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ANALYSIS OF ENVIRONMENTAL FACTORS AFFECTING METHANE PRODUCTION FROM HIGH-SOLIDS ORGANIC WASTE

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ABSTRACT

A simple model developed from the Gompertz equation was used to describe the cumulative methane production curve in the batch culture. By using this model, three key parameters, namely methane production rate, potential and lagphase time, in a cumulative methane production curve were exactly estimated based on the experimental data. The results indicate that each gram of dry organic waste of a sludge cake, meat, carrot, rice, potato and cabbage had a methane production potential of 450, 424, 269, 214, 203 and 96 mL, respectively. The methanogenic activity of these digesters decreased with a decrease in the moisture content. The moisture content threshold limit, at which the methanogenic activity dropped to zero, was found to be 56.6% for the sludge cake, but greater than 80% for meat, carrot and cabbage. In the high-solids sludge digestion, the relative methanogenic activity dropped from 100% to 53% when the moisture content decreased from 96% to 90%. The rate of methane production at moisture contents of 90% to 96% functioned in a pH range between 6.6 and 7.8, but optimally at pH 6.8, and the process may fail if the pH was lower than 6.1 or higher than 8.3. On the other hand, the methanogenic activity was dependent on the level of ammonium, NH_4^+, but not free ammonia, NH_3, indicating that the NH_4^+ was the more significant factor rather than the NH_3 in affecting the methanogenic activity of a well-acclimatized bacterial system. In the wide pH range of 6.5 to 8.5, the methanogenic activity decreased with the increase in the NH_4^+; dropped 10% at the NH_4^+-N concentration of 1670-3720 mg•L^{-1}, 50% at 4090-5550 mg•L^{-1} and dropped to zero at 5880-6600 mg•L^{-1}. However, the lagphase time was dependent on the NH_3 level, but not on NH_4^+, and when NH_3-N was higher than 500 mg•L^{-1}, a notable shock was observed. This suggests that the NH_3 level was the more sensitive factor than the NH_4^+ level for an unacclimatized bacterial system. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Ammonia; high-solids anaerobic digestion; lagphase time; methanogenic activity; methane fermentation; moisture content; organic fraction of municipal solid waste; pH; sewage sludge.

INTRODUCTION

Due to high human activity, the municipal solid waste, blended with sewage sludge, has increased year-by-year. This increase has led to the organic fraction of the municipal solid waste (OFMSW) being considered more as a resource, such as energy, than a waste material (Pipatti et al., 1995).

The methane fermentation of high-solids organic wastes, such as the OFMSW and dewatered sewage sludge, has been attempted as a new approach for developing a recycle and/or energy-producing type solid waste management system (Ghosh et al., 1984; Mata-Alvare et al., 1993; Kayhanian and Rich, 1996). To maintain a stable high-solids digestion process, the chemical nature, pH, volatile fatty acids, ammonia and moisture content have been considered to be the important environmental factors affecting the efficiency in the high-solids organic waste digestion (McCarty and McKinney, 1961; Koster and Lettinga, 1988; Speece, 1996).

During anaerobic fermentation, the organic refuse is converted to methane and carbon dioxide via a series of interrelated microbial metabolisms, including hydrolysis/fermentation, acetogenesis and methanogenesis (Barlaz et al., 1989). According to the studies on the anaerobic digestion of wastewater and sludge, the kinetics of methane fermentation strongly depends on the chemical nature of the organic matter. It has been elucidated that the rate-limiting step of the overall methane fermentation for sludge is hydrolysis; but for soluble sugars, methanogenesis is the rate-limiting step (Nagase and Matsuo, 1982; Henze and Harremoës, 1983).

The pH is known to influence enzymatic activity, because each enzyme is active within only a specific and narrow pH range and displays maximum activity at an optimum pH. Most methanogenic bacteria function in a pH range between 6.7 and 7.4, and the rate of methanogenesis may decrease if the pH is lower than 6.3 or higher than 7.8 (Bitton, 1994; van Haandel and Lettinga, 1994). The sharp drop in the rate below pH 6.3 may be related to the fact that methane formation proceeds at a slower rate than the production of organic acids (Koster, 1986); the sharp drop in the rate above pH 7.8 may be related to a shift in NH_4^+ to the toxic, unionized NH_3 form (Seagren et al., 1991).

