Effect of moisture content on anaerobic digestion of dewatered sludge: ammonia inhibition to carbohydrate removal and methane production

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Abstract The purpose of this study is to investigate the effect of moisture content on anaerobic digestion of dewatered sewage sludge under mesophilic condition. The moisture contents of sludge fed to reactors were 97.0%, 94.6%, 92.9%, 91.1% and 89.0%. The VS removal efficiency changed from 45.6% to 33.8%, as the moisture content of sludge fed to digester decreased from 97.0% to 89.0%. The carbohydrate removal efficiency also decreased from 71.1% to 27.8%. Methane production decreased when the moisture content of sludge was lower than 91.1%. The number of glucose consuming acidogenic bacteria was decreased from 3.13 10^6 to 3.13 10^8(MPN/mL) as the moisture content decreased from 91.1% to 89.0%. The numbers of hydrogenotrophic and acetoclastic methanogenic bacteria decreased by one order of magnitude when the moisture content was lower than 91.1%. The decrease in numbers of glucose consuming acidogenic bacteria and methanogenic bacteria was found to correspond to the decrease in the carbohydrate removal efficiency and the accumulation of propionic acid. Batch experiments showed that acetoclastic methanogenic bacteria were acclimated to high ammonia concentration, on the other hand, glucose consuming acidogenic bacteria were inhibited.

Keywords anaerobic digestion; dewatered sludge; moisture content; ammonia inhibition; acidogenic bacteria; methanogenic bacteria

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Introduction

An anaerobic digestion process is one of the most useful processes that can recover renewable energy from sewage sludge. However, in Japan, the number of sewage treatment plants employing an anaerobic digester has not increased in the last ten years in spite of the increase of sewage treatment plants. This is because the small-scale sewage treatment plants have been mainly constructed nowadays in Japan. From an economical point of view, it is generally considered that the use of an anaerobic digester is not always profitable in such small-scale sewage treatment plants. In the sludge treatment system to be suggested in this study, the dewatered sludge discharge from the small scale plants is collected to the plant in which an anaerobic digester is installed and methane gas can be recovered effectively (Figure1). If a methane production rate and a solid removal rate of a dewatered sludge anaerobic digestion (moisture content <90%) are close to those of the conventional sewage sludge anaerobic digestion (moisture content >97%), anaerobic digestion of dewatered sludge would permit higher organic loads and the design of smaller volume digesters.

Sewage sludge generally contains a high amount of proteins that release ammonia. Ammonia is an essential nutrient for anaerobic bacteria, however, if the concentration exceeds a threshold level, it inhibits methane production rapidly (Koster and Lettinga, 1984, 1988; Hashimoto, 1986). To maintain a stable methane production above the level requires the acclimation of methanogenic bacteria to high ammonia concentrations. Velsen (1979) reported that methanogenic bacteria acclimated to an ammonia nitrogen concentration of 2420 mg-N/L could produce methane immediately without any lag phase at the ammonia nitrogen concentration of over 3000 mg-N/L. Acclimation of methanogenic bacteria to

