Methanogenic Activity and Repression of Hydrogen Sulfide Evolved During High Rate Thermophilic Methane Fermentation of Municipal Solid Waste

IKBAL, YUEQIN TANG, TORU SHIGEMATSU, SHIGERU MORIMURA, and KENJI KIDA*

Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kumamoto University
2-39-1 Kurokami, Kumamoto City, Kumamoto 860-8555, Japan

Abstract

High rate thermophilic methane fermentation using a gas circulation-type reactor was explored for its potential to reduce the volume of and make beneficial use of municipal solid waste (MSW). Treatment without addition of mineral nutrients allowed for a maximum TS volumetric loading rate of only 1 g/l·d. However, with addition of mineral nutrients, a high TS volumetric loading rate of 8 g/l·d was achieved. At a TS loading rate of 1 g/l·d, the methanogenic activity with addition of mineral nutrients was 4-fold higher than that without mineral nutrients. The concentrations of coenzymes F₄₃₀ and corrinoids were 0.12 μmol/g-VSS and 0.07 μmol/g-VSS, respectively, at a TS volumetric loading rate of 8 g/l·d. Carbon recovery at each TS volumetric loading was nearly 100%, and about 50% of the carbon in raw waste was converted to methane gas. The degradation efficiency of lipid, protein, holocellulose and lignin were 90.7%, 59.4%, 91.9% and 66.0 %, respectively. To repress the evolution of hydrogen sulfide, air was supplied to the anaerobic reactor, by which the concentration of hydrogen sulfide in biogas was reduced nearly 100% when air at 7.5% of the amount of evolved biogas was used.

Key words: High rate thermophilic methane fermentation, municipal solid waste, methanogenic activity, carbon recovery, degradation of waste components, repression of hydrogen sulfide evolution.

INTRODUCTION

In Japan, the discharge of general waste increases annually. At present, the amount of the waste disposal has reached 50,000,000 ton/year and food processing waste alone has reached 16,000,000 ton/year, which is more than 30% of the total general waste. Utilization of food processing waste is greatly neglected and only about 0.3% is composted. The greater part of this waste is disposed of by landfilling after incineration. Municipal solid waste (MSW) is a significant part of the total waste, however it is difficult to recycle due to its water content of about 80%. Due to its high water content, it cannot self-burn and when burned with other wastes, the burner temperature is lowered, results in production of dioxins. In addition, in Japan there is not enough land for landfilling nor for building incineration facilities. Therefore, effective methods for volume reduction and reuse of MSW are urgently needed.

Studies on methane fermentation of MSW have been reported. Owens et al. studied the conversion of several MSW fractions to methane and Ten Brummeler et al. conducted dry anaerobic digestion of MSW. Lagerkvist et al. investigated the effects of adding cellulolytic enzymes to MSW during anaerobic treatment. Recently, Li et al. investigated thermal recycling of MSW by

* Corresponding Author
methane fermentation and noted the effects of volumetric loading rate on the mesophilic and thermophilic anaerobic digestions processes under the conditions of adding Co²⁺, Ni²⁺ and Fe²⁺. We had already reported the influence of Co²⁺ and Ni²⁺ on anaerobic treatment of wastewater, from which a 4-fold increase in reaction rate was achieved with additions of mineral nutrients. The maximum TOC loading rate achieved mesophilic and thermophilic methane fermentation of shochu distillery wastewater were 24 and 48 g/l·d, respectively.

For the present study, we conducted high rate thermophilic methane fermentation of MSW and investigated the following items: (a) the influence of mineral nutrients Co²⁺, Ni²⁺ and Fe²⁺ on methanogenic activity and the amounts of coenzymes involved; (b) the carbon balance; (c) the degradation efficiency of organic components and (d) the repression of hydrogen sulfide production.

**MATERIALS AND METHODS**

**Synthetic municipal solid waste** Table 1 shows the composition of the synthetic MSW used in this study. It contained fruits, vegetables, meat & fish and staple foods. These materials were mashed using a mixer and tap water was added to obtain a final total solids (TS) concentration of 10%. In the case of MSW with addition of mineral nutrients, Ni²⁺, Co²⁺ and Fe²⁺ were added at concentrations of 4.57 mg/l, 4.57 mg/l and 61.11 mg/l, respectively, to accelerate the methane fermentation rate.

