Anaerobic digestion of sewage sludge of municipal wastewater was investigated using inhibitors to determine the relationship between methanogenesis and sulfate reduction. During anaerobic incubation of the sludge without inhibitors, CH₄ was actively produced, and there were no volatile fatty acids, the intermediary products of anaerobic degradation of organic matter, in the sludge. Inhibition of methanogenesis by chloroform resulted in the accumulation of H₂ and volatile fatty acids such as acetate and propionate. Adding sulfate to the sludge did not affect CH₄ production, but there was much sulfate reduction. In the sulfate-supplemented sludge, the inhibition of methanogenesis also caused acetate accumulation, but not the longer-chain fatty acids or H₂. Inhibition of sulfate reduction by molybdate did not affect either CH₄ production or the concentrations of the intermediary products. But the inhibition of both methanogenesis and sulfate reduction caused the accumulation of acetate, longer-chain fatty acids and H₂.

These results indicate that the enhancement of sulfate reduction in the sludge is supported by the acceleration of electron flow in the ecosystem and apparently does not retard methanogenesis.

A large amount of sewage sludge rich in organic matter is produced in municipal wastewater treatment facilities. Many attempts have been made to develop viable systems to use the biomass resource. Since organic matter is decomposed to CH₄ and CO₂, anaerobic digestion is a practical way to convert sewage sludge into an energy source.

Organic matter is digested anaerobically by many species of bacteria forming a complex community characteristic of anaerobic environments. To control the biogas production, the ecological roles of the bacteria with specialized functions in the anaerobic process need to be understood.
Methanogenesis and sulfate reduction are terminal stages in the anaerobic degradation process. These reactions are known to compete with each other for the electron donors in various environments. In the presence of sulfate at available levels, sulfate reduction generally dominates over methanogenesis due to differences in kinetic and thermodynamic properties (1–5). Also, the accumulation of hydrogen sulfide, the end-product of sulfate reduction, inhibits methanogenesis (6). Thus, sulfate reduction is thought to be one of the essential factors affecting methanogenesis. But in an anaerobic digester slurry of cattle waste, a nutritionally rich environment, sulfate reduction does not compete with methanogenesis even in the presence of sulfate at mM levels (7, 8). It is of interest to know whether sulfate reduction competes with methanogenesis in sewage sludge of municipal wastewater, which is also nutritionally rich.

In the present study, anaerobic digestion of sewage sludge of municipal wastewater was investigated to determine the relationship between methanogenesis and sulfate reduction.

MATERIALS AND METHODS

Sampling of municipal sewage sludge. Sewage sludge was sampled from the bottom of an anaerobic digester, where CH₄ production was actively proceeding, in the Wastewater Treatment Center of Tsuruoka City in Yamagata Prefecture. The sludge sample was stored at 7–8 °C in 500-ml polyethylene bottles sealed tightly with caps until use. The CODₘₙ of the sludge sample was about 3,200 ppm and the pH was about 7.4. In general, acetate (about 0.8 mM) and propionate (about 0.3 mM), but no other volatile fatty acids, were present in the sludge sample at detectable levels. The concentrations of sulfate and sulfide were less than 0.05 mM and about 1.5 mM, respectively.

Anaerobic incubation of sewage sludge. The sludge was incubated at 30 °C for 24 h under N₂ to activate the microbial activity, then 0.0005% (v/v) chloroform and/or 5 mM sodium molybdate were added to the sludge. Some of the samples were supplemented with 10 mM sodium sulfate under N₂. Ten-ml portions of the sludge with or without the inhibitors and/or sodium sulfate were distributed under N₂ to test tubes (18 x 180 mm) which had been flushed with N₂. The tubes were tightly sealed with butylrubber double stoppers and then incubated at 30 °C on a reciprocal shaker (70 rpm).

