal concentrations were measured were measured by atomic absorption spectrometer (WYG 2200, Wayee, China).

The microbial populations in selected reactors (reactor 3.0 and the control) were analyzed following the published report (9,10). The genomic DNA was extracted using Power Soil DNA Isolation Kit (MO BIO Laboratories, USA) according to the manufacture's protocols. Polymerase chain reaction (PCR) amplification was carried out using the primer pairs 515F/806R and Arch349F/Arch806R for bacteria and archaea, respectively. PCRs were performed in a total volume of 50 μL containing 6 μL 10× Ex Taq Buffer, 6 μL dNTPs, 0.6 μL BSA, 0.3 μL Ex Taq, 1.2 μL forward primer, 1.2 μL reverse primer, and 1 μL template DNA. The PCR amplification was performed under the following thermocycling steps: initial denaturation at 94°C for 5 min, followed by 31 cycles of denaturing at 94°C for 30s, annealing at 52°C for 30s, and extension at 72°C for 45s, followed by a final extension at 72°C for 10 min. Each sample was amplified in triplicate. The resulting PCR products were pooled and purified.

After purification, a mixture of the 16S rRNA PCR products was used for sequencing on the Illumina Miseq platform according to the manufacturer's instructions which was performed by a commercial company (Magigen, Guangdong, China). Sequencing was performed using a 2 × 250 paired-end (PE) configuration, and image analysis and base calling were conducted using the MiSeq Control Software on the MiSeq instrument.

RESULTS AND DISCUSSION

Reactor performance Removal efficiency of COD in the AMD-dosed reactors (reactors 3.0 and 5.0) was improved to 71.5 ± 3.1% and 61.7 ± 1.8% (Fig. 1), respectively, which was significantly higher than for the control and reactor 1.0. At the same time, the removal efficiency of SO_4^{2-} were 67.7 ± 3.5%, 91.5 ± 1.8% and 90.1 ± 2.4% in reactors 1.0, 3.0 and 5.0, respectively, which approximated the reported data in the biological treatment process.

As shown in Fig. 2, methane production yield from the landfill leachate was significantly enhanced by the addition of AMD. A maximum methane yield of 100 mL/g initial COD was achieved in reactor 3.0 which was significantly higher compared to the other reactors. On the other hand, the methane production rate was also significantly influenced by the addition of AMD. Firstly, the reduction of SO_4^{2-} in the AMD would consume electron donors which could be used for methane production. Secondly, the generated S^{2-}/HS^- would have a negative toxicity on MPA. This mainly depends on the equilibrium between HS^- and soluble H_2S, which is pH dependent (11). The system pH increased rapidly to above 8.0 and fluctuated around 8.6 (Fig. S1). The main component is the less toxic dissociated HS^-(Fig. 3). This partially decreased the inhibition or toxic effect of soluble sulfur compound on the MPA and SRB. Thirdly, the heavy metal in the AMD would had a negative inhibition on the biological activity in the systems. However, this inhibition effect could be weakened by the precipitation of generated CO_3^{2-} and S^{2-}. As listed in Table S1, the concentrations of Fe, Ni, Mn, Cu, and Zn at the end were quite low and the removal efficiencies were close to 99% (Table S2). XRD analysis results confirmed the component of precipitates were $CuFeS_2$ and $CaCO_3$ (Fig. S2). Anticipated precipitates of $MgCO_3$, ZnS and MnS were not found which could attributed to lower crystallinity. Results showed that heavy metal was able to be removed efficiently in the simultaneous treatment of the mixture.

These could explain the methane production in the reactors. In the reactors 3.0 and 5.0, accumulation rate and final concentration of HS^- were both lower compared to reactor 1.0. This meant a lower inhibition effect on the methanogenic microorganisms caused by HS^-. Because of the relative high concentration of heavy metal in the reactor 5.0 (Table S2), the methane production was also lower compared to reactor 3.0 (Fig. 2). With the inhibition of HS^- and heavy metals, the methane production in reactor 1.0 was low compared to reactors 3.0 and 5.0.

HS^-/S^{2-} generated in the sulfate reduction process still residues in the effluent which might be helpful for the nitrogen removal (12,13). A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process has been developed based on the sulfur cycle for the biological nitrogen removal process (13). The effluent could be treated further by anoxic and aerobic processer to remove the ammonium and the residue S^{2-}. S^{2-} in effluent could be

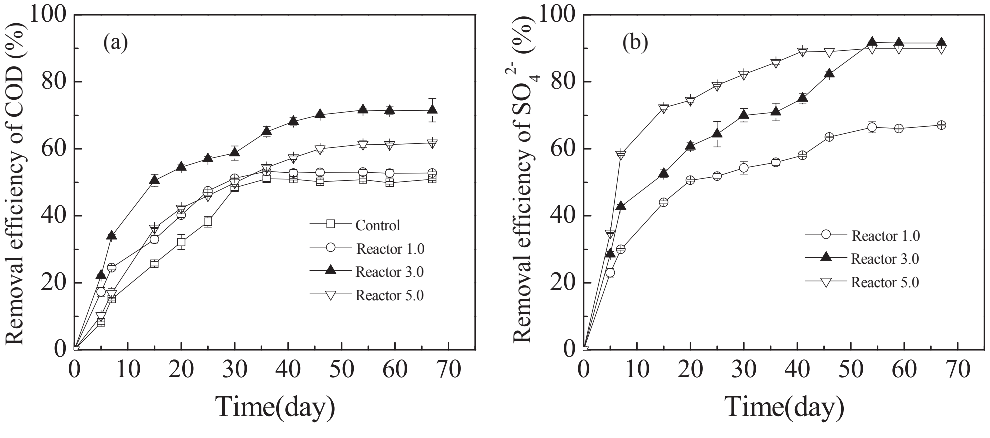

FIG. 1. Removal efficiency of COD and SO_4^{2-} in the reactors.

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