



Effect of increasing salinity on biogas production in waste landfills with leachate recirculation: A lab-scale model study



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ABSTRACT

The effects of salinity on anaerobic waste degradation and microbial communities were investigated, in order to propose an appropriate leachate recirculation process in a waste landfill in a tropical region. A salt concentration of 21 mS cm⁻¹ of electrical conductivity (EC) did not affect waste degradation, but a salt concentration of 35 mS cm⁻¹ of EC inhibited CH₄ generation. A higher salt concentration of 80 mS cm⁻¹ of EC inhibited not only CH₄ and CO₂ generation, but also degradation of organic compounds. The bacterial and archaeal community compositions were affected by high salinity. High salinity can exert selective pressure on bacterial communities, resulting in a change in bacterial community structure. Ammonium caused strong, dominant inhibition of biogas production in the salt concentration range of this study. Quality control, especially of ammonium levels, will be essential for the promotion of waste biodegradation in landfills with leachate recirculation.

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1. Introduction

Appropriate management of waste landfills is required worldwide, including in developing countries. Early stabilization of landfills and reduction of environmental pollution by leachate is recognized as an important issue [1]. In most developing countries, landfill leachate is treated in stabilization ponds [2]. Leakage or overflow of untreated leachate to the surrounding environment is a concern, with large volumes of landfill leachate being produced by extensive landfill areas with high seasonal rainfall. Leachate recirculation in landfill bodies is an attractive technology that can reduce the volume of leachate and attenuate pollutants in the leachate by degradation in the landfill body [3,4]. Leachate recirculation is a process known to enhance the biodegradation of organics in waste and leachate, especially in arid regions, since it contributes moisture and extends the retention time [1,5–7]. Recently, a decrease in the biodegradation rate in the dry season in the tropics was reported [8], and Sanphoti et al. [9] reported that leachate recirculation with supplemental water enhanced stabilization in a simulated landfill reactor in a tropical region. In this context, leachate recirculation in waste landfills in tropical regions might improve both the handling of leachate in the rainy season and enhance the degradation of wastes in the dry season.

Landfill leachate typically contains not only a high concentration of organic matter but also salts, ammonium, and metals [10–14]. Sodium, potassium, and ammonium are often detected as major inorganic components in landfill leachate (sodium, up to 10,930 mg L⁻¹; potassium, up to 2243 mg L⁻¹; ammonium, up to 13,000 mg L⁻¹; electrical conductivity (EC), 3–41 mS cm⁻¹) [10–14]. Salt accumulation in landfill bodies can result from repeated leachate recirculation [15], and high salinity and ammonium are known to affect biological processes, including anaerobic digestion [16–19]. However, there are only a few reports on the effect of salinity on waste biodegradation with leachate recirculation [20,21]. To the best of our knowledge, this is the first report on the effect of the accumulation of complex inorganic matter, including salts, ammonium, and metals, on anaerobic waste degradation and microbial communities, as a possible result of leachate recirculation. An evaluation of the impact of salt concentration on microbial activity and community composition should lead to a greater understanding of biogas generation as an end-point reaction.

The purpose of this study was to evaluate the influence of salt accumulation on biogas production and microbial communities with the application of leachate recirculation technology to tropical developing countries in mind.

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2. Materials and methods

2.1. Waste extract and culture media

Organic waste is major component (41–63%) of municipal solid waste (MSW) in tropical developing countries [22,23]. Currently, the most common MSW disposal method in tropical sites is direct landfilling. Degradation of organic wastes in landfills is primarily a result of anaerobic biodegradation processes. Therefore, an anaerobic degradation test using synthetic organic waste was conducted. Synthetic organic waste was made of mixed dog food, rice, and compost (7:7:6 [wet weight]). The synthetic organic waste was mixed at a solid-liquid ratio of 9 by blender and then centrifuged twice (10,000g, 4 °C, 20 min). The supernatant was used as the waste extract. Characteristics of the waste extract are shown in Table 1. Culture medium was added to each test system to provide essential elements for bacterial reactions. The composition of the culture medium was as follows [(final concentration, mg L⁻¹): KH₂PO₄ (270), K₂PO₄ (1120), EDTA (EDTA·2Na) (1), MgCl₂·6H₂O (100), NH₄Cl (530), FeCl₂·4H₂O (10), CoCl₂·6H₂O (2), MnCl₂·4H₂O (0.5), NiCl₂·6H₂O (0.14), Na₂SeO₃ (0.12), AlCl₃·6H₂O (0.09), H₃BO₃ (0.05), (NH₄)₆Mo₇O₂₄·4H₂O (0.05), CaCl₂·2H₂O (0.04) and HCl (37.7%) 0.001 mL].