**Anaerobic sludge** Mesophilic anaerobic digested sludge was provided by Kumamoto Hokubu sewage works (Kumamoto City, Japan).

**Anaerobic treatment of municipal solid waste** Figure 1 shows a schematic diagram of the MSW anaerobic treatment system. The reactor with a working volume of 5 l was a gas circulation-type, and was made of acrylic resin. 5 l of anaerobic digested sludge liquor was added into the reactor and the MSW was treated by the draw-and-fill method. The temperature was thermostatically maintained at 53°C by circulation of heated water through a water jacket. The liquid in the reactor was agitated continuously by evolved gas using a pump (P-1). The evolved gas was channeled into a
Methanogenic Activity and Repression of Hydrogen Sulfide Evolved During Methane Fermentation of Municipal Solid Waste

The effects of TS volumetric loading rate on the treatment efficiency were studied by changing the draw-and-fill volume. The operation at each TS loading rate was maintained for two to three weeks to obtain reliable data.

**Determination of methanogenic activity**

The methanogenic activity at each TS volumetric loading rate during steady-state operation was calculated based on the specific gas evolution rate. The specific gas evolution rate (ml/h·g-VSS) was determined according to the method of Kida *et al.* as follows. 0.2 ml of sodium acetate solution (292.85 mg/l) was added into a vial with a stirring bar to give a final concentration of 70 mM. The vial was sealed with a rubber stopper and an aluminum cap and then a needle was penetrated into the rubber stopper to allow for N₂ purging to create anaerobic conditions. A 10 ml aliquot of culture broth in the reactor was transferred to a vial and the vial was connected to a volumetric pipette using a vinyl tube immersed in a thermostatted water-bath as shown in Fig. 2. The gas evolution rate was then measured during incubation at 53°C under agitation at 100 rpm. The methanogenic activity was calculated as:

\[
\text{Methanogenic activity (ml/h/g-VSS)} = \frac{\text{gas evolution rate (ml/h/10 ml)}}{\text{concentration of sludge (g-VSS/10 ml)}}
\]

**Isolation and quantification of corrinoids**

For analysis of corrinoids, a 50 ml aliquot of culture broth in the reactor was centrifuged at 8000 x g for 30 min. Corrinoids in the precipitate were extracted in cyanic form according to the method of Nishio *et al.* The precipitate was re-suspended in 50 ml of extraction buffer. 0.5 M acetate buffer containing KCN at a concentration of 0.02% VSS of the precipitate and then corrinoids were extracted by incubation at 105°C for 20 min. The resulting extract was clarified by two sets of centrifugations at 8,000 x g for 30 min and then at 27,000 x g for 30 min. Extracted corrinoids were then partially purified using an Amberlite XAD-2 (Organo CO. Ltd., Tokyo) column (14.5 mmD x 300 mm) equilibrated with distilled water. The corrinoids adsorbed on the column were washed with distilled water and then eluted with methanol. The concentrations of corrinoids in the sample were estimated in unit of cobalt as determined by using an atomic absorption spectrophotometer (AA-6600G, Shimadzu, Kyoto) with vitamin B₁₂ solutions as standards.

**Isolation and quantification of F₄₃₀**

F₄₃₀ was extracted from the precipitate after centrifugation of a 50 ml aliquot of culture broth from the reactor following the method of Kida *et al.* as follows. The precipitate was re-suspended in 50 ml of distilled water and F₄₃₀ was extracted by incubation at 120°C for 120 min. The resulting extract was clarified by two sets of centrifugation at 8,000 x g for 30 min and then at 27,000 x g for 30 min. The pH of the clarified extract was adjusted to 4.0 with a 1 N HCl solution and was introduced into an Amberlite XAD-2 column equilibrated with 0.1 M acetate buffer (pH 4.0). F₄₃₀ adsorbed on the column was washed and eluted in the same manner as above. F₄₃₀ concentrations in the sample were estimated in units of nickel as determined by atomic absorption spectrophotometry.

