ACCELERATIVE STABILIZATION OF SOLID WASTE IN ANAEROBIC/AEROBIC LAB-SCALE LANDFILL BIOREACTORS

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ABSTRACT
This study investigated microbial population dynamics and performance in lab-scale conventional, anaerobic, and aerobic landfill reactors. Each reactor (2.35 L) was loaded with 1.5 kg of synthetic municipal solid waste. The conventional reactor was operated without leachate recirculation and aeration, but the other reactors used leachate recirculation at 200 mL/d and without aeration (anaerobic bioreactor) or with aeration at 2 L/min (aerobic bioreactor). The respective final waste volumes on day 138 of the conventional, anaerobic, and aerobic reactors were approximately 75, 65, and 60% of the initial volumes. Leachate recirculation in the anaerobic bioreactor accelerated biochemical reactions and promoted methane production. However, leachate from the anaerobic bioreactor showed TOC and NH_4-N concentrations that were as high as those of the conventional reactor. Aeration lowered leachate production and methane concentration and decreased organic matter in the solid waste and leachate. The MPN value of *amoA* gene reached 10^5 MPN-copies/g-dry in the aerobic bioreactor, where nitrogen was removed from the solid waste and leachate.

KEYWORDS
aeration, DNA monitoring, landfill bioreactor, leachate recirculation, solid waste

INTRODUCTION
Conventional landfills have been operated without moisture control in the waste. In such operations, buried wastes decompose slowly, thereby necessitating long-term management of landfills. The most common method to hasten biodegradation is to add supplemental water and/or recirculate leachate to the waste. Such landfills with moisture control are designated as bioreactor landfills. Methane production and solid-waste stabilization are accelerated in anaerobic bioreactor landfills (Barlaz et al., 1990). Aerobic bioreactor landfills with aeration provide more rapid biodegradation of organic matter, mostly into CO_2 and water, reduce methane production, and enhance ammonia-nitrogen removal (Borglin et al., 2004; Mertoglu, et al., 2006).

Many investigations of landfill stabilization have evaluated physical and chemical characteristics of solid wastes and/or landfill emissions. However, several studies have specifically investigated microorganisms in bioreactor landfills (Huang, et al., 2004; Mertoglu et al., 2006). Heterotrophic bacteria play a fundamental role in the biodegradation process. Fungi also decompose organic matter, especially cellulose residues. Ammonia-nitrogen (NH_4-N) is converted to nitrate-nitrogen (NO_3-N) via nitrite-nitrogen (NO_2-N) in aerobic conditions. The *amoA* gene coding for a subunit of ammonia monooxygenase is a marker for ammonia-oxidizing bacteria (Rotthauwe et al., 1997). During the denitrification process, NO_3-N is converted into gaseous nitrogen in anaerobic conditions. The *nirK* encoding a cytochrome cd_1 nitrite reductase and *nirS* genes encoding a Cu-containing nitrite reductase are markers for denitrifying bacteria (Zumft, 1997).

This study investigated the effects of leachate recirculation and aeration on landfill stabilization and characteristics of leachate and gases generated from lab-scale bioreactor landfills. Population dynamics of microorganisms in the landfills were also studied by monitoring eubacterial 16S rDNA, fungal 18S rDNA, *amoA*, *nirS*, and *nirK* genes.

MATERIALS AND METHODS

Three acrylic cylindrical bioreactors of 10 cm diameter, 30 cm height, and 2.85 L in total volume were constructed. The three reactors were maintained at 28°C. Deionized water, simulating rainfall, was added to all reactors using peristaltic pumps. The first reactor was operated without leachate recirculation and aeration (conventional reactor). The second reactor was operated with leachate recirculation at 200 mL/d using a peristaltic pump and without aeration (anaerobic bioreactor). The third reactor was controlled with leachate recirculation at 200 mL/d and aeration at 2 L/min for creating a microaerobic condition (aerobic bioreactor). The sludge cakes collected from a wastewater treatment plant in Osaka, which treats mainly domestic wastewater were minced and mixed with wood chips and dry dog food as bulking agents at a dry weight ratio of 2:2:1. The synthetic solid waste of 1.5 kg was compacted into 2.35 L of each reactor (640 kg/m^3 density).

