Artificial Fe-Type Zeolite Enhances Methane Production in Anaerobic Digestion under Ammonium-Rich Conditions

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Abstract

In this study, the effects of Fe-type, Ca-type, and low-sodium-level Ca-type artificial zeolites, made from waste coal ash, on methane production and the methanogen communities under ammonium-rich conditions were investigated. Methane production with Fe-type zeolite was higher than that with the other types of zeolites, and was about four times higher than that without zeolite. Acetate was not accumulated during anaerobic digestion of ammonium-rich organic waste with Fe-type zeolite. The methanogen community which were analysed by real-time polymerase chain reaction (PCR) with Fe-type zeolite was almost the same as that with Ca-type zeolite. These results showed that quantities of methanogen have not relation with the volume of methane production. A comparison of methane production with Fe-type zeolite and that with Fe ions or Ca ions in anaerobic digestion under ammonium-rich conditions confirmed that Fe-type zeolite was the most effective in enhancing methane production, Ca ions addition was not enhancing the methane production contrary. Fe-type zeolite is considered to play two roles in anaerobic digestion; namely, supplying iron to the anaerobic microbes, and decreasing the ammonium concentration.

Keywords: Ammonium, Anaerobic digestion, Artificial zeolite, Fe, Methane

1. INTRODUCTION

Anaerobic digestion of organic wastes has the advantages of relatively low sludge production, low energy consumption, and methane production, with the result that this process is widely studied and used¹⁾. Organic waste usually contains nitrogenous compounds such as proteins, which are converted to ammonium by anaerobic digestion. Inhibition of anaerobic digestion of organic wastes, such as from livestock and dewatered sewage sludge, is often caused by a high ammonia

concentration²⁻⁴⁾. A high NH_4^+ concentration is toxic for anaerobes⁵⁻⁷⁾. McCarty reported that $\text{NH}_4^+\text{-N}$ concentrations in excess of 3000 mg/l could be expected to be toxic at any pH value⁸⁾. Hobson and Shaw reported that 235 mmol (3290 mg N/l) NH_4^+ completely prevented the growth of *Methanobacterium formicicum* in pure culture⁹⁾.

To avoid NH_4^+ inhibition, organic wastes are usually diluted with water and carbon-rich materials before digestion; however, dilution requires a large reactor and increases the cost of treatment. For this reason, NH_4^+

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removal during anaerobic digestion is necessary for efficient digestion of waste having a relatively high organic concentration.

Other than dilution studies, there are only a few reports on reducing ammonia inhibition; for example, the magnesium ammonium phosphate (MAP) process¹⁰⁾ and the lighted upflow anaerobic sludge blanket method¹¹⁾. Some zeolites and clays are able to absorb NH_4^+ by cation exchange reaction, and several papers have reported on the application of zeolite and clay to reduce inhibition by ammonia during anaerobic digestion¹²⁻¹⁴⁾. Angelidaki et al. (1993) used bentonite clay for anaerobic thermophilic digestion of cattle manure¹³⁾. Milan et al. (2001) used a natural zeolite, consisting of a mixture including clinoptilolite, mordenite, and montmorillonite, for the anaerobic digestion of piggy waste¹²⁾. Hansen et al. (1999) used glauconite for the anaerobic digestion of swine manure¹⁴⁾. Tada et al. (2005) reported that natural mordenite enhanced methane production in ammonium-rich organic waste during anaerobic digestion, and that Ca ions released from the natural mordenite had the effect of improving methane production under ammonium-rich conditions¹⁵⁾. These studies revealed that the addition of clay and zeolite improved methane production; however, the mechanism by which zeolite enhances anaerobic digestion has not yet been actively discussed.

In this study, several types of artificial zeolite made from waste coal ash were used to investigate the effects of zeolites on methane production and the archaeal community under ammonium-rich conditions.

2. MATERIALS AND METHODS

2.1 Seed sludge and zeolites Anaerobically digested and dewatered sludge was collected from a sewage treatment plant in Ibaraki Prefecture, Japan, and stored at 4°C. The sludge was mixed with distilled water and stirred anaerobically for 10 days at room temperature before being used as the seed sludge for the anaerobic digestion.