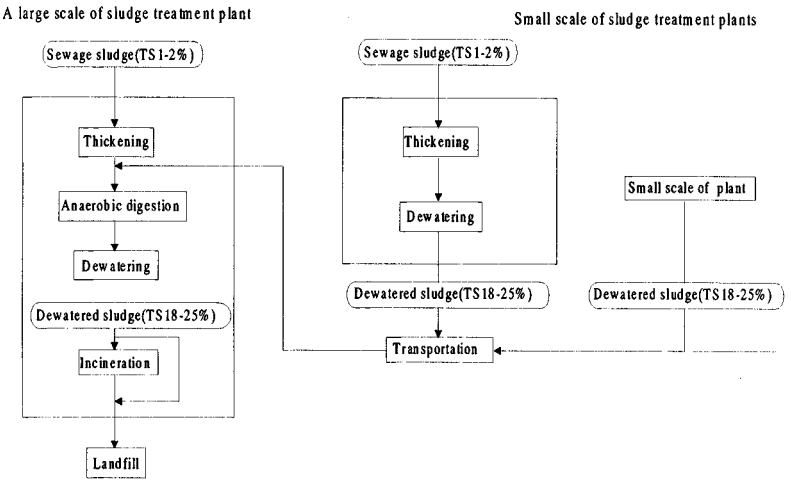

Figure 1 Scheme of the sludge treatment system to be suggested from this study

ammonia has been investigated by many researchers (Parkin, 1983; Koster and Lettinga, 1988; Robbins, 1989).

It is known that ammonia does not inhibit methane production in conventional anaerobic digestion of sewage sludge (moisture content >97%), because the ammonia nitrogen concentration is under 1000 mg-N/L. However, if the protein removal rate of dewatered sludge anaerobic digestion (moisture content <90%) are close to that of the conventional sewage sludge digestion (moisture content >97%), ammonia nitrogen concentration will be over the level in which an inhibition to the methane production occurs. The acclimation of methanogenic bacteria to ammonia concentration is essential to the successful operation of a dewatered sludge anaerobic digestion.

The purpose of this study is to investigate the effect of moisture content on a dewatered sludge anaerobic digestion under mesophilic condition, and the effect of high ammonia concentrations on acidogenesis and methanogenesis.

Materials and methods

Substrate and seed sludge

The dewatered sewage sludge was sent from the sludge treatment plant in the Kawasaki city, Japan. The dewatered sludge was adjusted to the target moisture content with tap water and mixed in a blender. The sludge was filtered through a 4-mm mesh net before fed into reactors as a substrate. The sludge was kept at 4ºC in a substrate tank.

The moisture contents of the sludge fed to reactors were 97.0%, 94.6%, 92.9%, 91.1% and 89.0%, and the average CODs of the sludges used were 37.2g/L, 67.0g/L, 88.0g/L, 110g/L and 136g/L, respectively. The dewatered sludge contained total carbohydrate 23.2%, total protein 29.8%, lipid 34.7% and total VFAs 6.5% based on COD. The seed sludge was obtained from the mesophilic sludge digester in the sewage treatment plant located in Yamagata city.

Continuous experiment

Figure 2 shows a schematic diagram of a completely mixed anaerobic digester. The reactors were maintained at 3.5ºC by hot water circulation in a water jacket surrounding the reactor.

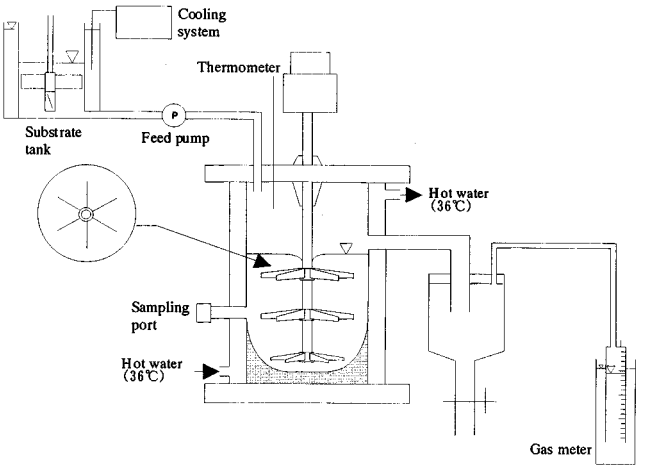

Figure 2 Schematic diagram of a completely mixed anaerobic digester

Substrate was fed four times per day. Digested sludge was drawn two times per day from a sampling port. The sludge retention time (SRT) was 14 days. These reactors were operated for about two months.