2.2. Anaerobic waste degradation test under different salt concentrations

Continuous leachate recirculation can cause an accumulation of high concentration of inorganic matter in a landfill. Five experimental conditions, A to E, included different salt concentrations. A salt mixture was used to simulate salt accumulation by leachate recirculation. The salt mixture was made based on the concentrations of inorganic matter in the waste extract (NaHCO₃, K₂CO₃, CaCl₂·2H₂O, FeSO₄·7H₂O, MgSO₄·7H₂O, ZnSO₄·7H₂O, MnCl₂·4H₂O, and NH₄Cl) (Table 2). Condition A was prepared as a control, and the salt mixture was not added. Conditions B–E had concentrations of inorganic matter from the waste extract that were 1, 50, 100, and 200-fold as much as in condition A. Forty milliliters of the waste extract was combined with culture medium, and then the salt mixture was added to each of the conditions. Subsequently, anaerobic sludge (mixed liquor suspended solids [MLSS], 13.6 g L⁻¹) was inoculated into the mixture and the mixture was adjusted to a pH of 7.4–7.6 and a total volume of 80 mL. The sludge was taken from anaerobically digested food waste in a lab-scale reactor. The mixture was added to a 100-mL vial, flushed with nitrogen gas to provide anaerobic conditions, and incubated (100 rpm, 35 °C) for 28 days. The ECs in conditions A–E were 4, 5, 21, 35, and 80 mS cm⁻¹, respectively. Condition A was conducted in quadruplicate, and the other conditions were conducted in duplicate. A blank experiment of each condition using deionized water instead of the waste extract was also

Table 1
Characteristics of the waste extract.

Parameter	Concentrations (mg L ⁻¹)
TS	8380
COD	10200
TOC	3550
TN	70
Calcium	22.4
Potassium	36.8
Sodium	45.9
Iron	0.8
Magnesium	7.5
Manganese	0.3
Zinc	0.5

Table 2

Salt mixture simulating salt accumulation by leachate recirculation (final concentration, mg L⁻¹).

	A	B	C	D	E
NH ₄ Cl	0	110	5500	11000	22000
CaCl ₂ ·2H ₂ O	0	40	2000	4000	8000
K ₂ CO ₃	0	33	1700	3300	6600
NaHCO ₃	0	85	4300	8500	17000
FeSO ₄ ·7H ₂ O	0	2.0	100	200	400
MgSO ₄ ·7H ₂ O	0	38	1900	3800	7600
MnCl ₂ ·4H ₂ O	0	0.50	25	50	100
ZnSO ₄ ·7H ₂ O	0	1.1	55	110	220

performed. The volume and composition of gas produced were measured periodically. Dissolved organic carbon (DOC) was measured on days 0 and 28. Net gas generation and DOC removal efficiency were calculated as follows:

Net gas generation (mL) = gas generation in each condition (mL) – gas generation in the blank of each condition (mL)

$$\text{DOC removal efficiency(\%)} = \frac{\text{DOC}_i - \text{DOC}_e}{\text{DOC}_i} \times 100$$

where DOC_i = concentration of DOC on day 0, and DOC_e = concentration of DOC on day 28.

The specific gas generation rate was calculated using a modified Gompertz equation [24]. A statistical analysis was performed by ANOVA.