Three types of artificial zeolites, Fe-type, Ca-type, and low-sodium-level Ca-type, made from waste coal ash (Maeda Co., Ltd., Tokyo,

Table 1 Properties of the artificial zeolites used in the experiments

	pH	CEC (cmol/kg)	Na (cmol/kg)	K (cmol/kg)	Ca (cmol/kg)
Fe-type zeolite	8.9	240	83	14	229
Ca-type zeolite	10.0	258	109	17	310
Low sodium level Ca-type zeolite	9.8	258	32	11	285

Japan) and supplied in the form of powders (particle size: 20 μm) were used as additives in the sludge tests. It is reported that Ca ion enhanced the methane production from anaerobic digestion¹⁵⁾. The low-sodium-level Ca type zeolites were chosen for knowing effect for enhancing of methane production by Ca ion. The properties of the artificial zeolites used in the experiments are shown in Table 1.

2.2 Loading of batch reactors with zeolites

First, 150 ml of the sludge (10% dry matter) was placed in a 200 ml glass reactor. Glucose was added to the reactor at a final concentration of 2 g/l. A mineral solution containing KH_2PO_4 (16 mg/l), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (25 mg/l), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (25 mg/l), Fe-EDTA (5 mg/l), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (5 mg/l), and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (5 mg/l) was added at 200 ml/l to the reactor. NH_4Cl was used for the ammonium source. Four different experiments were designed: (A) high ammonium concentration (3000 mg N/l) without zeolite, (B) high ammonium concentration (3000 mg N/l) with addition of Ca-type zeolite, (C) high ammonium concentration (3000 mg N/l) with addition of Fe-type zeolite, and (D) high ammonium concentration (3000 mg N/l) with addition of low-sodium-level Ca-type zeolite. The zeolites were added as 5% (w/w) of the total liquid. The reactor was sealed with a rubber septum held in place by a plastic cover for the anaerobic conditions and a gas bag was connected to the bottle to measure the biogas volume. Nitrogen gas was added to remove the air from the glass reactor. Biogas production was periodically measured on the measuring scale of a plastic syringe. The reactors were then placed in a thermostated incubator at 35°C. Once every 3 weeks, 50 ml of digested sludge in the reactor was exchanged for new medium.

2.3 Addition of cations To clarify the effect of the cations released from the Fe-type zeolite, Ca ions and Fe ions were added to the sludge. Twenty ml of the sludge (10% dry matter) was placed in a 50 ml glass vial, and 1 g of Fe-type zeolite was added to the anaerobically digested sludge. CaCl_2 was added at 950 mg Ca^{2+}/l . Fe citrate was added at 15 mg Fe^{3+}/l . Samples of the sludge were taken under the same ammonium-rich conditions as described in section 2.2. Five percent Fe-type zeolite, 950 mg Ca^{2+}/l , 15 mg Fe^{3+}/l , 950 mg Ca^{2+}/l + 15 mg Fe^{3+}/l , and no additive as the control were separately added to the vials, and incubated for 21 days at 35°C. Each vial was sealed with a rubber septum held in place by a thin aluminum cover for the anaerobic conditions, and a 50 ml-volume plastic syringe was inserted into the vial to measure biogas volume. Nitrogen gas was added to remove the air from the glass vial. The vials were then placed in a thermostated incubator at 35°C. After a 21-day digestion period, the $\text{NH}_4\text{-N}$ (nitrogen in NH_4 form) and methane concentrations were measured.

Standard deviations were evaluated by error propagation from triple applications of the sample.

2.4 Analytical methods The concentrations of NH_4^+ , Ca^{2+} , Mg^{2+} , Na^+ , and K^+ were determined using an ion chromatograph (DX120; Dionex Corp., Sunnyvale, CA, USA) and an IonPac CS12A column (Dionex) with a methyl-sulfonic acid solution (18 mmol) as the mobile phase. The samples for ion chromatography were separated into supernatant and sediments by centrifuge (10,000 rpm, 10 min). The concentrations of organic acid were determined using the ion chromatograph (DX120) and an IonPac ICEAS1 column (Dionex) with a heptafluorobutyric acid solution (1 mmol) as the mobile phase. The composition of the biogas was determined using a gas chromatograph (GC-8A; Shimadzu Corp., Kyoto, Japan) with a thermal conductivity detector equipped with a steel column (3 m $\times$ 3 mm) packed with Porapak Q (Shinwa Chemical Industries, Ltd., Kyoto, Japan) at 90°C, and argon was used for the carrier gas

with a gas flow pressure of 100 kPa. Biogas was sampled from the gas bag connected to the bottles or the top of the vials using a syringe attached to a needle. The volume of methane was obtained by the volume of increased biogas multiplied by the concentration of methane detected by gas chromatograph.