Water is essential for methane fermentation as the nutrients for the microorganisms must dissolve in water before they can be assimilated (Forster and Wase, 1989). The moisture content may not only aid in bacterial movement, but is also known to influence the mass transport limitation on a high-solids bed and the balance between volatile fatty acids (VFAs) production by acidogenic bacteria and the conversion of acids to methane by methanogenic bacteria (Ghosh, 1985).

The objective of this study was to investigate the influences of the chemical nature, pH, moisture content and ammonia in the high-solids anaerobic digestion. For this purpose, a simple model was applied to systematically analyze the methane production rate, potential and lagphase time during the organic wastes decomposition. The effects of the environmental factors on methane production were then quantitatively evaluated for the well-acclimated and unacclimatized bacterial systems based on a series of batch experiments.

MATERIALS AND METHODS

Seed sludge and sludge cake. The seed sludge used in this study was taken from a ten-liter laboratory digester, operated at a temperature of 37 ± 1 °C and a HRT of 20 days. This digester has been run for over one year and a half by feeding it with sludge cake. The solids concentration in the digester was maintained at 3-4 wt.%. The sludge cake was conditioned with lime and obtained from a municipal sewage treatment plant in Sendai City, Japan.

Experimental apparatus and procedure. The experiments were performed under a mesophilic condition of 37 °C using a glass bottle of 120 mL volume as the high-solids organic waste digester. All operations proceeded under an atmosphere of 80% N_2 and 20% CO_2 using the Hungate technique (Miller and Wolin, 1974) to assure an anaerobic condition. A rotary cell culture apparatus with a rotation speed of 1.5 rpm was used for mixing the contents of the digester. The biogas production was determined by glass syringes of 5-50 mL using Owen's approach (1979). The experimental procedures used in this study are outlined as follows.

(i) *Effects of chemical nature and moisture contents*: A total of six different organic matters, including meat, cabbage, carrot, rice, potato and sludge cake was used as the main components. Except for rice, the particle size of each individual solid waste was about 2 cm. For each vial, forty grams of the seed sludge and a proper quantity of organic matter (Table 1) were added, and then filled to 80 grams with deoxygenated incubation media (Table 2, Li and Noike, 1992).

Table 1. The experimental conditions used for studying the effects of chemical nature and moisture contents.

Organic waste	Moisture contents of digesters (%)						
Sludge cake	97.2	94.4	91.6	88.8	86.0	83.2	77.7
Meat	96.0	94.0	92.2	90.0	88.0	86.0	-
Cabbage	98.1	97.8	97.3	97.0	96.6	96.3	95.5
Carrot	98.0	97.6	97.2	-	96.3	95.9	95.0
Rice	97.1	95.8	94.4	93.0	91.6	90.3	87.5
Potato	88.6	87.3	85.9	84.5	83.1	87.8	79.0

(ii) *Effects of pH and moisture content*: A total of twenty-eight digesters with various pH and moisture contents was simultaneously operated. For each digester, forty grams of the seed sludge and a proper quantity of sludge cake were added to adjust the moisture contents from 90% to 96% and then filled to 80 grams with deoxygenated distilled water. The pH of the digester content was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 with concentrated HCl or NaOH.

(iii) *Effects of pH and ammonia*: A total of sixty digesters with different pH and NH_4^+ concentrations was simultaneously operated. For each digester, forty grams of the seed sludge and an appropriate quantity of sludge cake and NH_4Cl were added to adjust the moisture contents to 96% and the NH_4^+-N ranged from 100 to 6000 mg•L^{-1}, and then filled to 80 grams with deoxygenated distilled water. The pH of the digester content was adjusted to 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 with concentrated HCl or NaOH.

