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Note

Mesophilic and Thermophilic Methane Fermentations of Slop Waste

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Mesophilic and thermophilic methane fermentations of slop waste from molasses were studied under semi-continuous and continuous conditions using a 5-l jar fermentor, and a small increase of gas production and loading of volatile solids (VS) were observed in the continuous thermophilic fermentation.

Gas production of the semi-continuous mesophilic (37°C) and thermophilic (55°C) fermentations were 42.0 l (CH_4, 52–64%) and 64.8 l (CH_4, 52–68%), respectively, from 1 l of slop waste for 3 days' incubation. Gas production and COD_{Mn} reduction rate constants of the semi-continuous mesophilic fermentation were 0.203–0.610 day^{-1} and $1.80–3.00\times10^{-4}$ day^{-1}. These rate constants for thermophilic fermentation were 0.189–0.523 day^{-1}, and $2.18–3.00\times10^{-4}$ day^{-1}. These rate constants of continuous thermophilic fermentation were 0.297–0.460 day^{-1} and $1.50–2.98\times10^{-4}$ day^{-1}. Maximum loading of VS in the thermophilic fermentations was 24 g per l per day, and the gas evolution from 1 g of VS was 1.36 times larger than that of the mesophilic fermentation.

Slop waste is one of the waste products in alcohol manufacture from molasses. It contains large quantities of organic materials and has a large chemical oxygen demand (COD). Anaerobic lagoon systems have been used to treat slop waste in Thailand instead of anaerobic fermentation systems. If an anaerobic fermentation system can be used for treatment of slop waste, large amounts of methane gas would be recovered for use in industrial processes.

Clearly, thermophilic methane fermentation could take a load of slop waste 2.5 times larger than the loading in mesophilic fermentation, and it was also superior to mesophilic fermentation in a shorter fermentation period.[1–3] However, if a large volume of the sludge was used in both mesophilic and thermophilic fermentations, the same level of gas production was expected.[3]

In a previous paper, the superiority of thermophilic methane fermentation for treatment of agro-wastes was presented.[4] In this paper, methane fermentation of slop waste in semi-continuous mesophilic and thermophilic conditions and continuous thermophilic conditions was compared.

Materials and Methods

Methanogen sludge The mesophilic methanogen sludge was acclimatized from sludge obtained from an old lotus root field in Japan as described in our previous papers.[5–8] The thermophilic sludge was obtained from mud in a cow pasture in the Kamphaengsaen campus of Kasetsart University, Thailand, and acclimatized by intermittent addition of small amounts of cellulose, glucose, Polypepton (Daigo), and yeast extract for 2 months, followed by acclimatization by addition of small amounts of slop waste for one month at 55°C.[4]

Fermentation method The semi-continuous methane fermentation was done in a 5-l fermentor, which was controlled at 37°C or 55°C, equipped with 20 l of gas holder. The fermentor had four blades (5 cm in width) in the center of the tank. These impellers were placed at 1/3 of the height from the bottom of the vessels, and rotated at 150 rpm for 5 min after every 12 h of incubation. The fermentor has a diameter of about 25 cm and height of about 20 cm. There was 4.0 l mash in the fermentor, containing 1.5 l of acclimatized sludge and 2.5 l of the slop waste solution. The feeding materials were intermittently added through a hole on the top of the fermentor. After some of the incubated supernatant was removed as an effluent, slop waste was added to the mash as a new substrate. In the case of continuous fermentation using a 5-l fermentor, 1.5 l of the acclimatized sludge was added to the reaction mixture with total volume of 4 l at first. Gas evolution reached the same level during incubation for one month with 400 ml of slop waste added every day, and sandy silt contained in the mud was taken out almost completely.

Analytical methods Amounts of total sugar were measured by the phenol sulfuric acid method.[9] The total nitrogen content was measured by the micro-Kjeldhal method. Ash content was measured by weighing the residue after incineration at 600°C for 30 min. COD_{Mn} was measured by the method described in JIS KO102.[10] The biomass content, total organic carbon (TOC), and the concentration of DNA in the sludge were measured by the method reported by Hashimoto *et al.*[11] Gas composition and volatile fatty acids in the mash were measured as described previously.[5]

Gas production and COD_{Mn} reduction rate The theoretical gas production value was calculated from the value of total organic carbon in the slop waste. The velocity of gas production was represented approximately by the first order equation described in our previous paper,[8]

$$\ln\frac{G-G_t}{G}=-kt \qquad (1)$$

and the rate constant could be calculated from values obtained from an equation. The COD_{Mn} reduction rate was represented by Eq. (2),

$$-\frac{dS}{dt}=K_L\cdot S_a\cdot(S-S^*) \qquad (2)$$

where S is the COD_{Mn} concentration in the mash, S^* is the concentration of undecomposable COD_{Mn} by the microbes, S_a is the concentration of sludge, which was conveniently represented by the concentration of anaerobes calculated from the concentration of DNA-biomass and TOC-biomass in the mash. K_L is the rate constant of COD_{Mn} reduction. Equation (2) is integrated,

$$S=S^*+(S_0-S^*)e(-K_L\cdot S_a\cdot t) \qquad (3)$$

where S_0 is the COD_{Mn} concentration in the mash at the starting point.