2.3. Anaerobic waste degradation test under different inorganic concentrations

The effects of various concentrations of inorganic matter (sodium, potassium, and ammonium) on biogas generation were evaluated. Six batch experiments were conducted with each salt (NaHCO₃, K₂CO₃, and NH₄Cl) in the same manner as described above. Conditions Na5, K5, and AM5 were prepared at a final concentration of 5000 mg L⁻¹ of each ion (Na⁺, K⁺, and NH₄⁺). Also, conditions Na8, K8, and AM8 were prepared to final concentration of 8000 mg L⁻¹ of each ions. Forty milliliters of the waste extract was combined with culture medium, and then the each salt was added to each of the conditions. Subsequently, anaerobic sludge (MLSS, 13.6 g L⁻¹) was inoculated into the mixture and the mixture was adjusted to a pH of 7.4–7.8 and a total volume of 80 mL. The mixture was added to a 100-mL vial, flushed with nitrogen gas to provide anaerobic conditions, and incubated (100 rpm, 35 °C) for 28 days. The ECs in conditions Na5, Na8, K5, K8, AM5, and AM8 were 14, 28, 12, 20, 39, and 59 mS cm⁻¹, respectively. The ratio of free ammonia to total ammonium in conditions AM5 and AM8 (pH 7.4) were estimated to be 3% based on the equation described by Hansen et al. [25]. A blank experiment of each condition using deionized water instead of the waste extract was also performed. The volume and composition of gas produced were measured periodically. Net gas generation was calculated as described above.

2.4. Molecular analysis of microbial community structure

The effects of salt accumulation that can result from leachate recirculation on the community structure of bacteria and archaea were evaluated by comparing the communities in condition A (control) and condition E, which exhibited the strongest inhibition of biogas generation. Total DNA was extracted from 400 μL of the liquid sample in conditions A and E using an ISOIL kit (NIPPON GENE, Tokyo, Japan). The crude DNA extract was purified and concentrated by Montage PCR (Millipore, Bedford, MA, USA) and amplified by PCR using a primer set for the eubacterial 16S rRNA gene, 10F (5'-CAG AGT TTG ATC CTG GCT CAG-3') and 1492R (5'-

GGT TAC CTT GTT ACG ACT T-3'), and a primer set for the archaeal 16S rRNA gene, A109f (5'-ACK GCT CAG TAA CAC GT-3') [26] and ARC1059 (5'-GCC ATG CAC CWC CTC T-3') [27]. The PCR reaction mixture consisted of 2 mM MgCl₂, 2 μL of 10 × Taq Gold buffer, 1.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, New Jersey, USA), 200 μM each dNTP, 1 μM each primer, 5 μL of DNA template, and autoclaved water to complete 20 μL. PCR amplification was performed using a Mastercycler Gradient (Eppendorf Japan, Co. Ltd., Tokyo Japan) as follows: initial denaturation at 95 °C for 15 min, approximately 26 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 3 min, and a final extension at 72 °C for 7 min. The PCR products were purified using Montage PCR and cloned into the pT-7Blue vector (Novagen, San Diego, CA, USA) by DNA ligation kit (Takara Bio, Otsu, Japan). The ligated vectors were transformed into *Escherichia coli* DH5α competent cells (Takara Bio, Otsu, Japan) and incubated (37 °C, overnight) on Luria-Bertani plates containing 50 μg mL⁻¹ ampicillin and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). The white colonies were picked (blue/white selection) and checked for insert size by colony PCR with the M13 primer set. The positive PCR products were purified using Exo SAP-IT (Affymetrix, Cleveland, Ohio, USA), and sequencing was performed using DTCS Quick Start Master mix (Beckman Coulter, Brea, CA, USA) and a CEQ8000 Genetic Analysis System (Beckman Coulter, Brea, CA, USA). A homology search was conducted using DDBJ BLAST (<http://blast.ddbj.nig.ac.jp/blastn?lang=en>), and the diversity of the bacterial community was calculated using the Shannon-Weaver index [28].

2.5. Analytical procedures

Total solids (TS) in liquid solution were measured according to JIS K0102 [29]. Chemical oxygen demand (COD) was measured by the HACH procedure #8000 [30]. Total organic carbon (TOC), DOC, and total nitrogen (TN) were analyzed by a TOC-V/TNM-1 automatic TOC/TN analyzer (Shimadzu Co., Kyoto, Japan). For the DOC analysis, liquid samples from days 0 and 28 were centrifuged (8000g, 10 min) and filtered (0.45 μm). Inorganic waste extract was measured by an iCAP6300 inductively coupled plasma-optical emission spectrometer (Thermo Fisher Scientific K. K., Yokohama, Japan). The CH₄ and CO₂ contents were measured by a GC-2014 gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a thermal conductivity detector and a packed column (2.0 m, 3.0 mm i.d., Shincarbon ST, Shinwa Chemical Industries, Kyoto, Japan).