2.5 Quantification of immobilized microbes using real-time PCR analysis

DNA was extracted from the liquid of four types (A, B, C, D) in the reactors after completion of the experiments. Real-time polymerase chain reaction (PCR) analysis was conducted using an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) and a TaqMan® Universal PCR Master Mix (Applied Biosystems). The amplifying primer set S-P-MArch-0348-S-a-17 and S-D-Arch-0786-A-a-20 and the double dye probe S-P-MArch-0515-S-a-25 were used for the methanogens^{16,17}. The amplifying primer set S-F-Msaet-0387-S-a-21 and S-F-Msaet-0573-A-a-17 and double dye probe S-F-ZMsaet-0540-A-a-31 were used for the *Methanosaeta* spp.¹⁷. The amplifying primer set S-F-Msar-0450-S-a-19 and S-F-Msar-0588-A-a-20 and double dye probe S-F-Msar-0544-A-a-31 were used for the *Methanosarcina* spp.¹⁸. The amplifying primer set S-F-Mbac-0398-S-a-20 and S-F-Mbac-0578-A-a-22 and double dye probe S-F-ZMbac-0526-A-a-33 were used for the *Methanobacterium* spp.¹⁸. The real-time PCR amplification followed a three-step PCR (40 cycles) with 20 s denaturation (95°C), 20 s annealing (55°C), and 120 s elongation (72°C). Quantitative measurements by real-time PCR were conducted in quadruplicate.

3. RESULTS

3.1 Effect of Fe-type zeolite on methane production from batch reactors

Figure 1 shows the cumulative methane production from the batch reactors with and without zeolite addition. The methane production from the reactor with Fe-type zeolite addition was higher than those with Ca-type zeolite, with low-sodium-level Ca-type zeolite, and without zeolite. The methane production with Ca-type zeolite addition showed no improvement compared to that without

Fig. 1 Methane production from reactors with and without artificial zeolites. The closed circles show methane production without additives, the open squares show that with Fe-type zeolite addition, the open triangles show that with Ca-type zeolite addition, and the closed squares show that with low-sodium-level Ca-type zeolite addition.

zeolite. The methane production with low-sodium-level Ca-type zeolite addition was 1.36 times higher than that without zeolite at day 80. The cumulative methane production with Fe-type zeolite addition was approximately 4 times higher than that without zeolite at day 80.

3.2 Cation concentrations Table 2 shows the ion concentrations of the reactors after anaerobic digestion. With zeolite addition, the ammonium ion concentration lowered by about 500 mg/l compared to that without zeolite. The amount of decrease was almost the same among the different zeolites. The Fe concentrations with Ca-type zeolite and low-sodium-level Ca-type zeolite additions were less than those with Fe-type zeolite

Table 2 Ion concentrations of the reactors after anaerobic digestion

	NH ₄ -N (mg/l)	Na ⁺ (mg/l)	Ca ²⁺ (mg/l)	K ⁺ (mg/l)	Fe ³⁺ (mg/l)	pH
Before experiment	1127	15	47	30	<0.5	7.2
Fe-type zeolite	1067	733	937	257	32	7.0
Ca-type zeolite	1047	1129	753	130	<0.5	7.2
Low sodium level	986	257	1406	100	<0.5	7.1
Ca-type zeolite						
No additives	1581	36	50	16	21	6.5

addition and with no additive. Fe-type zeolite also released Ca²⁺ ions. The Ca²⁺ ion concentration with the zeolite additions was 10 times higher than that without zeolite. The pH value without zeolite was 6.5, whereas that with the zeolite additions was almost 7.

3.3 Organic acid concentrations Figure 2 shows the concentrations of organic acids after anaerobic digestion. The acetate concentrations with the zeolite additions showed a larger decrease compared to those without zeolite. In particular, the acetate concentration with Fe-type zeolite addition was very low compared to those with Ca-type and low-sodium-level Ca-type zeolite additions. The propionate concentrations with the zeolite additions did not differ significantly. The butyrate concentrations with the zeolite additions were lower than those without zeolite. The butyrate concentration with Fe-type zeolite addition was less than half of those with Ca-type zeolite and low-sodium-level Ca-type zeolite additions. The lactate concentrations with the zeolite additions were higher than those without zeolite.