Slop waste Slop waste was produced by alcoholic fermentation of molasses in on a laboratory scale. The medium consisted of 25 g of dry yeast (Oriental Yeast Co. Ltd.), 125 ml of molasses, 0.5 g of KH_2PO_4, and 2.0 g of $(NH_4)_2HPO_4$ in a total volume of 500 ml. The pH of the broth was adjusted to 6.8. The molasses was fermented in a one-l bottle. As carbon dioxide was removed from the broth during fermentation, the weight of the bottle decreased by about 50 g in 72 h of incubation at 30°C. The broth was centrifuged for 15 min at 5,000 rpm, and the supernatant, which usually contained 10–13% ethyl alcohol by weight, had the alcohol evaporated under reduced pressure. After evaporation, the solution was boiled once. The slop waste was kept in a refrigerator.

Chemicals All chemicals were of commercial grade.

Results and Discussion

Composition of slop waste Chemical composition of the slop waste is shown in Table 1. Amounts of total solids, volatile solids, and total sugar in the sample were 11.5%, 9.8%, and 3.45%. The pH of the slop waste was 5.1, and it was neutralized to pH 7.0 before use for methane fermentation.

Semi-continuous methane fermentation of slop waste A semi-continuous methane fermentation was examined with serial addition of slop waste from 30 ml to 2,000 ml for 3 to 6 days of incubation at 37°C or 55°C. Gas evolution, COD_{Mn} reduction, TOC decrease, total sugar consumption, pH, and the volume percentages of methane in the gas evolved from the broth with addition

Table 1. Analytical data for slop waste.

Total organic carbon (TOC)	70,000 ppm
Chemical oxygen demand (COD_{Mn})	60,500 ppm
Inorganic carbon (IOC)	115 ppm
Total solid	11.5 %
Volatile solid	9.76%
Total sugar	3.45%
Total nitrogen	0.16%
Ash	2.01%
SO_4^{--}	0.20%
pH	5.1