Batch experiment

Batch experiments were performed in 120 mL total capacity serum bottles. Serum bottles contained 80 mL of the mixture composed of 20 mL of seed sludge from anaerobic digester fed with sewage sludge (moisture content 89.0%), 60 mL of a stock solution, comprising $NaHCO_3$; 6g/L, glucose; 3g/L or sodium acetate; 2g/L and varying amounts of NH_4Cl. The pH in stock solution was adjusted to 8.0–8.1 with 1N HCl and 1N NaOH. All the operations were conducted while the samples were gassed with an atmosphere of N_2 using the Hungate technique to assure an anaerobic condition. The serum bottles were stopped with butyl rubber plugs and sealed with aluminium caps, and incubated in a water bath at 35ºC under a shaking speed of 80 (strokes/minute). Biogas production was measured with glass syringes with the volumes of 5 to 50 mL, using the Owen's approach (Owen, 1979). Glucose concentration was measured by the phenol-sulfuric acid method with glucose as a standard.

Analytical methods

Total solids (TS), volatile solids (VS), chemical oxygen demand (COD_{Cr}), volatile fatty acids (VFA) and pH were analyzed according to *Standard Methods* (APHA, 1992). Carbohydrate concentration was measured by the phenol sulfuric acid method with glucose as a standard. Protein was determined by Lowry-Folin method using bovine serum albumine as a standard. Lipid concentration was extracted by the Bligh-Dyer method, subsequently dried and weighed. The percentages of methane, carbon dioxide, and nitrogen in digestion gas were analyzed by a gas chromatograph (Shimadzu GC-6A) equipped with a thermal conductivity detector and a 1.5m stainless steel column filled with activated carbon. Helium was used as a carrier gas under a pressure of 0.75 kg/cm^2. The temperatures of the injection port and the column were 100ºC and 70ºC, respectively. The concentrations of individual volatile fatty acids were determined by a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector and a 2.0-m glass column filled with Greensorb.

The temperatures at the injection port and the column were 190ºC and 140ºC, respectively. Helium was used as the carrier gas with a pressure of 1.5 kg/cm^2. In addition, hydrogen gas and air were used under the pressure of 0.6 kg/cm^2. Ammonia was determined using a ion chromatograph (Shimadzu CDD-6A) equipped with a conductivity detector and a column (Shimpack IC-C3). The temperature at the column was 40ºC. Oxalic acid (2.5 mM) was used as an eluent solution. The flow rate was adjusted to maintain 1.0 mL/min.

The numbers of glucose consuming acidogenic bacteria, peptone consuming acidogenic bacteria, hydrogenotrophic methanogenic bacteria and acetoclastic methanogenic bacteria in each continuous digester were counted according to Most Probable Number (MPN) method using 10-fold dilutions and five tubes per dilution. All tubes contained 9 mL of pre-reduced liquid culture medium. The substrates were glucose (3 g/L), peptone (3 g/L), H_2/CO, (4/1; 1.5 atm) and sodium acetate (1 g/L). Fermentative bacteria and methanogenic bacteria were incubated at 35ºC for 14 days and 28 days, respectively. The presence of acidogenic bacteria was determined by turbidity. The presence of methanogenic bacteria was determined by the detection of methane in the gas.

Table 1 Performance for anaerobic digestion of dewatered sludge at various moisture content

	Moisture content of dewatered sludge fed to reactors (%)				
Parameter	**97.0**	**94.6**	**92.9**	**91.1**	**89.0**
pH	7.4	7.5	7.7	7.8	8.1
TS (%)	1.85	3.93	5.04	6.65	8.35
VS (%)	1.23	2.50	3.26	4.44	5.69
Methane production rate (mL/L-reactor/day)	450	880	1240	1420	1700
Methane yield (mL/g-VSS added)	280	310	330	300	290
Total-COD(g/L)	19.8	37.5	50.5	66.7	84.5
Carbohydrates (g/L)	2.38	5.18	6.50	14.4	21.5
Proteins (g/L)	5.94	11.2	14.8	18.0	19.1
Lipids (g/L)	1.79	3.36	4.49	5.33	7.74
Total ammonia nitrogen (mg/L)	710	1370	1820	2480	3100
Acetic acid (mg/L)	72	214	103	253	232
Propionic acid (mg/L)	41	63	51	260	858

