



ECOLOGICAL ANALYSIS OF THE BACTERIAL SYSTEM IN A FULL-SCALE EGG-SHAPED DIGESTER TREATING SEWAGE SLUDGE

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ABSTRACT

A full-scale investigation was conducted to evaluate the treatment performance and the bacterial system in an egg-shaped digester treating sewage sludge. The experiment was continued over one year to measure the methanogenic activities using various substrates and the population levels of various trophic groups of methanogenic bacteria, as well as the degradation of each component of organic matters. A total of 10 full-scale egg-shaped sludge digesters fed with concentrated sludge were investigated in this study. At a retention time of 30 days and mesophilic condition of 36 °C, the average reduction efficiencies of carbohydrates, proteins and lipids, which are the three main components in raw sludge, were 74.2%, 55.2% and 42.2%, respectively. As a result, the removal efficiencies of volatile solids (VS) and total COD_{cr} reached 54.2% and 60%, respectively, and 1 m³ influent concentrated sludge produced 23.3 m³ digestion gas in which methane content was about 60%. The comparison between the specific methanogenic activity and the actual specific methane production rate in the digester suggests that the retention time of the digester could be reduced from 30 days to 15 days without affecting their performance. In addition, both the hydrogen and acetate-consuming methanogenic bacteria were enumerated on the order of 10⁷ MPN/ml.

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KEYWORDS

Anaerobic digestion, activity, bacterial population, carbohydrate, degradation, egg-shaped digester, lipid, methanogenic bacteria, protein, sewage sludge,

INTRODUCTION

Sewage sludge usually contains 1-2% of total solids with over 80 percent of organic fraction. Because the sludge is not only biologically degradable, but also a good material for methane fermentation, anaerobic digestion technology has been worldwide used as a typical unit process for the stabilization of raw sludge and for energy recovery from sewage. However, the conventional anaerobic sludge digestion has some problems which have limited its applications. Scum formation and low efficiency in VS reduction and energy production are well-known problems (Ghosh, 1990; Li and Noike, 1992). Several new processes have been reported for the upgrading of sludge digestion, including thermal and/or alkali pretreatment,

two-phase process, and digester shape modification. Of them, as a practical approach to improve the mixing performance in the digester, egg-shaped anaerobic digester has been developed to treat the concentrated sewage sludge. In Japan, more than 50 egg-shaped digesters have been built for the sludge treatment since the first installation in 1983. The treatment performance and the bacterial system in those full-scale digesters, however, have not been elucidated.

The objective of this study was to clarify the bacterial ecology as well as the treatment performance of full-scale egg-shaped sludge digesters. The experiment was conducted over a year to measure the metabolic activities and the population levels of various trophic groups of anaerobic bacteria, as well as the degradation characteristics of each component of organic matters. A total of 10 full-scale digesters were investigated in this study.

MATERIALS AND METHODS

Schematic of the sludge treatment plant investigated in this study

Figure 1 illustrates the flow chart of the sludge treatment plant investigated during the study. This plant is located in the city of Yokohama and involves a process sequence of thickening, digestion, dewatering, and incineration. The sludge to be treated was received from the five wastewater treatment plants in the northern part of Yokohama City. This is a typical centralized sludge treatment system in Japan. A total of 10 egg-shaped digestion tanks with individual working volume of 6800 m³ were run under mesophilic condition (36 °C) to stabilize sludge and produce biogas. The raw sludge, which has a concentration of around 2 percents (20g/L), is concentrated to about 5 percents (50g/L) by means of centrifugal thickener, and then the concentrated sludge is fed to the digester by pump. The biogas produced from the digester is beneficially used as fuel for power generation and as auxiliary fuel for incinerators. In addition, waste heat from power generation is used to warm the influent sludge through a heat exchanger and as a heat source for air conditioning equipment in the plant.