The DNA extracted using a bead beating kit was serially diluted ten-fold at each step, and three samples of each dilution were subjected to most probable number–polymerase chain reaction (MPN-PCR). The primers EUBf-933 (5'-GCACAAGCGGTGGAGCATGTGG-3') and EUBr-1387 (5'-GCCCGGGAACGTATTCACCG-3') were used for MPN-PCR targeting 16S rDNA. The primers EF4 (5'-GGAAGGGRTGTATTTATTAG-3') and Fung5 (5'-GTAAAAGTCCTGGTTCCCC-3') were used for 18S rDNA. The primers amoA-1F (5'-GGGGTTTCTACTGGTGGT-3') and amoA-2R (5'-CCCCTCKGSAAAGCCTTCTTC-3') were used for *amoA*. The primers NIRK-F (5'-TCATGGTGCTGCCGCGKGACGGA-3'), NIRK-R (5'-GAACTTGCCGGTKGCCCAGAC-3') and NIRS-F3 (5'-CCTAYTGGCCGCCRCART-3'), NIRS-R3 (5'-CGTTGAACTTRCCGGT-3') were used for *nirK* and *nirS*, respectively.

RESULTS AND DISCUSSION

Water balance in landfill reactors

The moisture content in the solid waste increased from 62% on day 0 to 70–75% on day 7 by the supplemental water addition. After day 13, the moisture contents in the anaerobic and aerobic bioreactors with leachate recirculation were higher than those in the conventional reactor. The cumulative volume of leachate produced from the two bioreactors was lower than that from the conventional reactor. The moisture content of the solid waste was less than the field capacity and adsorption of the recirculated leachate was continuous. The moisture content after day 108 and cumulative leachate production of the aerobic bioreactor were slightly lower than those of the anaerobic reactor, which is inferred to result from the evaporative effects of aeration.

Solid waste characterization

Figure 1 shows characteristics of the solid waste in the landfill reactors. The organic matter content in the anaerobic bioreactor was comparable to that of the conventional reactor in the first 72 days, but a drastic decrease was observed by day 138. The aerobic bioreactor showed a rapid reduction in the organic matter content during the experimental period. The T-N contents in the conventional and anaerobic reactors increased considerably from 700 mg/kg on days 0–1,700 and 1,900 mg/kg on day 138, respectively.

These increases in the T-N content resulted from the greater removal of carbonaceous components than nitrogenous components. On the other hand, the T-N content in the aerobic bioreactor dropped sharply after day 75 and reached 300 mg/kg on day 138, indicating effective nitrogen removal.

Furthermore, anaerobic and aerobic bioreactors showed rapid settling of solid wastes. The respective waste volumes on day 138 were approximately 75, 65, and 60% of the initial volume of the conventional, anaerobic, and aerobic reactors.

Leachate characterization

Figure 2 shows characteristics of leachate generated from the landfill reactors. The pH values of leachate from the conventional and anaerobic reactors were less than 7.0 until day 70, which reflects the accumulation of fermented acids. Thereafter, the pH value increased to over 7.0 and

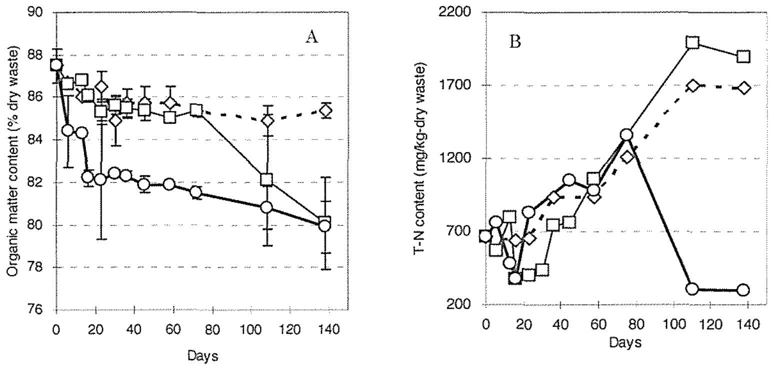

Figure 1 Characteristics of solid waste in landfill reactors. (A) Organic matter content. (B) T-N content. Symbols for reactors: ◊, conventional; □, anaerobic; ○, aerobic. Error bars show the standard deviation.

stabilized at 7.5–8.0. Leachate of the aerobic bioreactor showed pH values higher than 7.0 through the experimental period. Aeration limits the anaerobic fermentation reactions, thereby producing large amounts of acid and considerably reducing the pH value. The TOC concentrations in leachate of the conventional and anaerobic reactors were very high, although gradual decreases were observed in the methanogenesis phase. On the other hand, the aerobic bioreactor showed a rapid decrease in the TOC concentration.