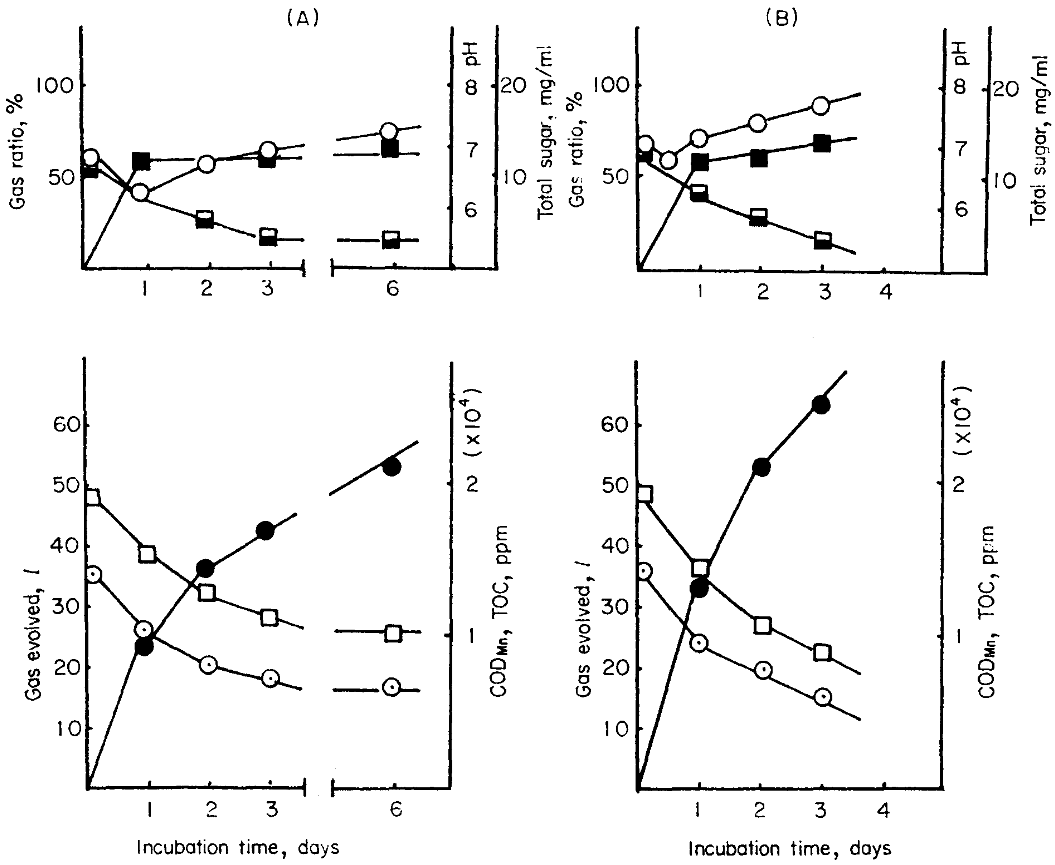

Fig. 1. Mesophilic and thermophilic methane fermentations of slop waste.
A, mesophilic fermentation (37°C); B, thermophilic fermentation (55°C)
—●—, gas evolution; —⊙—, COD_{Mn} concentration; —□—, TOC concentration; —⬓—, total sugar concentration; —■—, CH_4; —○—, pH.

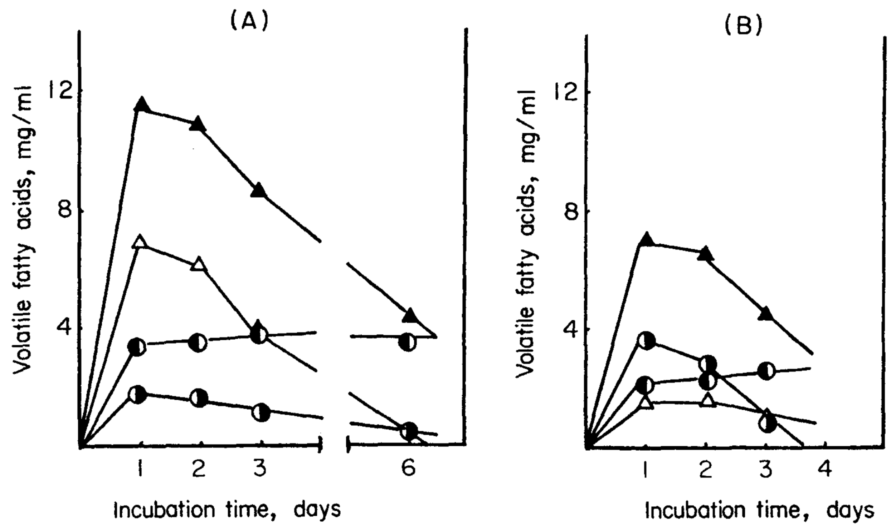

Fig. 2. Accumulation of volatile fatty acids in the broth of mesophilic and thermophilic methane fermentations of slop waste.
A, mesophilic fermentation (37°C); B, thermophilic fermentation.
—▲—, total volatile fatty acids; —△—, acetic acid; —◑—, butyric acid; —◐—, propionic acid.

Table 2. Gas evolution, COD_{Mn}, TOC, and total sugar concentrations in the mash for methane fermentation.

Fermentation	Slop waste added (ml)	Gas evolution[a] (l) Incubation (days)				TOC (ppm)		COD_{Mn} (ppm)		Total sugar (mg/ml)	
		1	2	3	6	Start	End[b]	Start	End[b]	Start	End
Semi-continuous mesophilic	30	1.8	2.0	—	—	500	200	500	170	0.40	0.15
	60	3.6	5.0	—	—	1,100	600	1,000	370	0.80	0.25
	125	5.8	7.8	7.9	—	2,200	1,100	2,100	800	1.40	0.40
	250	11.2	14.6	15.5	—	4,600	2,300	4,200	1,670	3.20	0.70
	500	16.0	23.2	30.0	—	9,300	4,900	8,400	3,600	6.00	1.40
	1,000	24.0	36.0	42.0	54.0	19,000	10,500	14,000	6,550	12.0	2.60
Thermophilic	30	1.6	2.0	—	—	700	350	600	175	0.40	0.15
	60	3.3	5.0	—	—	1,400	700	1,100	370	0.85	0.25
	125	6.0	7.0	8.0	—	2,300	1,100	2,000	745	1.50	0.40
	250	11.7	13.5	15.5	—	4,800	2,500	4,500	1,560	3.20	0.80
	500	23.4	30.5	32.5	—	9,500	5,000	8,400	3,130	6.20	1.60
	1,000	33.4	54.0	64.8	—	19,000	9,900	14,000	6,050	12.3	2.80
	2,000	38.0	62.4	84.0	124.0	38,000	19,800	28,000	12,200	26.0	6.00
Continuous thermophilic	240	12.0				7,500[c]	5,500[d]	8,000[c]	3,300[d]	3.90[c]	1.90[d]
	320	15.6				9,500	6,200	9,500	4,200	4.60	2.20
	480	23.5				11,000	7,400	11,500	5,500	5.80	2.90
	550	27.0				12,000	9,500	12,000	5,920	6.40	3.40
	650	30.0				14,000	10,500	13,200	6,700	7.20	3.60
	750	32.0				15,500	11,500	14,500	8,590	7.90	4.20
	950	33.0				18,000	14,000	17,000	11,500	9.50	5.50