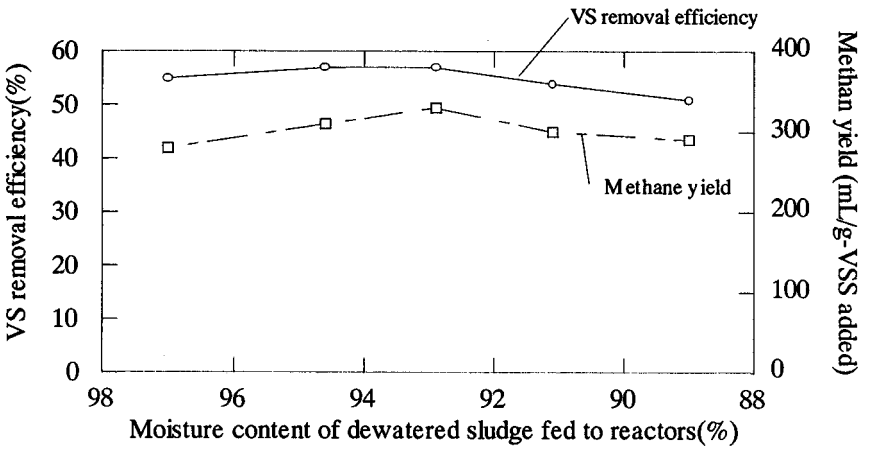

Figure 3 Effect of moisture content of dewatered sludge fed to reactors on VS removal efficiency and methane yield

Results and discussion

Figure 3 shows the effect of moisture content of dewatered sludge fed to reactors on VS removal efficiency and methane yield. In order to consider the volatization of NH_4HCO_3, the calculation of VS removal efficiencies (Beall, 1998) was modified by adding NH_4HCO_3 concentration to TS concentration in influent and effluent, respectively (Matsunaga, 1999). NH_4HCO_3 concentration was calculated on the basis of ammonium ion concentration. VS removal efficiencies and methane yield were varied from 51 to 57% and from 280 to 330 (mL/g-VSS added), respectively.

Figure 4 shows the effect of a moisture content of substrate on removal efficiencies of individual matter. The carbohydrate removal efficiency decreased from 71.1% to 27.8% as the moisture content of dewatered sludge fed to digester decreased from 97.0% to 89.0%. The protein removal efficiency increased from 30.7% to 42.0%. The lipid removal efficiencies were varied from 50% to 60%. The results indicated that the decrease of moisture content of substrate caused inhibition of carbohydrate degradation and activation of protein degradation.

The highest methane yield was obtained when the moisture content of substrate was 92.9%. The total ammonia nitrogen concentration increased from 710 to 3100 mg-N/L as the moisture content of substrate decreased from 97.0% to 89.0%. Koster (1984) reported that ammonia nitrogen had relatively strong effect on the metabolism of the acetoclastic

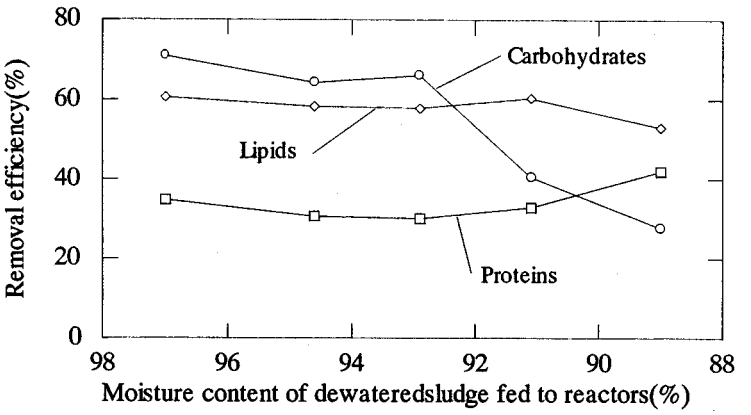

Figure 4 Effect of moisture content of dewatered sludge fed to reactors on removal efficiencies of the individual organic composition