Analytical methods. CH₄, CO₂, H₂ and the volatile fatty acids were analyzed by gas chromatography using a Hitachi 163 Gas Chromatograph, as reported previously (7). Gas was sampled from the headspace of the incubation tube by inserting the needle of a pressure lock syringe (Precision Sampling Co.) through the butylrubber double stopper, and then injected into the gas chromatograph. Samples for fatty acid analysis were deproteinized with 24% (w/v) metaphosphoric acid in 5 N H₂SO₄ and then injected into the gas chromatograph. The CODₘₙ was
Anaerobic digestion of sewage sludge in the presence or absence of inhibitors

Effects of chloroform and molybdate, specific inhibitors of methanogenesis and sulfate reduction (11, 12), on anaerobic digestion of the sludge were first examined without the sulfate supplementation.

CH$_4$ production during anaerobic incubation of the sludge with or without inhibitors is shown in Fig. 1. Without the addition of chloroform, CH$_4$ production proceeded actively, and about 27 mmol/l of CH$_4$ was evolved during 8 days of incubation. Adding 0.0005% (v/v) of chloroform drastically reduced the CH$_4$ accumulation during the incubation period to only about 2 mmol/l. Adding 5 mM molybdate did not significantly affect CH$_4$ production. The production of CO$_2$ was reduced, only slightly, in the presence of chloroform (data not shown). As also shown in Fig. 1, H$_2$ was transiently accumulated only in the presence of chloroform, irrespective of the addition of molybdate. The H$_2$ increased to about 0.27 mmol/l after 4 days and decreased thereafter.

The pH of the sludge was between 7.3 and 7.5 during the incubation period,

![Graph showing CH$_4$ and H$_2$ accumulation during anaerobic incubation of sewage sludge.](image)
irrespective of the addition of inhibitors (data not shown). Any decrease in sulfate concentration by sulfate reduction could not be measured in practice, since the sulfate concentration in the sludge was too low.

Concentrations of volatile fatty acids changed during incubation of the sludge with or without inhibitors (Fig. 2). Without the addition of chloroform, concentrations of volatile fatty acids decreased to undetectable levels after 2-4 days. But in the sludge with chloroform, acetate, propionate, butyrate and isovalerate increased after 8 days to about 9.5, 1.8, 0.3 and 0.4 mM, respectively. In the sludge with both chloroform and molybdate added, acetate, propionate and butyrate also increased after 8 days to about 6.5, 1.5 and 0.4 mM, respectively.

**Anaerobic digestion of sewage sludge supplemented with sulfate in the presence or absence of inhibitors**

Since sulfate concentration in the sludge was too low to investigate the relation of sulfate reduction to methanogenesis, 10 mM sulfate was added to the sludge, and the effects of the inhibitors on anaerobic digestion were examined. As shown in Fig. 3, CH$_4$ production was not affected by the added sulfate. CH$_4$ production was drastically reduced by adding chloroform, but it was not significantly affected by adding of molybdate. The production of CO$_2$ was slightly reduced only in the presence of chloroform (data not shown). H$_2$ accumulated only in the sludge with both chloroform and molybdate (Fig. 3). The level of H$_2$ increased to about 0.4 mmol/l after 4 days, and decreased thereafter.

Figure 4 shows the effects of the inhibitors on sulfate reduction in the sulfate-supplemented sludge. Sulfate reduction proceeded markedly without the addition of molybdate. Sulfate reduction proceeded somewhat more in the sludge with
Adding molybdate completely blocked sulfate reduction during the incubation period.