Analytical method. The percentage of CH_4 and CO_2 in biogas was analyzed using a gas chromatograph (Shimadzu 8A) equipped with a thermal conductivity detector (TCD) and a 2 m stainless column packed with activated carbon (60/80 mesh). The operational temperatures of the injection port, the oven and the TCD were 140, 120 and 140 °C, respectively. Helium was used as the carrier gas at a flow rate of 30 mL•min^{-1}. The concentrations of the VFAs were determined by a second gas chromatograph of the same model, equipped with a frame ionization detector (FID) and a 2 m glass column packed with KOCL-FM (60/80 mesh). The operational temperatures for the injection port, the oven and the FID were 160, 140 and 160 °C, respectively. Before the analysis of the VFAs, phosphoric acid was added to control the pH of the samples. The concentrations of the total solids (TS) and volatile solids (VS) were determined using a 10-mL sample in the 105 °C and 550 °C ovens, respectively, according to the procedures described in the Standard Methods (APHA et al., 1992). The pH of the samples was determined by a pH meter.

Table 2. Medium composition of bactch culture.

Components	Concentration	
KH_2PO_4	0.4	g•L^{-1}
K_2HPO_4	0.4	g•L^{-1}
NH_4Cl	1.0	g•L^{-1}
Mineral solution(a)	10.0	mL
Vitamin solution(b)	10.0	mL
$NaHCO_3$	4.0	g•L^{-1}
$MgCl_2$•$6H_2O$	0.21	g•L^{-1}
Cysteine HCl•H_2O	0.5	g•L^{-1}
Na_2S•$9H_2O$	0.25	g•L^{-1}
Resazurine	0.002	g•L^{-1}
pH	7.0-7.2	

(a) Contains, in grams per liter of distilled water: nitrotriacetic acid, 4.5; $FeCl_2$•$4H_2O$, 0.4; $CoCl_2$•$6H_2O$, 0.12; $Alk(SO_4)_2$, 0.01; NaCl, 1.0; $CaCl_2$, 0.02; Na_2MoO_4, 0.01; $MnCl_2$•$4H_2O$, 0.10; H_3BO_3, 0.01; $CuSO_4$•$5H_2O$, 0.01; $NiCl_2$•$6H_2O$, 0.02.

(b) Contains, in milligrams per liter of distilled water: biotin, 2; foric acid, 2; pyridoxine HCl, 10; thiamine HCl, 5; riboflavin, 5; nicotinic acid, 5; DL-calcium pantothenate, 5; vitamin B12, 0.1; *p*-aminobenzoic acid, 5; lipoic acid, 0.5.

Assay methods. The three equations used to assess the effects of the environmental factors on high-solids organic waste digestion are outlined as follows.

(i) The cumulative methane production data in the batch culture were fitted with the modified Gompertz equation (Lay et al., 1996).

$$M = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \tag{1}$$

where M is the cumulative methane production (mL), t is the incubation time (day), λ is the lagphase time (day), P is the methane production potential (mL), R_m is the methane production rate (mL•d^{-1}), and e is exponential 1.

(ii) The relationships between pH and the methane production rate on a certain moisture content were described by a modified Haldane equation (Tang et al., 1989; Antoniou et al., 1990; Mayo and Noike, 1994).

$$R = \frac{K_m \cdot [H^+]}{[H^+] + K_{OH} + \dfrac{[H^+]^2}{K_H}} \tag{2}$$

where R is the specific methane production rate (mL•gVS^{-1}•d^{-1}), K_m is the maximum specific methane production rate (mL•gVS^{-1}•d^{-1}), and the K_{OH} and K_H are rate constants [M].

(iii) The effects of NH_4^+ or NH_3 on methanogenic activity at a certain pH condition was described using a mathematical model proposed by Han and Levenspiel (1988).

$$R = R_0(1 - \frac{C}{C*})^n \tag{3}$$

where R_0 is the specific methane production rate without adding NH_4^+ or NH_3 (mL•gVS^{-1}•d^{-1}), C is the NH_4^+ or NH_3

concentration (mg•L^{-1}), C* is the critical NH_4^+ or NH_3 concentration above which the reaction cannot proceed (mg•L^{-1}), and n is a constant.

Here, the function of the "Solver" in Microsoft Excel version 5.0 (Trade secret, Soft-Art, Inc.), by converging the residual sum of the square between the experiment and the estimation to a minimum value was used to estimate the parameters of these models. All parameters were judged by the diagnosis procedure according to the approach reported by Wen et al. (1994).