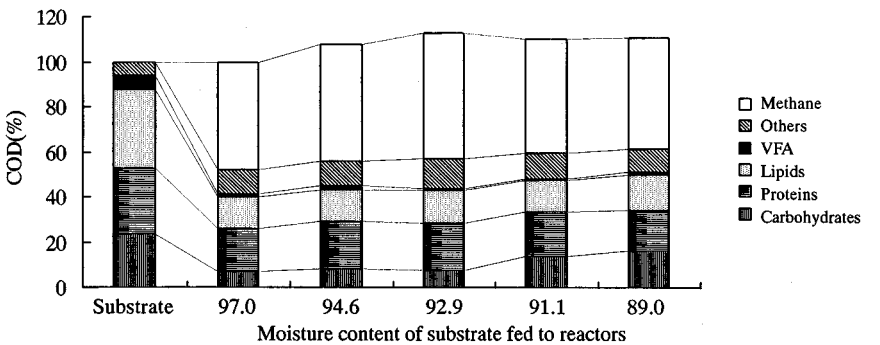

Figure 5 Material balance based on COD in each digester

Table 2 Conversion factor based on COD

Parameter	Conversion factor
Carbohydrate	1.067 mg-COD/mg-glucose
Protein	1.240 mg-COD/mg-protein
Lipid	2.880 mg-COD/mg-lipid
Acetic acid	1.066 mg-COD/mg-acetic acid
Propionic acid	1.512 mg-COD/mg-propionic acid
Butyric acid	1.816 mg-COD/mg-butyric acid
Valeric acid	2.037 mg-COD/mg-valeric acid
Methane	2.857 mg-COD/mL-CH_4

methanogenic bacteria than on that of the hydrogenotrophic methanogenic bacteria when the ammonia nitrogen concentration was over the threshold level of about 1700 mg-N/L. Table 1 shows that total VFA concentrations in all digesters were lower than 1000 mg/L. It seems that the low acetic acid concentration was due to the result of the acclimation of acetoclastic methanogenic bacteria to high ammonia-nitrogen concentrations by gradually decreasing moisture content of substrate. On the other hand, hydrogenotrophic methanogenic bacteria were slightly influenced by the high ammonia nitrogen concentration when the moisture content of substrate was 89.0%, because the concentration of propionic acid was 858mg/L. Angelidaki and Ahring (1993) showed a higher sensitivity of the acetoclastic compared to the hydrogenotrophic methanogenic bacteria. In contrast, Wiegant and Zeeman (1986) reported that ammonia (>3500 mg-N/L) inhibited hydrogenotrophic methanogenic bacteria, but acetoclastic methanogenic bacteria was not influenced by ammonia concentration up to 4500 mg-N/L. Our results is similar to the results by Wiegant and Zeeman (1986), because both studies were performed using the sludge acclimated to high ammonia condition. The former paper reached opposite results because the sludge may not be acclimated to high ammonia condition.

Figure 5 show the material balance based on COD in each digester. At all the moisture content conditions, the conversion efficiencies of organic matter into methane were varied from 44% to 50%. Though substrate contains 6.5% total VFAs based on COD, total VFAs in all the digested sludges used were lower than 1%. These results indicate that methanogenesis was not a rate limiting reaction.

Carbohydrate removal efficiencies were decreased when the moisture content of substrate was under 91.1%. At the condition, the conversion efficiency of substrate into methane also decreased. It seemed that the decrease in methane yields were caused by the decrease in carbohydrate removal efficiency.