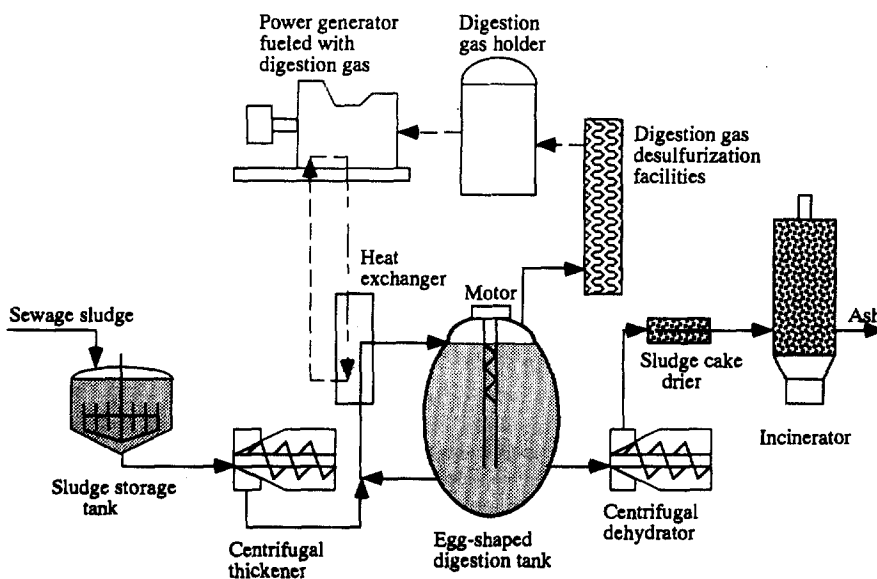


Figure 1 Flow chart of the sludge treatment plant investigated in this study

Measurement of specific methanogenic activity

The anaerobic digestion of sewage sludge is a complex biological process composed of four steps: (1) hydrolysis of particulates to soluble single molecules, (2) acidogenesis producing volatile fatty acids, (3) acetogenesis producing acetate and hydrogen/formate from propionate, butyrate, (4) methanogenesis producing methane from acetate and hydrogen/formate. In order to understand the specific methanogenic activities (SMA) of the digester content for degrading the typical organic matters in each step, a series of batch experiments were performed at 36 °C using serum vial of 120 ml. For each SMA measurement, 40 mL of digester sludge and 40 ml substrate media was added to the vial under an atmosphere of 80% N₂ and 20% CO₂, an anaerobic condition. The individual substrate used for the SMA measurement included, respectively, formate and acetate as typical substrates for methanogenesis step, propionate and butyrate as typical substrates for acetogenesis step, glucose (carbohydrate), peptone (protein) and linseed oil (lipid) as typical substrates for acidogenesis and the raw sludge as the substrate for hydrolysis step. Initial concentrations of these substrates in the bial were 2000 mg-COD/L and all activities were determined by measuring methane production using the glass syringe technique of Owen *et al* (1979).

Enumeration of methanogenic bacteria

The most-probable-number (MPN) method was used for the enumeration of methanogenic bacteria (MPB). The MPN tests were run in screw-capped glass tubes with butyl-rubber stoppers containing the media for the growth of MPB. The modified anaerobic techniques (Balch *et al*, 1979; Mackie and Bryant, 1981; Chartrain and Zeikus, 1986) for the preparation of media were applied in this study. Each liter of the basal medium for MPB contained: KH₂PO₄, 0.4g; K₂HPO₄, 0.4g; NH₄Cl, 1.0g; MgCl₂·6H₂O, 0.21g; NaHCO₃, 6.0g; yeast extract, 0.2g; Na₂S·9H₂O, 0.25g; sodium cysteine.HCl.H₂O, 0.5g; mineral solution (Li and Noike, 1989) and vitamin solution (Balch *et al.*, 1979), 10 ml each. The organic substrate added to the basal medium were, respectively, 3.0 g sodium acetate for acetate-consuming MPB and H₂/CO₂ (80/20) gas for hydrogen-consuming MPB. The medium was dispensed into tubes under anaerobic condition and the head space contained a gas mixture of N₂/CO₂ (80/20), except for the MPN test for the hydrogen-consuming MPB; in that case, the gas phase was replaced with H₂/CO₂ (80/20) gas at 2 atm of pressure after the inoculation. All tubes were incubated at 36 °C for one month and then the growth of MPB was interpreted on the basis of presence of methane in the gas phase of the tube.