RESULTS AND DISCUSSION

Characteristics of chemical nature and effect of moisture content in high-solids organic waste fermentation. To assess the effects of the chemical nature and moisture content on the high-solids organic waste digestion, Eq. (1) was applied to estimate the methane production potential, lagphase time and rate during the decomposition of each individual organic waste. As an example, Figure 1 shows the fitting curves using the best values of the parameters and Eq. (1). Both the curve fitting and statistical analysis demonstrated that Eq. (1) was suitable to describe the progress of cumulative methane production on anaerobic digestion. The results indicate that the methane production potentials of meat, cabbage, carrot, rice, potato and sludge cake are 424, 96, 269, 214, 203 and 450 mL•gVS^{-1}, respectively. It reveals that these organic wastes could be divided into three groups: (i) meat and sludge cake; (ii) carrot, rice and potato; and (iii) cabbage. The main chemical components of the group (i) are proteins and lipids; while for the groups (ii) and (iii) are starch and cellulose, respectively (George et al., 1993; Patricia, 1988). Apparently, the methane production potential of the organic wastes depended on their chemical nature. In addition, a good relationship between moisture content and lagphase time was obtained for each organic waste decomposition, indicating that the lagphase time decreased with the increase in the moisture content, as shown in Figure 2. It has been reported that the period of the lagphase was mainly influenced by the initial concentrations of microorganisms and substances stimulating the growth of bacteria (Lankford et al., 1966). In this study, because the same quantity of seed sludge and the same size of the samples were used, the change in lagphase time for the same kind of solid waste was caused by the change in moisture content.

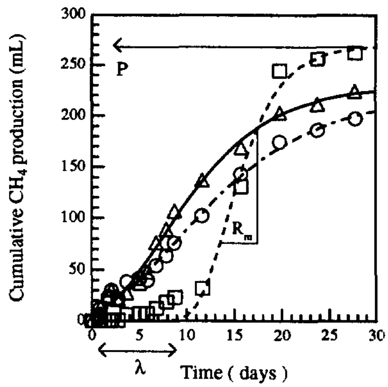

Fig. 1. The nonlinear regression fit of the modified Gompertz equation on the cumulative methane productions obtained from the solid beds.

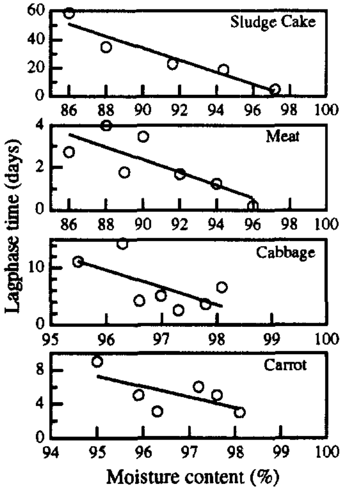

Fig. 2. Effects of the moisture content on the lagphase time of the solid wastes.

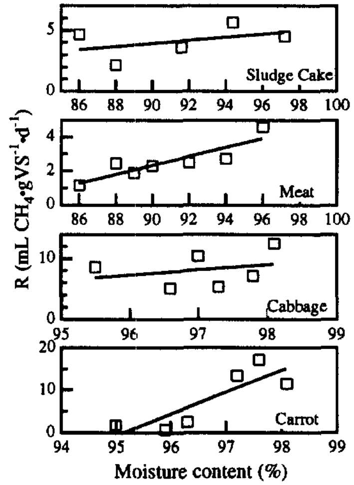

Fig. 3. Effects of the moisture content on the specific methane production rate of the solid wastes.

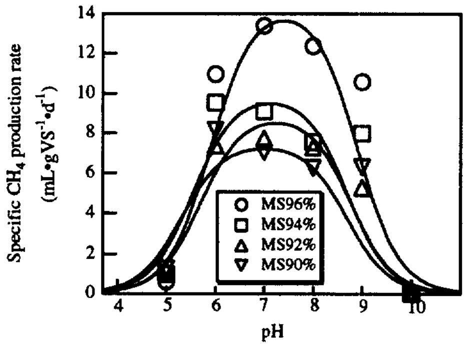

Fig. 4. Specific methane production rate of the digester sludge over the pH range of 5 to 10 and the moisture contents of 90% to 96%.