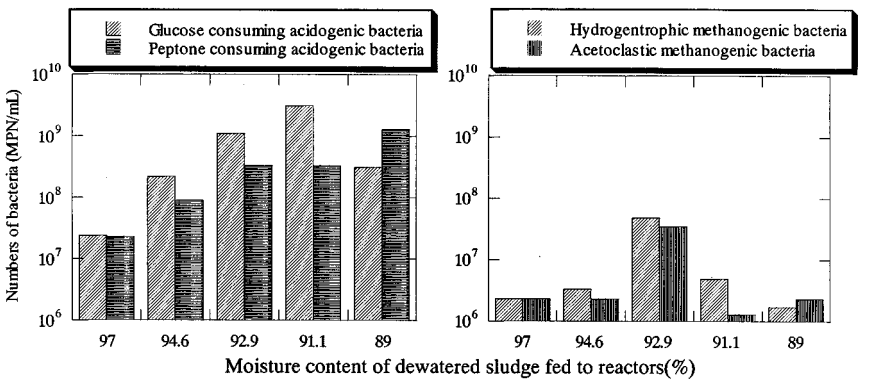

Figure 6 Effect of moisture content of dewatering sludge fed to reactors on the bacterial populations

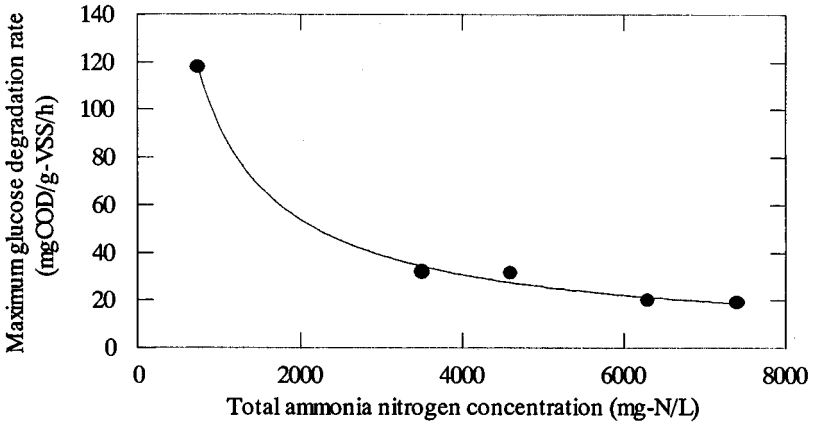

Figure 7 Effect of total ammonia nitrogen concentration on maximum glucose degradation rate

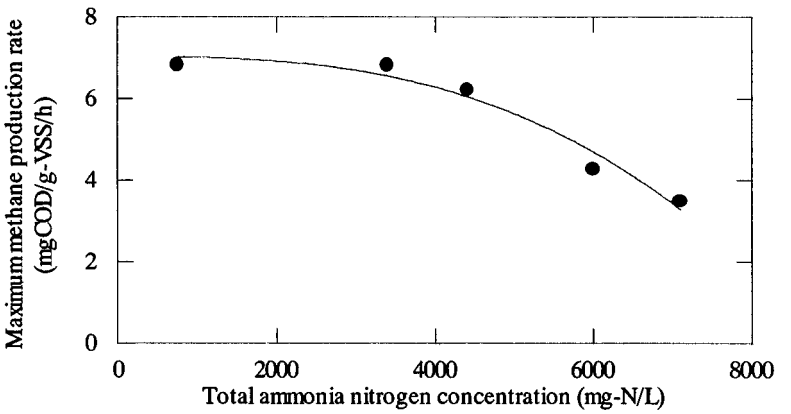

Figure 8 Effect of total ammonia nitrogen concentration on maximum methane production rate

Figure 6 shows the effect of moisture content of dewatered sludge fed to the reactors on the bacterial populations. The number of peptone consuming acidogenic bacteria increased from 2.3×10^7 to 1.3×10^9 (MPN/mL), as the moisture content decreased from 97.0% to 89.0%. However, the number of glucose consuming acidogenic bacteria decreased from 3.1×10^9 to 3.1×10^8 (MPN/mL), when the moisture content decreased from 91.1% to 89.0%. The decrease in number of glucose consuming acidogenic bacteria and the increase in that of peptone consuming acidogenic bacteria correspond to the decrease in carbohydrate removal efficiency and the increase in protein removal efficiency in the continuous experiments, respectively.