Changes in concentrations of volatile fatty acids during incubation of the sulfate-supplemented sludge are shown in Fig. 5. Without the addition of inhibitors, acetate at low concentrations (0.3–0.8 mM) was the sole detectable volatile fatty acid. Acetate increased to about 12 mM after 8 days of incubation with chloroform alone. Isovalerate was also accumulated in the sludge with chloroform, while propionate and butyrate, which were accumulated in the sludge without sulfate supplemented, were not accumulated. Volatile fatty acids did not accumulate in the sludge with molybdate alone. The concomitant addition of chloroform with molybdate during the incubation period greatly increased the concentrations of acetate, propionate, butyrate and isovalerate to about 6.0, 1.7, 0.5 and 0.5 mM, respectively.
Methanogenesis and sulfate reduction are the terminal steps of the electron flow in the anaerobic digestion of organic matter and are thought to compete with each other for the utilization of electron donors. In sulfate-rich environments, such as marine sediments, sulfate reduction is dominant, but methanogenesis is dominant in environments such as freshwater sediments, where sulfate is limited (1, 2, 13–17).

In the present study, CH4 was actively produced during the anaerobic incubation of sewage sludge, but there were no significant amounts of intermediary volatile fatty acids. Thus, anaerobic digestion in this sludge apparently proceeded steadily without being limited by its terminal steps.

The inhibition of methanogenesis by chloroform caused the accumulation of H2 and volatile fatty acids, in particular acetate and propionate. Since the sludge was anaerobically digested with a batch treatment and sulfate was limited, the contribution of sulfate reduction to the anaerobic digestion appeared to be minimal when sulfate was not supplemented. That molybdate did not significantly affect the anaerobic digestion was consistent with this interpretation. It was expected that without the sulfate supplement, the inhibition of methanogenesis would result in the accumulation of substrates for methanogenesis. Generally 60 to 80% of CH4 is thought to come from acetate (18, 19), and the remainder mostly from H2 and CO2. Present results, however, showed that propionate and other longer chain fatty acids accumulated together with acetate and H2 when methanogenesis was inhibited. It is known that the degradation of propionate and other longer-chain fatty acids to
acetate and $H_2$ by the $H_2$-producing syntrophic bacteria is an essential step in anaerobic degradation (20, 21). Many of the $H_2$-producing syntrophic bacteria can grow only at a very low $H_2$ concentration, and their growth depends strictly on the presence of $H_2$-utilizing bacteria, such as methanogenic bacteria and sulfate reducing bacteria (22–28). The accumulation of longer-chain fatty acids and $H_2$ when methanogenesis is blocked suggests that the degradation of longer-chain fatty acids by the syntrophic bacteria is inhibited as a result of the accumulation of $H_2$. In the present sludge, methanogenic bacteria may use acetate and also $H_2$ as the electron donor, playing a role as a main $H_2$-scavenger, when sulfate is not supplemented.

The sulfate supplementation did not significantly affect $CH_4$ production, although sulfate reduction proceeded markedly. In addition, the inhibition of sulfate reduction did not affect the anaerobic digestion. These results indicate that methanogenesis in the sulfate-supplemented sludge proceeds practically without suppression by sulfate reduction. In the sulfate-supplemented sludge, longer-chain fatty acids and $H_2$ were accumulated together with acetate only when both methanogenesis and sulfate reduction were inhibited. Thus, it seems that mainly acetate is used in the methanogenesis in the sulfate-supplemented sludge, and that sulfate reduction utilizes $H_2$ and longer-chain fatty acid, perhaps propionate, and competes with methanogenesis for the utilization of $H_2$.

In nutritionally rich environments, such as the digester sludge of cattle waste, sulfate reduction is known to proceed without suppressing methanogenesis even in the presence of sulfate at mM levels (7, 8). The present study shows that in anaerobic digestion of municipal sewage sludge, as well as in the digester sludge of cattle waste, sulfate reduction and methanogenesis do not practically compete with each other, while these reactions share the role of $H_2$-scavengers.

In conclusion, electron flow to sulfate reduction in the sludge with sulfate at available levels can be supported apparently without retarding electron flow to methanogenesis. In the presence of sulfate at available levels, total reducing power distributed to methanogenesis is, however, finally reduced, when compared with that in the absence of sulfate.

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REFERENCES