[a] cumulative volume; [b] after 3 days of incubation; [c] influent; [d] effluent.

of 1 l slop waste are shown in Fig. 1 and 2. Gas evolution and concentrations of COD_{Mn}, TOC, and total sugar in the reaction mixture are summarized in Table 2. In the mesophilic fermentation, gas evolution increased to 30 l in proportion to the addition from 30 ml to 500 ml of slop waste. Also, it increased to 64.8 l in proportion to the addition from 30 ml to 1 l of slop waste for the thermophilic fermentation. In the case of the thermophilic fermentation with addition of 2 l of slop waste, gas evolution was observed even at 6 days similarly to the mesophilic fermentation with addition of 1 l of slop waste. However, gas evolution from the broth with 2-l slop waste addition increased to 84 l for 3 days of incubation and 124 l after 6 days of incubation in the thermophilic fermentation. There were great differences between thermophilic and mesophilic fermentations in the accumulation of volatile fatty acids, as shown in Fig. 2. In the mesophilic fermentation, acetic acid (7 mg/ml) was mainly accumulated at the first day of incubation. On the other hand, butyric acid (3.1 mg/ml) was accumulated in the reaction mixture with thermophilic fermentation. In both fermentations, propionic acid was accumulated after 2 days of incubation. The formation, accumulation, and use of volatile fatty acids in the reaction mixtures seem to be governed by the characteristic nature of both sludges.

Though the decreases of COD_{Mn}, TOC, and total sugar concentrations in these fermentations had the same pattern, gas evolution in the thermophilic fermentation were superior to the mesophilic fermentation from over 500 ml of slop waste. There were significant advantages in thermophilic fermentation in the shorter retention times and higher loading above 500 ml of slop waste.

Gas production and COD_{Mn} reduction

rates in semi-continuous mesophilic and thermophilic fermentations The theoretical gas volume evolved, G, from 1 l of slop waste was calculated to be 135 l based on the amount of TOC in the slop waste. Gas production rates were calculated from slopes of lines drawn from the data of gas evolution ratio, $(G-G_t)/G$, for the first day of incubation. G_t is the gas volume evolved for t days of incubation. The gas production rate constant, k, was calculated to be 0.203–0.610 day^{-1} for mesophilic fermentation and 0.189–0.544 day^{-1} for thermophilic fermentation based on the volumes of gas evolved in the first day of incubation. Gas production rate constants are summarized in Table 3.

When the data on the COD_{Mn} reduction rate represented by Eq. (2) were plotted, with $\ln (S-S^*)/(S_o-S^*)$ as the ordinate and $S_a t$ as the abscissa, the rate constants of COD_{Mn} reduction, K_L were obtained from slopes of the lines in Eq. (3). The concentration of the sludge (0.8 g/l) was adopted as S_a for calculation of the COD_{Mn} reduction rate, because TOC-biomass and DNA-biomass concentrations of the sludge of mesophilic fermentation were 0.35 g/l and 20 mg/l, respectively. During the fermentation, the value of S_a was nearly constant. The relationship between the concentration of DNA-biomass and TOC-biomass were represented by the equation, (DNA) = 0.0512 (TOC) + 7.5, by the method by Hashimoto *et al.*[8,11] Concentration of anaerobes (CM) in the sludge were estimated by the following relationship; (CM) = 2.286 (TOC).[8] The COD_{Mn} reduction rate constants of mesophilic fermentation were $1.80–3.00 \times 10^{-4}$ day^{-1} (Table 3). The S_a value of thermophilic fermentation was also 0.8 g/l, because the concentration of TOC-biomass in the sludge was 0.35 g/l, and the

Table 3. Gas production and COD_{Mn} reduction rate constants of mesophilic and thermophilic fermentation of slop waste.