The numbers of hydrogenotrophic and acetoclastic methanogenic bacteria varied from 10^6 to 10^8 (MPN/mL), when the moisture content decreased from 97.0% to 89.0%. The numbers of hydrogenotrophic and acetoclastic methanogenic bacteria decreased by one order of magnitude when the moisture content was lower than 91.1%. The number of hydrogenotrophic methanogenic bacteria decreased from 4.9×10^6 to 1.7×10^6 (MPN/mL), as the moisture content decreased from 91.1% to 89.0%. Hydrogenotrophic methanogenic bacteria play an important role in anaerobic digestion by keeping the hydrogen concentration low enough to make it thermodynamically possible for the conversion of propionic acid into acetic acid and hydrogen which are substrate for methanogenic bacteria. The

accumulation of propionic acid was observed when the moisture content was 89.0%. On the other hand, the numbers of acetoclastic methanogenic bacteria were almost the same when the moisture content varied from 91.1% to 89.0%. This observation indicates that the acetoclastic methanogenic bacteria presented in the digester had relatively strong resistance to high ammonia concentration. Wiegant and Zeeman (1986) reported that acetoclastic methanogenic bacteria acclimated to high ammonia concentration, but hydrogenotrophic methanogenic bacteria did not. This report was found to correspond to the results in this study.

In order to understand the effect of ammonia concentration on the glucose degradation and on the acetoclastic methanogenic reaction by anaerobic microflora incubated at an ammonia nitrogen concentration of 3100 mg-N/1, batch experiments were performed at various ammonia nitrogen concentrations. Figure 7 and Figure 8 show the effect of total ammonia nitrogen concentration on maximum glucose degradation rate and maximum methane production rate. Maximum glucose degradation rates and maximum methane production rates at each ammonia nitrogen concentration were calculated by fitting using the Gompertz equation (Zwietering, 1990).

As the concentration of ammonia nitrogen increased from 740 to 3500 mg-N/L, glucose degradation rate significantly decreased. This result in the batch experiments was approximately similar to the decrease in carbohydrate renewal efficiencies in continuous experiments. Therefore, it seems that the accumulation of ammonia had inhibitory effect on the glycolytic pathway via which glucose hydrolyzed from carbohydrates was degradated. Robbins (1989) reported that the appreciable inhibitory effect on glucose utilization rate was not observed. This is because the glucose utilization rate was low even at normal ammonia concentration.

Methane production rate from acetic acid was almost equivalent among the ammonia nitrogen concentration of 750, 3400 and 4400 mg-N/L. It was clear that the acetoclastic methanogenic bacteria had resistance to the high ammonia concentration. This was the result of sufficient adaptation of the anaerobic microflora to the high ammonia nitrogen concentration of 3100 mg-N/L in the digester.

Conclusions

1. The carbohydrate removal efficiencies was decreased from 71.1% to 27.8% as the moisture content of dewatered sludge fed to digester decreased from 97.0% to 89.0%.
2. The decrease in the number of glucose consuming acidogenic bacteria was found to correspond with the decrease in the carbohydrate removal efficiency in continuous experiments.
3. Acetoclastic methanogenic bacteria was acclimated to an ammonia nitrogen concentration of 3100 mg-N/L, but hydrogenotrophic methanogenic bacteria was not.
4. The decrease in carbohydrate removal efficiency was caused by an inhibition of ammonia on glycolytic pathway. Glucose consuming acidogenic bacteria had not acclimated to high ammonia concentration in this experimental period.

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