Table 3. Summary of kinetic parameters of K_m, K_{OH} and K_H for the Haldane equation at various moisture content.

Moisture (%)	K_m (CH_4mL•gVS^{-1}•d^{-1})	K_{OH} [M]	K_H [M]	pH Optimum	r^2
96	14.5	8.0×10^{-9}	8.0×10^{-6}	6.6	0.897
94	10.0	3.5×10^{-9}	5.0×10^{-6}	6.9	0.810
92	9.0	5.0×10^{-9}	6.0×10^{-6}	6.8	0.892
90	7.5	2.0×10^{-9}	5.0×10^{-6}	7.0	0.815

Similar results were obtained for the high-solids bed (Ghosh, 1984) and the sanitary landfill (Barlaz, 1988), indicating that the moisture content is an important factor on stimulating methane production. On the other hand, as shown in Figure 3, the specific methane production rate, R, increased with the increase of moisture content in the range conducted in this study. In Figure 3, the moisture content threshold limit, MSL, on the methane production of sludge cake, meat, carrot and cabbage occurred at 57%, 80%, 88% and 95%, respectively. Among them, the MSL of sludge cake, 57%, was close to the value of 60% for methane fermentation in a solid-bed (Ghosh, 1984) and a sanitary landfill (Barlaz, 1987). However, in this study, the MSL for meat, carrot and cabbage were significantly higher than 60%. This significant difference may determine the effect of the chemical nature, such as the degradation characteristics and pH buffer ability of the organic wastes. Because lime was used as the coagulant aid for sludge dewatering, the digester of sludge cake had a high capacity to buffer organic acids and reduced the inhibition of VFAs. However, the pH values of digesters for meat, carrot and cabbage were, respectively, 5.0, 4.8 and 5.2. At such condition, the methane production was inhibited by pH and a high level of organic acids. Consequently, the MSL of the sludge cake was mainly caused by the moisture content; the MSL of meat, carrot and cabbage was related to the organic acids.

Influences of pH and moisture contents on methane production in high-solids sludge digestion. As shown in Figure 4, the specific methane production rate, R, of the digesters over the pH range of 5 to 10 and the moisture contents of 90% to 96% were obtained in order to examine the influences of pH and moisture content on the high-solids sludge digestion. The R data were fitted with Eq. (2) and the best values of the kinetic parameters, K_m, K_{OH} and K_H, are listed in Table 3. Subsequently, the "optimum" and "acceptable" pH ranges at which the R dropped 5% and 20%, respectively, were calculated based on Eq. (2), and the results are listed in Table 4. An examination of Table 4 reveals that the methane production in the high-solids sludge digestion at moisture contents of 90% to 96% may proceed at a high rate when the pH is maintained at the optimal pH of 6.8 and decreases if the pH is lower than 6.6 or higher than 7.8, whereas the process may fail if the pH is lower than 6.1 or

Table 4. The "acceptable" and the "optimum" pH range in the high-solids sludge digestion.

Moisture	Acceptable pH range		Optimum pH range	
(%)	Acidic	Alkaline	Acidic	Alkaline
96	6.4 -	8.4	6.9 -	8.0
94	6.1 -	8.2	6.5 -	7.7
92	6.2 -	8.2	6.7 -	7.8
90	5.8 -	8.2	6.3 -	7.7

higher than 8.3. Accordingly, the "Equi-activity" lines were obtained for the plot of pH from 4.5 to 10.0 versus moisture contents inside the digesters from 90% to 96%. As shown in Figure 5, when the digesters were maintained at the optimal pH, the relative methanogenic activity decreased from 100% to 53% and the moisture content decreased from 96% to 90%. Under each moisture content condition, when the pH changed from optimum to the acidic condition of 5.6 or to an alkaline condition of 8.7, the relative methanogenic activity dropped from 100% to 36%. Also, the pH range for maintaining the high activity became narrower with the decrease in the moisture content. That is, to obtain a high rate of methane production, the moisture content in the digester must be maintained at above 96%, otherwise the methanogenic activity may decrease.