Analytical methods

Standard methods (APHA, 1992) were used for the measurement of COD_{cr} and total solids (TS), volatile solids (VS), suspended solids (SS), volatile suspended solids (VSS), alkalinity and pH. Carbohydrate concentration was measured by the anthrone-sulfuric acid method using glucose as the standard (SGRMM, 1979). Protein was determined by Lowry's method (Lowry *et al.*, 1951) using albumin as the standard. Lipids concentration was measured by the Bligh-Dyer method (SGRMM, 1979). The percentage of methane, carbon dioxide and air in digestion gas were analyzed by a gas chromatograph (Shimadzu) equipped with a thermal conductivity detector and a 1.5-m stainless steel column filled with activated carbon. Helium was used as the carrier gas with a pressure of 1.5 kg/cm². The temperature of the injection port and detector were 140 °C and 120 °C, respectively. Concentrations of individual volatile fatty acids (VFA) were determined by a second gas chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector and a 1.5m (length) x 5mm (ID) glass column filled with Greensorb. The temperature of the injection port and detector were 190 °C and 170 °C, respectively. Helium was used as the carrier gas with a pressure of 1.5 kg/cm². In addition, hydrogen gas and air were used at the pressure of 0.6kg/cm².

RESULTS AND DISCUSSION

Treatment performances

Fig.2 illustrates the long period variation of several typical operation parameters, including (a) influent TS concentration, (b) removal efficiencies of TS and VS, (c) alkalinity, (d) pH, (e) biogas production ratio of one of the 10 digesters operated at the retention time of 30 days. The influent TS concentration fluctuated between 40 to 60 g/L with an average of 50 g/L (Fig.2a). The pH in digester ranged from 7.1 to 7.7 with