Nitrogen compounds contained in leachate are mainly in the form of ammonium. The NH_4-N concentration in leachate of the conventional and anaerobic reactors remained very high during the operation period, but it was markedly lower in leachate of the aerobic bioreactor after day 50. A slightly lower NO_3-N concentration in leachate from the aerobic bioreactor was also observed than that from the others. High NO_3-N concentrations in leachate from the conventional and anaerobic reactors suggested that nitrification proceeded even in the conventional and anaerobic reactors. The NO_3-N concentration in leachate from the aerobic bioreactor was low in spite of the rapid decrease in the NH_4-N concentration, suggesting that simultaneous nitrification and denitrification processes readily occurred.

Landfill gas characterization

The CO_2 concentration of the conventional and anaerobic reactors was 10–30%. In contrast, the CO_2 concentration in the aerobic bioreactor was less than 0.2% through the experiment because of forced aeration. Methane production was observed after day 70 from both conventional and anaerobic reactors, suggesting that these reactors had shifted from the acidogenesis phase to the methanogenesis phase. The maximum percentages of methane were recorded as 27.6% on day 117 in the conventional reactor and 78.5% on day 134 in the anaerobic bioreactor. After day 140, methane concentrations decreased rapidly, suggesting that the two reactors were in transition from the methanogenesis phase to the stabilization phase. Methane concentrations in the aerobic bioreactor were less than 0.1% through the experiment.

Population dynamics of microorganisms

Figure 3 shows MPN values of DNA as indicators of microbial populations in the landfill reactors. No remarkable difference was apparent in the MPN value of eubacterial 16S rDNA in the three reactors. The 16S rDNA increased from about 10^6 MPN-copies/g on day 0 to 10^9 MPN-copies/g on day 45 with decreases in the organic matter content (Fig. 1A), then decreased nearly to the original level of 10^6 MPN-copies/g on day 138.

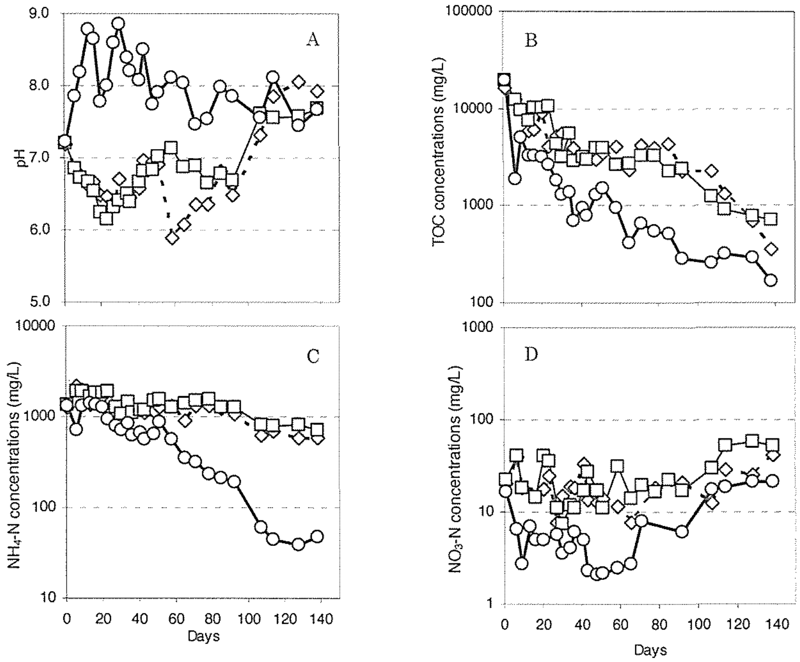

Figure 2 Characteristics of leachate generated from landfill reactors. (A) pH. (B) TOC concentration. (C) NH_4-N concentration and (D) NO_3-N concentration. Symbols for reactors: ◊, conventional; □, anaerobic; ○, aerobic.