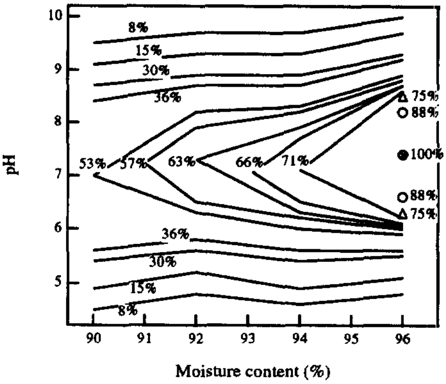

Fig. 5. Expression of the equivalent relative methanogenic activity on the "pH-Moisture" graph.

Effects of NH_4^+ and NH_3 under various pH levels. Many studies on the ammonia inhibition or toxicity in anaerobic digestion processes have been reported (McCarty, 1964; van Velsen, 1979; Koster and Lettinga, 1988). McCarty (1964) reported that ammonia can be presented in the form of ammonium ion or as dissolved ammonia gas, depending on the pH level. Consequently, the ammonia inhibition/toxicity on sludge not only simply considers the levels of ammonia but also those of the ammonium and free ammonia (Kroeker et al., 1979). Additionally, many attempts reported that the NH_4^+ was an important inhibitor on methanogenic bacteria (McCarty, 1964; van Velsen, 1979; De Baere et al., 1984). Koster and Koomen (1988) confirmed that NH_3 was a more powerful inhibitor than NH_4^+ according to the methanogenic activity. It has also been reported that after a long period of acclimation under a proper concentration of ammonia, sewage sludge can produce methane (Koster and Lettinga, 1988; van Velsen, 1979). In this study, to elucidate the effects of NH_4^+ and NH_3 during the high-solids sludge digestion, a nonlinear regression was used to fit a model described by Eq. (3), between pH 6.5 and 9.0. The

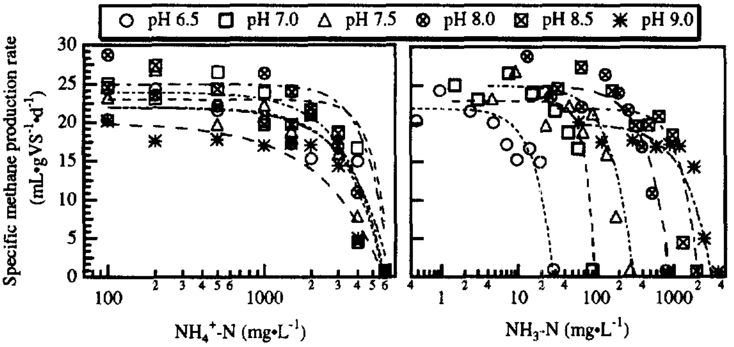

Fig. 6. The influences of NH_4^+ and NH_3 on the specific methane production rate at various levels of pH.

Table 5. Summary of parameters of C*, n, C_{10} and C_{50} for the best fitting using Eq. (3).

pH	n		C* (mg·L⁻¹)		C_{50} (mg·L⁻¹)		C_{10} (mg·L⁻¹)	
	NH_4^+-N	NH_3-N	NH_4^+-N	NH_3-N	NH_4^+-N	NH_3-N	NH_4^+-N	NH_3-N
6.5	1.9	1.9	5875	28	4086	20	1759	8
7.0	4.0	3.7	6600	97	5550	80	3711	52
7.5	1.8	1.6	6000	300	4082	195	1670	71
8.0	3.0	1.5	6500	890	5159	567	3017	199
8.5	1.7	2.1	6500	2000	4323	1438	1678	668
9.0	1.1	1.3	5900	3003	3141	1778	727	526

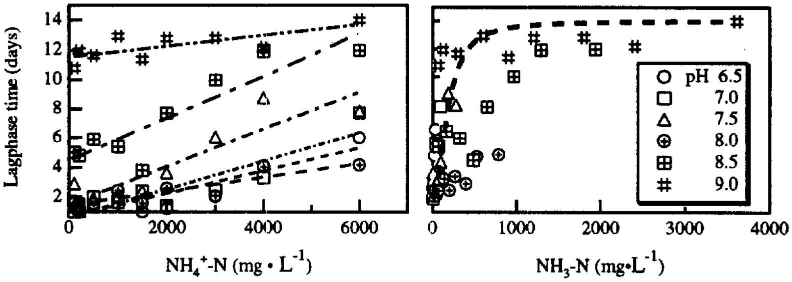

Fig. 7. The influences of NH_4^+ and NH_3 on the lagphase time.