3. Results and discussion

3.1. Effect of salt concentrations on biogas generation

Fig. 1 shows the cumulative CH₄ and CO₂ generation in the anaerobic waste degradation test under different salt concentrations. Conditions D and E exhibited significantly lower CH₄ generation than the control condition A ($p < 0.01$). No difference in CH₄ generation was observed among conditions A–C. In contrast, the cumulative CO₂ generation in condition E was lower than that in the control condition A ($p < 0.01$). There were no difference in CO₂ generation among conditions A–D. A high salt concentrations, such as that of condition D (EC 35 mS cm⁻¹), inhibited biogas generation; however, the salt concentration in condition C (EC 21 mS cm⁻¹) did not affect the generation of biogas. Specific CH₄ and CO₂ generation rates obtained from the anaerobic waste degradation test are shown in Fig. 2. The highest specific CH₄ and CO₂ generation rates were observed in condition C. The salt concentration in condition D decreased the specific CH₄ generation rate. Also, the higher salt concentration in condition E clearly decreased both the specific CH₄ and CO₂ generation rates. These

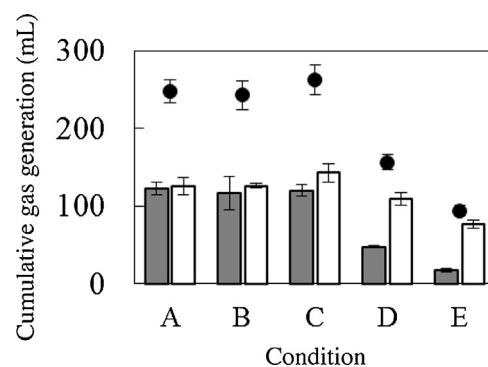


Fig. 1. Comparisons of cumulative generation of CH₄ (■), CO₂ (□), and biogas (CH₄ and CO₂) (●) in different salt concentrations after 28 days. The ECs in conditions A–E were 4, 5, 21, 35, and 80 mS cm⁻¹, respectively. Vertical bars indicate standard deviations (A, n = 4; others, n = 2).

results suggested that biogas generation might be enhanced at the optimal salt concentration of condition C (21 mS cm⁻¹). The DOC removal efficiency in condition E was significantly lower than the control ($7 \pm 3\%$) ($p < 0.01$), while that in conditions B–D were almost the same as the control condition A ($p > 0.01$) (Table 3). Higher salt concentrations inhibited not only biogas generation but also degradation of organic compounds. This indicated that the high salinity in condition E negatively affected the whole methane fermentation process from hydrolysis, to volatile fatty acid (VFA) formation, acetate production, and final biogas production. The salt concentration in condition D (EC 35 mS cm⁻¹) had a negative effect on CH₄ generation, but it did not affect DOC removal and CO₂ generation. This suggested that final biogas generation might be strongly inhibited, rather than hydrolysis, VFA formation, and acetate production, by this level of salinity. Under this condition, CO₂ generation by VFA formation might have occurred.

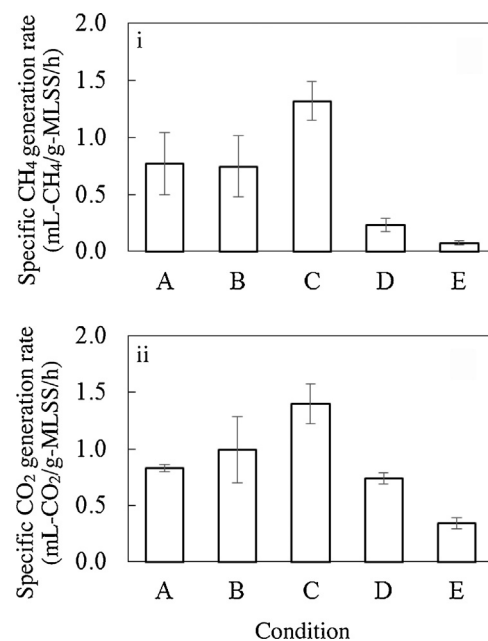


Fig. 2. Comparison of specific CH₄ (i) and CO₂ (ii) generation rates in different salt concentrations. The ECs in conditions A–D, and E were 4, 5, 21, 35, and 80 mS cm⁻¹, respectively. Vertical bars indicate standard deviations (A, n = 4; others, n = 2).