**Other analytical methods**

All of the following parameters of the culture solution in the reactor, except for total solids (TS), total volatile solids (TVS), suspended solids (SS) and volatile suspended solids (VSS) were analyzed in supernatants obtained after centrifugation at 10,000 rpm for 10 min. TS, TVS, SS and VSS were analyzed in accordance with Standard Methods®. S-TOC was analyzed by using a TOC auto analyzer.
(TOC-500, Shimadzu, Kyoto) according to the testing methods for industrial wastewater, JISK0102-1986. Volatile fatty acids (VFAs) were analyzed as described previously. NH₄⁺ was measured by using the HACH method (HACH Co., World Headquarters, USA). The methane content of the biogas was measured by gas chromatography using a thermal conductivity detector (TCD) (KOR-2G, GL Science Co., Tokyo) with a packed column (Porapack Q, GL Science Co., Tokyo). Hydrogen sulfide was measured by using Kitagawa precision gas detector tubes (Komyo Kitagawa, Tokyo). The protein content of MSW was analyzed as 6.25 x nitrogen content, and the nitrogen was determined by using a CHN (carbon, hydrogen, nitrogen) coder MT-3 (Yanako-Tokyo). Lignin, holocellulose and lipid were analyzed in accordance with JIS (Japan Industrial Standards) P 8008-1976, P 8010-1976 and P 8012-1976, respectively.

RESULTS AND DISCUSSION

Influence of mineral nutrients on performance of anaerobic digestion Figure 3 shows the quality of effluent and the efficiency of treatment as a function of TS volumetric loading rate. The MSW was first fed into the reactor at a TS volumetric loading rate of 1 g/l·d. At this loading rate, stable conditions could be achieved without addition of mineral nutrients. The concentration of VFA was low and pH was also stable at about 7.5. However, when the TS volumetric loading rate increased to 2 g/l·d, the concentrations of TOC and VFA increased sharply to 5680 g/l and 11383 mg/l (acetate, 7,979 mg/l; propionate, 1,966 mg/l; butyrate, 786 mg/l; valerian, 652 mg/l), respectively. An accumulation of VFA in the reactor caused a decrease in pH to about 6 and was accompanied with a decrease in gas evolution rate, even though the digestion efficiency of VSS remained high (more than 80%). These results indicated that the digestion process did not continued completely to the gasification step, but mostly stopped after the acidification process. The maximum TS volumetric loading rate without addition of mineral nutrients was thus only 1 g/l·d. At this condition, the methanogenic activity was 1.06 ml/h/g-VSS.

In contrast, in the case of treatment with addition of mineral nutrients, the process remained stable when the TS volumetric loading rate was increased to 8 g/l·d (Fig. 3). At a TS volumetric loading rate of 6 g/l·d or less, the concentration of VFA was very low (ca. 400 mg/l). When TS volumetric loading rate was increased to 8 g/l·d, the concentrations of TOC and VFA increased to 2,800 mg/l and 3,000 mg/l and reached to plateau, respectively; however, the pH in the reactor was stable for more than two weeks at this condition. The increased amount of VFA did not interfere with the stability of anaerobic process. At all of the TS volumetric loading rates studied, the digestion efficiency of VSS was high, being about 83% and the pH was also stable at about 7.5. The gas evolution rate increased nearly linearly with an increase in TS volumetric loading rate to a maximum of 7,000 ml/l·d at the highest TS volumetric loading rate of 8 g/l·d. In the case of addition of mineral nutrients, the digestion of MSW proceeded uninhibited to the gasification process through acidification of organic matter.

From the above, the maximum TS volumetric loading rate with addition of mineral nutrients was 8 g/l·d. This value was 8-fold higher than that without addition of mineral nutrients. The hydraulic retention time (HRT) at this TS volumetric loading rate was very short (ca. 12 d).