Fig. 2 Organic acid concentrations in the reactors on the final day of operation. The closed bars show the concentrations without additives, the diagonally shaded bars show those with Fe-type zeolite addition, the open bars show those with Ca-type zeolite addition, and the dotted bars show those with low-sodium-level Ca-type zeolite addition.

Fig. 3 Concentrations of methanogens in the reactors measured by real-time PCR. The open bars show *Methanosarcina* spp., the diagonally shaded bars show *Methanobacterium* spp., and the dotted bars show *Methanosaeta* spp. The bars indicate standard deviations.

3.4 Archaeal community Figure 3 shows the results of real-time PCR. The concentrations of *Methanosarcina* spp. with and without zeolite ranged from 1.2×10^8 to 8.0×10^9 copies/ml, those of *Methanobacterium* spp. ranged from 7.8×10^8 to 3.9×10^9 copies/ml, and those of *Methanosaeta* spp. ranged from 8.4×10^5 to 4.8×10^8 copies/ml. The concentrations of *Methanosarcina* spp. with zeolite were higher than that without zeolite, and that with the Ca-type zeolite addition was highest than that with Fe-type zeolite addition and with low-sodium-level Ca-type zeolite. The concentrations of *Methanobacterium* spp. with zeolite were lower than that without zeolite, but that were not different among zeolites addition condition. The concentrations of *Methanosaeta* spp. with low-sodium-level Ca-type zeolite additions was lower than other reactors.

3.5 Effect of Fe and Ca ions on methane production

Figure 4 shows the methane production during 21 days of anaerobic digestion with Fe³⁺ or Ca²⁺ ion addition. With the addition of Fe-type zeolite, methane production was higher than that with Ca²⁺ and Fe³⁺ ions. Comparing the methane production between the Fe³⁺ ion addition and the Ca²⁺ ion addition, methane production with the Fe³⁺ ion addition was enhanced to a greater extent than that with the Ca²⁺ ion

Fig. 4 Effect of iron and calcium ions on methane production under ammonium-rich conditions (3000 mg N/l). The open squares show methane production without additives, the open circles show that with Fe-type zeolite addition, the open triangles show that with the addition of Fe³⁺ and Ca²⁺ ions, the closed rhombuses show that with the addition of Fe³⁺ ions, and the crosses show that with the addition of Ca²⁺ ions. The bars indicate standard deviations.

Table 3 Organic acid concentration after 21 days of anaerobic digestion with Fe³⁺ or Ca²⁺ ion addition

	Acetate (mg/l)	Propionate (mg/l)	Butyrate (mg/l)	NH ₄ -N (mg/l)
No additives	741	117	204	1531
Addition of Fe-type zeolite	0	0	0	1095
Addition of Fe ³⁺ & Ca ²⁺	1.9	156	0	1538
Addition of Fe ³⁺	3.7	184	0	1552
Addition of Ca ²⁺	674	78	267	1571

addition. Table 3 shows the organic acid concentration after 21 days of anaerobic digestion with Fe³⁺ or Ca²⁺ ion addition. The acetate concentration without zeolites and with Ca²⁺ ions was 100 times higher than those with Fe-type zeolite, Fe³⁺ and Ca²⁺ ions, and Fe³⁺ ions. The pH values of each condition except without no addition were almost 7.

4. DISCUSSION

The results (Fig. 2) obtained for acetate concentration without zeolite addition showed

that ammonium inhibition during anaerobic digestion could lead to acetate accumulation. Poggi-Varaldo (1991) reported that the specific acetate-uptake rate in anaerobic digestion was affected by the concentration of un-ionized ammonia¹⁹⁾. The ammonium ion concentration decreased with the addition of zeolites (Table 2). Only Fe-type zeolite enhanced methane production (Fig. 1). Tada et al. (2005) reported that Ca^{2+} ions released from natural mordenite improved methane production from ammonium-rich organic waste during anaerobic digestion¹⁵⁾. However, the present artificial Ca-type zeolite did not improve methane production (Fig. 1). It is considered that differences in the exchanged ions and crystal structures of the zeolites caused the different results. The total carbon of the glucose added to the reactor was 0.28 g. The theoretical methane production from glucose added to the reactor was 261 ml. The methane production with Fe-type zeolite addition was 1.5 times higher than the theoretical value. These results suggest that Fe-type zeolite enhanced self-degradation of the anaerobically digested sludge.