Table 3

DOC removal efficiency in each experimental condition after 28 days (A, n=4; others, n=2).

Test systems	Mean (%)	S.D.
A	85	4
B	79	2
C	85	1
D	84	4
E	7	3

3.2. Effect of salt concentration on microbial communities

The effects of salt concentration on bacterial and archaeal communities were evaluated by comparing the communities in the control (condition A) and in condition E (Figs 3 and 4), which exhibited the strongest inhibition of biogas generation. The phyla *Bacteroidetes* and *Firmicutes*, which are known indicators of anaerobic conditions, were dominant in all samples in both conditions (Fig. 3i). These phyla are also major constituents of waste samples and leachate in landfills [31,32]. After 14 days of incubation, the relative abundances of phyla were different between conditions A and E. Condition A showed *Firmicutes* (32%), *Bacteroidetes* (21%), and *Actinobacteria* (16%) as the major phyla, while condition E showed *Bacteroidetes* (52%) and *Firmicutes* (22%) as the major phyla. To breakdown into order level, the orders *Clostridiales* (24%), *Bacteroidales* (10%), *Actinomycetales* (10%), and *Spirochaetales* (8%) were major constituents in condition A

(Fig. 3ii). In condition E, orders *Bacteroidales* (33%) and *Clostridiales* (18%) were dominant after 14 days. Shannon's diversity index for condition E ($H' = 1.8$) was lower than that for condition A ($H' = 2.2$), suggesting that the high salinity affected the diversity of bacterial communities even after 14 days.

After 28 days of incubation, the relative abundance of each phylum in condition A had changed from that at day 14; for instance, the phylum *Proteobacteria* increased and then replaced *Actinobacteria* (Fig. 3i). The community composition and dominant orders in condition A were also different between 14 and 28 days (Fig. 3ii). The order *Syntrophobacterales* (22%) was newly dominant after 28 days in condition A, but was not detected in condition E. Bacteria in the genus *Syntrophus*, belonging to the order *Syntrophobacterales*, are known as obligate hydrogen-producing acetogens, which exist in symbiotic association with methanogens. In contrast, the dominant phylum and order in condition E did not clearly change between 14 and 28 days. These results suggested that high salinity exerted a selective pressure on bacterial communities, resulting in changes in community structure over time.

An analysis of archaeal communities (Fig. 4) revealed that the genus *Metanosaeta*, whose members are known as acetic acid-utilizing methanogens, was dominant (85%) at day 0. *Methanospirillum* (3%) and *Methanobacterium* (9%), both known as hydrogen-utilizing methanogens, were also detected as minor genera at day 0. The relative abundance of the genus *Metanosaeta* decreased from 80% to about 50% in both conditions A and E after 28 days. The genus *Methanospirillum* increased to be the second most common