	Slop waste added (ml)	Gas production rate constant[a], k (day^{-1})	COD_{Mn} reduction rate constant[a], K_L ($\times 10^{-4}$ day^{-1})	Gas production (m^3 per kg COD_{Mn})[b]
Semi-continuous mesophilic	30	0.610	3.00	1.52
	60	0.610	2.38	1.98
	125	0.438	2.22	1.52
	250	0.421	1.94	1.53
	500	0.280	1.85	1.56
	1,000	0.203	1.80	1.41
Thermophilic	30	0.523	3.00	1.18
	60	0.544	3.00	1.60
	125	0.460	2.70	1.59
	250	0.444	2.38	1.32
	500	0.442	2.23	1.54
	1,000	0.290	2.20	1.55
	2,000	0.189	2.18	1.32
Continuous thermophilic	240	0.460	2.98[a]	0.624[a]
	320	0.450	2.82	0.734
	480	0.450	2.70	0.920
	550	0.453	2.68	1.11
	650	0.419	2.60	1.15
	750	0.380	1.94	1.23
	950	0.297	1.50	1.50

[a], calculated from one day gas evolution, [b], calculated from 3 days of incubation.

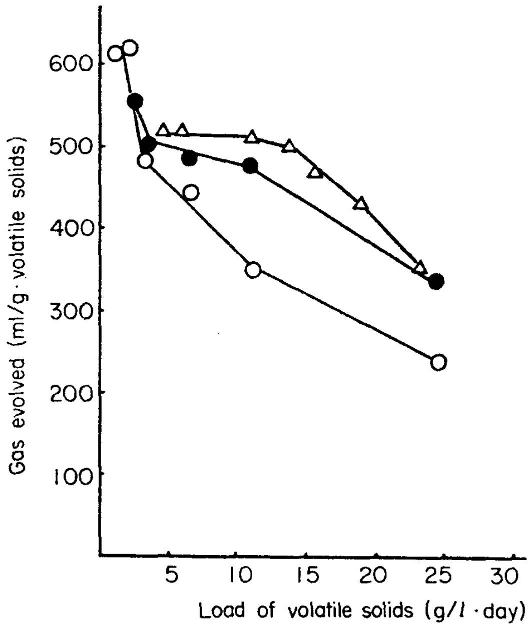

Fig. 3. Effects of volatile solids load on gas evolution in mesophilic and thermophilic methane fermentations.
—○—, semi-continuous mesophilic fermentation; —●—, semi-continuous thermophilic fermentation; —△—, continuous thermophilic fermentation.

concentration of DNA-biomass was 13 mg/*l*. The change of S_a value during the fermentation was 1.05 times at the highest value. The COD_{Mn} reduction rate constants of thermophilic fermentation were $2.18–3.00 \times 10^{-4}$ day^{-1}. Gas production per kg of COD_{Mn} was 1.41–1.98 m^3 in the mesophilic fermentation, and 1.32–1.60 m^3 in the thermophilic fermentation, as shown in Table 3. Gas production and COD_{Mn} reduction rate constants in the thermophilic fermentation were larger than those of mesophilic fermentation at higher loading (500 ml and 1,000 ml of slop waste addition in Table 3). The relationship between gas evolution and loading of volatile solids are shown in Fig. 3. These results were similar to the data reported by Torpey.[12] The maximum loading value in the thermophilic fermentation was 24 g/*l*·day of volatile solids. Apparently, thermophilic methane fermentation was superior to mesophilic fermentation at high loading and short retention time, and also produced a large volume of gas.

Continuous thermophilic methane fermentation Continuous thermophilic methane fermentation was done with 7 steps of supply from 240 ml to 950 ml of slop waste per day. Gas evolution was in the same range as semi-continuous thermophilic fermentations for one day of incubation, as shown in Table 2. Gas production and COD_{Mn} reduction rate constants were 0.297–0.460 day^{-1} and $1.50–2.98 \times 10^{-4}$ day^{-1}, as summarized in Table 3. Gas production per kg of COD_{Mn} was 0.624–1.50 m^3. The results between gas evolution and loading of volatile solids showed that continuous thermophilic methane fermentation was better than semi-continuous thermophilic and mesophilic methane fermentations (Fig. 3).

The results also indicated that the optimum conditions of gas production for mesophilic fermentation are at a lower loading than that of continuous thermophilic fermentation for limited retention time. The quantity of energy required to maintain the thermophilic fermentation is a function of both the size and type of operation. In Thailand, methane fermentation of slop waste was easily done at ambient temperatures, but thermophilic fermentation of slop waste could be supported to get a large volume of gas and more rapidly treat large volumes of slop waste.

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