The acetate concentration decreased with the addition of Fe^{3+} ions, but did not decrease with the addition of Ca^{2+} ions (Table 3). Furthermore, the methane production showed weak improvement with the addition of Fe^{3+} ions, although the ammonium ion concentration did not decrease (Fig. 4, Table 2). It has been reported that the addition of iron improved methane production from acetate²⁰⁾. Hoban et al. (1979) reported that the optimum soluble iron concentration was between 0.2 mM (11.17 mg/l) and 2 mM (111.7 mg/l) for conversion of acetic acid to methane²⁰⁾. In our results, the concentration of Fe^{3+} ions released from Fe-type zeolite after digestion was included in the optimum concentrations (Table 2). Fe-type zeolite addition could enhance biogas production through ammonium exchange and the release of Fe^{3+} and Ca^{2+} ions.

In the case of Fe-type zeolite, not only acetate but also propionate and butyrate were not detected after 21 days of anaerobic digestion (Table 2). Propionate and butyrate can be degraded only when acetate and especially hydrogen are effectively eliminated

by the methanogens. Van Schie et al. (1999) reported that *Syntrophomonas wolfeii*, known as a degrader of propionate and butyrate, adhered more on the Fe^{3+} surface of glass²¹⁾. It is considered that *S. wolfeii* increased at a higher rate with Fe-type zeolite addition than with the other zeolite additions.

It has been reported that *Methanosaeta* spp. decreased with increasing ammonium concentration (Sawayama et al., 2004). Angenent et al. (2002) reported that *Methanosarcina* decreased when the ammonia-N level increased to 3600 mg/l from 2000 mg/l²²⁾. The growth of *Methanosarcina barkeri* was resistant to ammonia in short-term studies comparing it with *Methanospirillum hungatei* and *Methanobacterium bryantii*⁶⁾. Methane production by *M. barkeri* with 200 mM NH_4Cl was inhibited to about 50% of that without NH_4Cl , and methane production by *M. bryantii* with 200 mM NH_4Cl was weakly inhibited by 10%. These results by Sprott et al. (1986) show that *M. barkeri* could grow under 200 mM NH_4Cl conditions, but the production of methane from the acetate was inhibited⁶⁾. In our results, the quantities of *Methanobacterium* spp. did not differ among the zeolite addition conditions, but the methane production was different among the conditions (Fig. 3). In addition, the acetate accumulation was observed in the reactor with Ca-type zeolite additions although the quantities of *Methanosarcina* spp. was highest in other reactors (Fig. 2). These results and reports suggest that not quantity of methanogen but methanogenic activity is affected by zeolite addition under ammonium-rich conditions. Gallert et al. (1998) reported that acetate and propionate were detected at ammonia concentrations higher than 1082 mg/l in mesophilic digestion²⁾. In the present study, the ammonium concentration decreased with Fe-type zeolite addition (1095 mg/l) (Table 3). Lay et al. (1998) reported that the methane production rate by differential $\text{NH}_4\text{-N}$ concentrations, the methane production were marked a sharp decline when the $\text{NH}_4\text{-N}$ concentration increasing more than 1200 mg/l²²⁾. In this study, the $\text{NH}_4\text{-N}$ concentration was decreased lower than 1200 mg/l by addition of zeolites. The removal of ammonium

ions by Fe-type zeolite prevents propionate accumulation during anaerobic digestion under ammonium-rich conditions.

5. CONCLUSIONS

The addition of Fe-type zeolite, which made from waste coal ash, is effective to enhance the methane production about four times higher than that without zeolite from anaerobic digestion under ammonium-rich conditions. Acetate was not accumulated during anaerobic digestion of ammonium-rich organic waste with Fe-type zeolite although that was with the other type zeolites. The methanogen community with Fe-type zeolite was almost the same as that with Ca-type zeolite. Comparing the addition of Fe-type zeolite and the addition of iron ions, the addition of Fe-type zeolite is more effective enhancing the methane production from anaerobic digestion under ammonium-rich conditions than the addition of iron ions.

Fe-type zeolite is considered to play two roles in anaerobic digestion; namely, supplying iron to the anaerobic microbes, and decreasing the ammonium concentration.

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