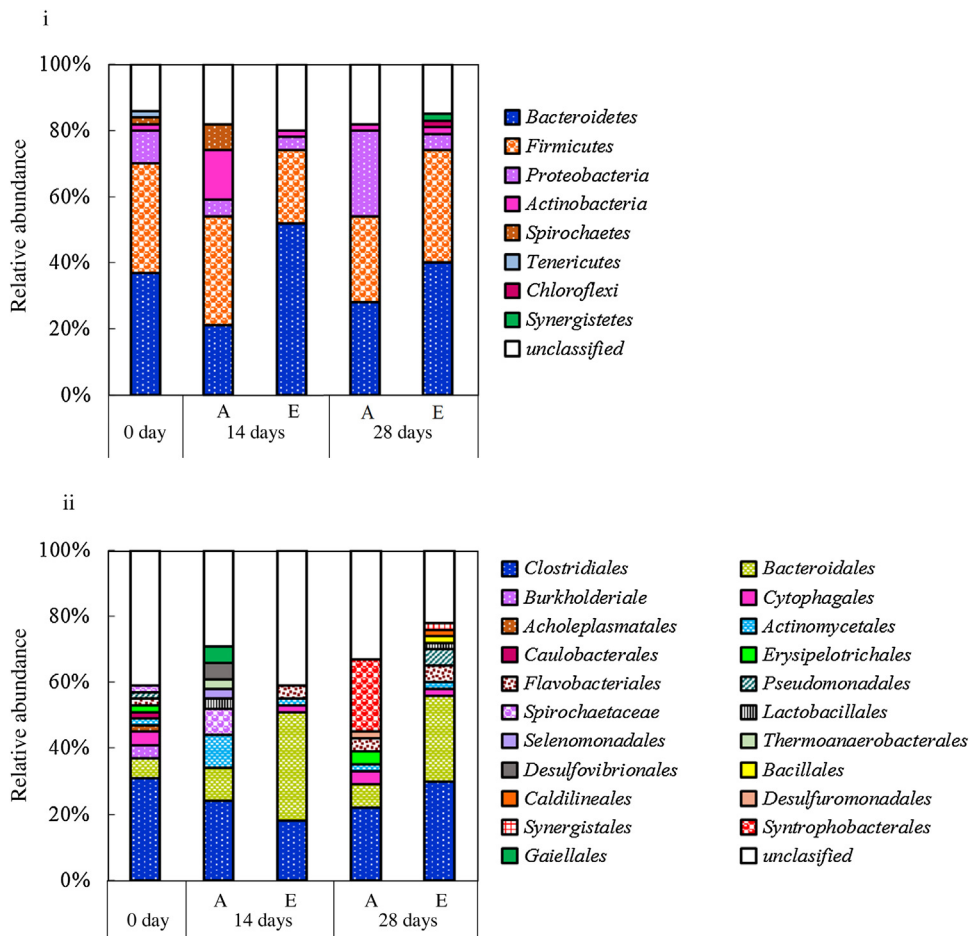


Fig. 3. Bacterial community composition at the levels of phylum (i) and order (ii) in conditions A and E on days 0, 14, and 28.

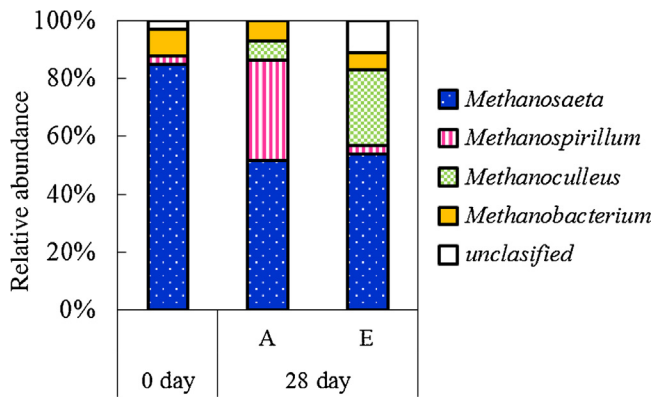


Fig. 4. Archaeal community composition at the genus level in conditions A and E on days 0 and 28.

(34%) in condition A, while *Methanoculleus* was second most common (26%) in condition E after 28 days. *Metanospirillum* is known to exist in symbiotic association with obligate hydrogen-producing acetogens. CH_4 generation would be expected to proceed by this kind of symbiotic association in condition A. The compositions of both bacterial and archaeal communities were affected by high salinity. This indicated that high salinity inhibited changes in the bacterial community and the formation of a symbiotic association between bacteria and archaea to produce CH_4 . The differences in bacterial and archaeal communities caused by salinity might have affected biogas production.

3.3. Effects of sodium, potassium, and ammonium concentrations on biogas production

The effects of various concentrations of sodium, potassium, and ammonium, which are often detected as major inorganic components of landfill leachate, on biogas production were evaluated. The results of biogas generation from the waste degradation test using various salt mixtures and individual inorganic ions (sodium, potassium, and ammonium) were analyzed (Fig. 5). There were no correlation between the concentrations of sodium or potassium and CH_4 or CO_2 generation (sodium: CH_4 [$r = -0.42$, $p > 0.01$]; CO_2 [$r = -0.32$, $p > 0.01$]; potassium: CH_4 [$r = -0.47$, $p > 0.01$]; CO_2 [$r = -0.53$, $p > 0.01$]). On the other hand, there was a strong negative correlation between the concentration of ammonium and both CH_4 and CO_2 generation (CH_4 [$r = -0.91$, $p < 0.01$]; CO_2 [$r = -0.84$, $p < 0.01$]). These results indicated that the effects of sodium and potassium on biogas production were weak, but ammonium caused strong, dominant inhibition of biogas production in the range of salt concentration assessed in this study. Ammonium is produced by biodegradation of organic nitrogen compounds, such as proteins, amino acids, and urea [33]. Ammonium in landfill leachate is a long-term pollutant of landfills [34], and more than 5000 mg L^{-1} of ammonium has been confirmed in real landfill leachate [13]. This study clearly showed that the concentration of ammonium is a key inhibitor of anaerobic waste degradation in landfills when leachate recirculation is applied. It should consider that anaerobic waste degradation in real landfills would be affected by other factors such as heterogeneity and flow paths of gas and liquid. Even though, a reduction in the concentration of ammonium is necessary for the enhancement of anaerobic waste degradation with leachate recirculation. The enhancement of anaerobic waste degradation will lead to accelerated stabilization of organic waste landfill.