The MPN value of the fungal 18S rDNA during the first 72 days in the aerobic reactor was higher than that in the other reactors, then decreased almost to the original level on day 138. The upper layer of the solid waste in the conventional and anaerobic reactors turned white at the end of the experiment, suggesting development of the fungal population. Correspondingly, the MPN values of 18S rDNA in the two reactors increased gradually during the experiment. The fungal population dynamics in the landfill bioreactors might be explained thusly: fungi became dominant in the reactors after degradation of most of the easily-degradable organic fractions because of their nutritional advantage over bacteria. Development of a fungal population that can decompose diverse chemicals is inferred to be important for accelerated stabilization of landfills.

The MPN value of the *amoA* gene remained high in the aerobic bioreactor, where effective nitrogen removal from both the solid waste and leachate was observed. These results showed that aeration is an important factor for development of the nitrifier population and effective removal of nitrogen in the solid waste and leachate. In the aerobic bioreactor, the increase in *amoA* genes mirrored the increase in the removal rate of NH_4-N in leachate and T-N content in solid waste.

The MPN values of *nirK* and *nirS* genes in the aerobic bioreactor, even in microaerobic conditions, were equivalent to those in the conventional and anaerobic reactors, suggesting that the predominant denitrifiers were facultative anaerobic bacteria. However, the MPN values of 16S rDNA, *nirK*, and *nirS* genes were sensitive neither to the difference of the moisture content nor to aeration, but rather reflected basic trends of the bacterial behavior in the landfills.

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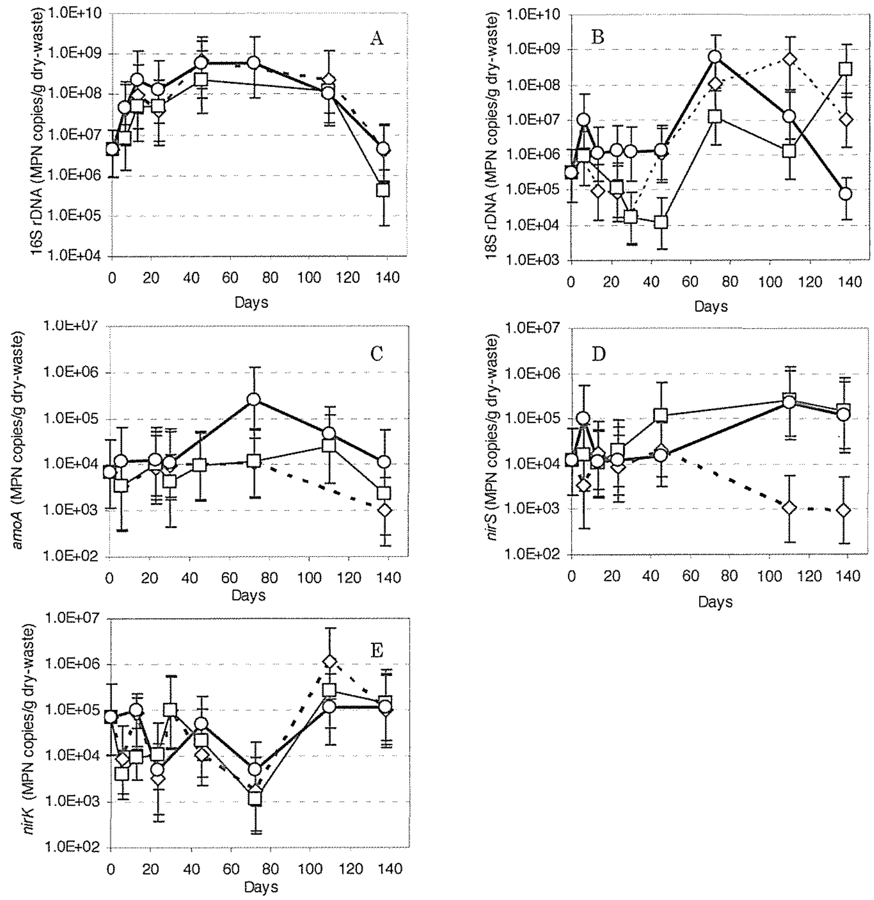

Figure 3 Population dynamics of microorganisms in landfill reactors: (A) Eubacterial 16S rDNA, (B) fungal 18S rDNA, (C) *amoA*, (D) *nirS*, and (E) *nirK*. Symbols for reactors: ◊, conventional; □, anaerobic; ○, aerobic reactors. Error bars show the 95% confidence interval.

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