Influence of loading rate and amount of
coenzymes on methanogenic activity
Under the conditions of adding mineral nutrients, the methanogenic activity at each TS volumetric loading rate was analyzed based on the specific gas evolution rate. The concentrations of coenzymes F430 and corrinoids in the biomass were also determined. Figure 4 shows the methanogenic activity, the concentrations of coenzymes F430 and corrinoids at each TS volumetric loading rate investigated. The methanogenic activity and the concentrations of F430 and corrinoids increased with an increase in the TS volumetric loading rate within the range of testing. At a TS volumetric loading rate of 8 g/l·d, the methanogenic activity and concentrations of F430 and corrinoids were 10.8 m/ll/h/g-VSS, 0.12 μmol/g-VSS and 0.07 μmol/g-VSS, respectively. These values were 1.5 times higher for methanogenic activity, 5 times higher for F430 concentration and 12 times higher for corrinoids concentration than those at the TS volumetric loading rate of 4 g/l·d. These results confirmed that the high concentrations of coenzymes F430 and corrinoids in the biomass caused the higher methanogenic activity. However, compared with the results obtained in our previous work, the concentrations of F430 and corrinoids in this experiment were lower. This might be due to the VSS in the MSW containing not only microorganisms but also residues of MSW, whereas the VSS in the acetate-containing synthetic wastewater contained only microorganisms.

Carbon balance in anaerobic digestion of municipal solid waste
The carbon balance of MSW in the anaerobic digestion process was investigated. The total carbon levels, namely in the raw waste, in the anaerobic treated liquid and in the biogas were calculated. As shown in Table 3, the carbon recovery at each TS volumetric loading rate was nearly 100%, and about 50% of carbon in the raw waste converted to methane gas. These results suggest that the biosynthesis of acetic acid in anaerobic treatment of MSW is the dominant reaction and subsequently acetic acid was converted to methane gas via acetyl-CoA and methyl CoM.

Degradation of MSW components in anaerobic digestion
The changes in waste components (lipid, protein, holocellulose and lignin) before and after anaerobic digestion were investigated and degradation efficiencies with respect to each component were determined at a TS volumetric loading rate of 6 g/l·d as shown in Table 4. The levels of lipid, protein, holocellulose and lignin in the SS of the raw waste were 23.7%, 21.65%, 39.8% and 13.7% respectively. These results show that the content of holocellulose in the MSW was higher than other components and the lignin content was low. The degradation efficiency of lipid, protein, holocellulose and lignin were 90.7%, 59.4%, 91.9% and 66.0 %, respectively. These results indicate that lipid and holocellulose are relatively easy to be

![Fig. 4 Effect of TS volumetric loading rate on methanogenic activity, concentrations of coenzymes F430 and corrinoids](image)

<table>
<thead>
<tr>
<th>TS volumetric loading rate (g/l·d)</th>
<th>Influent carbon (g/d)</th>
<th>Effluent carbon (g/d)</th>
<th>Carbon recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Biogas (CH₄, CO₂)</td>
<td>Residue (soluble + insoluble)</td>
</tr>
<tr>
<td>4</td>
<td>9.6</td>
<td>4.1 4.5</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>14.5</td>
<td>7.0 6.5</td>
<td>1.7</td>
</tr>
<tr>
<td>8</td>
<td>19.9</td>
<td>8.9 8.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 4 Quantity and degradation efficiency of each component of MSW

<table>
<thead>
<tr>
<th>Component</th>
<th>2-fold diluted MSW a (g)</th>
<th>Digested MSW (g)</th>
<th>Degradation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>0.237</td>
<td>0.022</td>
<td>90.7</td>
</tr>
<tr>
<td>Protein</td>
<td>0.216</td>
<td>0.087</td>
<td>59.4</td>
</tr>
<tr>
<td>Holocellulose</td>
<td>0.389</td>
<td>0.031</td>
<td>91.9</td>
</tr>
<tr>
<td>Lignin</td>
<td>0.137</td>
<td>0.046</td>
<td>66.0</td>
</tr>
<tr>
<td>Ash</td>
<td>0.021</td>
<td>0.012</td>
<td></td>
</tr>
</tbody>
</table>

a) The quantity of each component was calculated per 1 g of suspended solids from analytical values.

degraded by anaerobic digestion and that lignin, despite its putative resistance to biodegradation, could also be effectively degraded up to 66%. Conversely, the degradation efficiency of protein was only 60%. This might be the result of some of organic materials being used for growth of microorganisms which usually contain 50-70% protein as components14).