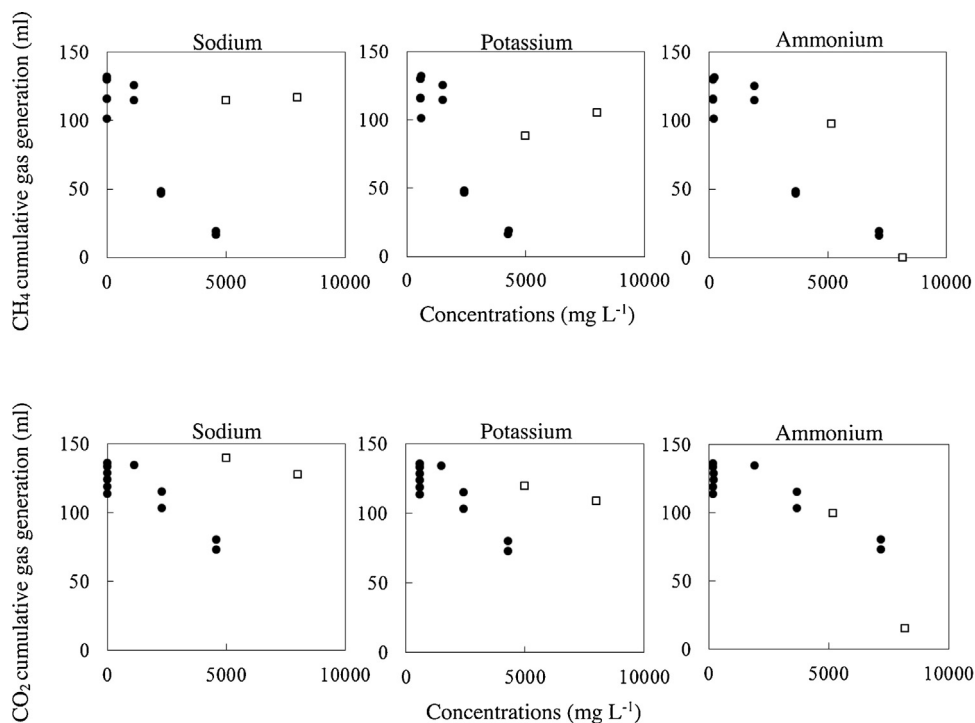


Fig. 5. Effects of sodium, potassium, and ammonium on cumulative generation of CH_4 and CO_2 gases. Test systems with salt mixture (●) and individual salts (sodium [NaHCO_3], potassium [K_2CO_3], and ammonium [NH_4Cl] (□)).

4. Conclusion

The effects of salt accumulation on biogas generation and microbial communities were evaluated using a batch test of anaerobic waste degradation under different salt concentrations. A salt concentration of 35 mS cm⁻¹ EC decreased CH₄, but not CO₂ generation. A higher salt concentration, 80 mS cm⁻¹ EC, inhibited not only CH₄ and CO₂ generation, but also the degradation of organic compounds. Although bacterial community structure changed over time in the control condition, the structure did not clearly change under a high salinity condition (EC 80 mS cm⁻¹). High salinity exerted selective pressure on bacterial communities to degrade organic waste under anaerobic conditions. The community structure of methanogenic archaea was also influenced by salinity. The effects of sodium and potassium on biogas production were weak, but ammonium caused strong, dominant inhibition of biogas production in the range of salt concentrations assessed in this study. These results indicate that quality control of leachate recirculation, especially of the ammonium concentration, is essential for the promotion of waste degradation in landfills that use this system. Findings from this study may contribute to improved stabilization of organic waste landfills with leachate recirculation.

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