best values for NH_4^+ and NH_3, which are listed in Table 5, were used to fit the data shown in Figure 6. In Table 5, the C_{10} and C_{50}, at which the R dropped 10% and 50%, respectively, were calculated based on Eq. (3) and the parameters of C* and n. The C_{10}, C_{50} and C* for NH_4^+-N in the pH range of 6.5 to 8.5 were 1670-3717, 4086-5550 and 5875-6600 $mg{\bullet}L^{-1}$, respectively, indicating that methanogenic activity began to significantly decrease at the NH_4^+-N of 1670-3717 $mg{\bullet}L^{-1}$, decreased 50% at 4086-5550 $mg{\bullet}L^{-1}$ and dropped to zero at 5875-6600 $mg{\bullet}L^{-1}$. However, the inhibition effect of NH_3 on the R strongly depended upon the pH level; for example, the C* of the NH_3-N at pH 8.0 was 890 $mg{\bullet}L^{-1}$, which was approximately 10 times that at pH 7.0 (Table 5). This evidence indicates that the NH_4^+ level was the more important factor, rather than the free NH_3 level, for illustrating the inhibition effect of ammonia on methanogenic activity in the well-acclimatized bacterial systems.

On the other hand, the lagphase time is a factor dependent on the acclimation period of microorganisms to a proper substrate and the environmental conditions in a batch culture (Lankford et al., 1966). However, due to lack of estimated method, the lagphase time has been neglected in most kinetic analyses of a batch experiment. In this study, the lagphase times for methane production at various pH and NH_4^+-N concentrations were estimated using Eq. (1), and the relationships between them are plotted in Figure 7. At each pH condition, the lagphase time increased with the increase in NH_4^+ level, but no clear relationship was found between the lagphase time and NH_4^+ for the entire data. This seems to suggest that the NH_4^+ level was not the conclusive factor affecting the lagphase time. However, as shown in Figure 7, when the lagphase times were plotted against their respective calculated NH_3-N concentrations, a good relationship was found. Apparently, the lagphase time in the batch experiment was dependent on the NH_3 level but not the NH_4^+. This suggests that the NH_3 level was the more sensitive factor than NH_4^+ for an unacclimatized bacterial system.

CONCLUSIONS

The principle conclusions derived from this investigation are as follows:

(1) The methane production potential of the organic fraction of municipal solid wastes depended upon their chemical nature. Each gram VS of the solid wastes of sludge cake, meat, carrot, rice, potato and cabbage had a methane production potential of 450, 424, 269, 214, 203 and 96 mL, respectively.

(2) The methanogenic activity of the digester decreased with the decrease in moisture content. The moisture content threshold limit, at which activity dropped to zero, was found to be 56.6% for sludge cake, but greater than 80% for meat, carrot and cabbage. In the latter cases, the methanogenic activity was inhibited by the high level of organic acids rather than moisture content.

(3) The rate of methane production in high-solids sludge digestion for moisture contents of 90% to 96% functioned in a pH range between 6.6 and 7.8, but optimally at pH 6.8, and the process may fail if the pH is lower than 6.1 or higher than 8.3. At optimum pH, the methanogenic activity in high-solids sludge digestion dropped from 100% to 53% when the moisture content decreased from 96% to 90%.

(4) Ammonium was the more significant factor rather than free ammonia in affecting the methanogenic activity of a well-acclimatized bacterial system. In the wide pH range of 6.5 to 8.5, the methanogenic activity decreased with an increase in NH_4^+-N; dropped 10% at the NH_4^+-N level of 1670-3720 $mg{\bullet}L^{-1}$, 50% at 4090-5550 $mg{\bullet}L^{-1}$ and dropped to zero at 5880-6600 $mg{\bullet}L^{-1}$. The lagphase time in the batch experiment was dependent on the NH_3 level, but not NH_4^+, and when the NH_3 was higher than 500 $mg{\bullet}L^{-1}$, a notable shock was observed.

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