Influence of hydrogen sulfide on the aceticlastic methanogen In anaerobic digestion, sulfide is an inhibitory substance. When the concentration of sulfide increases to more than 100 ppm, the process will generally fail15). In order to evaluate the influence of sulfide on anaerobic digestion, an experiment using an aceticlastic methanogen culture broth from a continuous cultivation reactor was conducted. Gas production rate rapidly decreased with an increase in the concentration of sulfide and it was nearly zero at a sulfide concentration of 300 mg/l (Fig. 5). With scale-up to a larger reactor with increased liquid depth, the concentration of soluble hydrogen sulfide will become higher, which will be detrimental to the process.

In this study, in order to reduce the concentration of hydrogen sulfide in the reactor, a high volumetric loading rate of TS has not been achievable.

In this study, in order to reduce the concentration of hydrogen sulfide in the anaerobic reactor, air at 10 % of the amount of biogas evolved was supplied continuously into the reactor. The results of this experiment conducted at a TS volumetric loading rate of 6 g/l-d are shown in Fig. 6. The concentration of hydrogen sulfide decreased from 680 ppm to 150 ppm within one day following the onset aeration and decreased to 10 ppm following four days. However, when the volume of air was reduced to 5%, the concentration of hydrogen sulfide sharply increased to 400 ppm. Judging from these results, an aeration volume of 5% did not interfere with the activity of the sulfate reducing bacteria. When the aeration volume was restored to 10%, the hydrogen sulfide concentration rapidly decreased to 20 ppm. After 10 days of aeration, the volume of air was adjusted to 7.5% of the amount of evolved biogas. At that time, because of a trouble of the reactor, the concentrations of hydrogen sulfide, TOC and VFA increased; however, after one week the concentration of hydrogen sulfide decreased to less than 10 ppm. Under these conditions, operation was conducted for more than four months and hydrogen sulfide, pH, digestion efficiency of VSS and methane content were...
Fig. 6 Effect of aeration on effluent quality, VSS degradation efficiency, gas evolution rate, and concentrations of CH<sub>4</sub> and H<sub>2</sub>S

maintained at less than 5 ppm, around 7.6, more than 82% and about 50%, respectively. The concentration of VFA also decreased to about 100 mg/l. The effect of aeration on the repression of hydrogen sulfide produced could be explained by the chemical oxidation of hydrogen sulfide. Another possible explanation is that the aeration caused a inhibition of production of hydrogen sulfide and/or a re-oxidation of hydrogen sulfide, because several sulfate-reducing bacteria have been demonstrated to oxidize sulfide to sulfite and sulfate in the presence of oxygen.

The mechanism of the repression of hydrogen sulfide evolution by air is still unknown. Clone analyses of sulfate reducing bacteria should be conducted. In addition, enzymes included in the SO<sub>4</sub><sup>2-</sup> reducing process should be studied on the expression level.

**CONCLUSION**

For the anaerobic treatment of MSW with additions of Co<sup>2+</sup>, Ni<sup>2+</sup> and Fe<sup>2+</sup>, the maximum TS volumetric loading rate observed in this study was 8 times higher than that without addition of mineral nutrients. The methanogenic activity and F<sub>co</sub> and corrinoid concentrations increased with increases in loading rate. At each TS volumetric loading rate, approximately 100% of the carbon in the raw waste could be recovered in treatment byproducts. Holocellulose and lipid in the MSW were easily degraded and lignin, though a recalcitrant material, was also effectively removed. However, the degradation efficiency of protein was apparently low, which might be due to the increase of microorganisms population in the reactor. When air at 7.5% of the amount of evolved biogas was supplied to the anaerobic reactor, the concentration of hydrogen sulfide decreased nearly 100% and could be maintained at less than 5 ppm. During aeration, methane fermentation had still good performance and the concentrations of TOC and VFA were maintained in low levels except for misoperation.

**REFERENCES**


(Submitted 2002. 8. 28)  
(Accepted 2002. 11. 30)