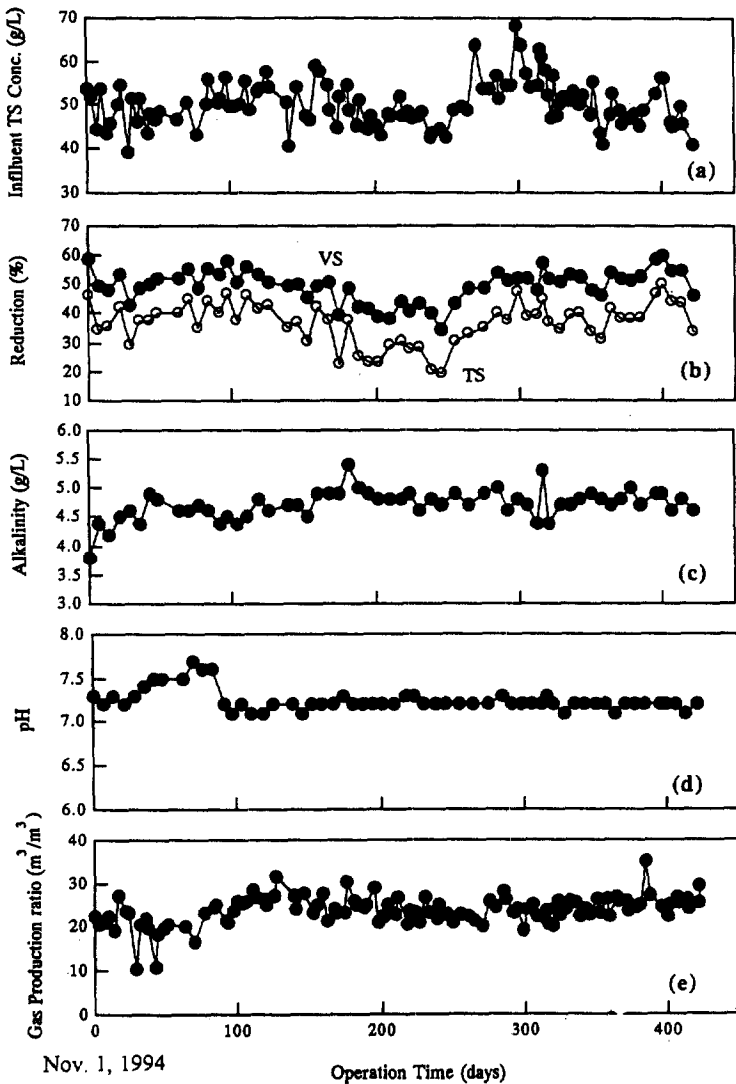


Figure 2 Long period variation of several typical operational parameters for the digester at a retention time of 30 days. (a) influent VS, (b) removal of TS and VS, (c) alkalinity, (d) pH of digester content, (e) biogas production ratio

an average of 7.3 (Fig.2b) while the alkalinity varied from 3700 to 4700 mg/L. The ratio of biogas production to influent sludge volume was within the range of 18 to 27 (m³/m³). The results of Fig.2 indicate that a stable operation for a long time was obtained. In addition, although the digesters have been continuously operated for over 13 years, there were no noticeable scum and foaming problems in all the egg-shaped digesters investigated.

Table 1 summarizes the average of all analytical data obtained from the ten full-scale digesters during one year of investigation. The environmental factors of pH, alkalinity and NH₄⁺-N concentration in the digesters varied, respectively, in the ranges of 7.1-7.4, 3380-4800mg/L and 890-1140mg/L which are favorable conditions for methane fermentation. As a result, the degradation of organic matter proceeded well and there was no accumulation of VFA in the digesters. As shown in Fig.3, the removal efficiencies of TS, VS, SS, VSS and total COD were 44.2%, 54.2%, 42.7%, 55.5% and 60.0%, respectively. 1 m³ of influent concentrated sludge produced 23.3 m³ of digestion gas containing 60% methane and 35% carbon dioxide. In addition, the H₂S concentration in the digestion gas ranged from 194 to 1494 ppm with an average of 665 ppm.

Table 1 Average of the experimental data for the mesophilic egg-shaped digester operated for one year at a retention time of 30 days

	Influent concentrated sludge		Digested sludge	
	Average	Range	Average	Range
Environmental factors				
pH	5.24	5.10 - 5.80	7.25	7.11 - 7.39
NH ₄ ⁺ -N (mg/L)	336	240 - 448	1060	890 - 1140
Alkalinity (mg/L)	500	312 - 693	3590	3380 - 4800
Sludge concentration				
TS (g/L)	47.6	43.2 - 55.6	28.0	23.6 - 32.8
VS (g/L)	37.1	30.5 - 46.7	17.3	14.8 - 20.1
SS (g/L)	45.0	38.0 - 54.7	26.0	20.4 - 32.0
VSS (g/L)	35.2	29.5 - 46.2	15.9	13.0 - 19.1
Total COD _{Cr} (g/L)	62.8	55.4 - 85.7	24.4	19.0 - 32.9
Soluble COD _{Cr} (g/L)	8.05	5.20 - 10.5	4.95	0.95 - 2.29
Chemical composition				
Total carbohydrates (g/L)	18.6	12.3 - 24.8	5.00	3.70 - 5.88
Total proteins (g/L)	12.6	9.70 - 18.0	6.96	4.93 - 8.58
Total lipids (g/L)	5.81	4.68 - 6.90	2.56	2.20 - 3.23
Soluble carbohydrates (g/L)	0.228	0.100 - 0.532	0.173	0.074 - 0.415
Soluble proteins (g/L)	0.387	0.157 - 0.768	0.320	0.150 - 0.692
Volatile fatty acids (mg/L)				
Acetate	1800	893 - 2500	46	ND - 46
Propionate	2300	868 - 4630	ND	ND
Butyrate	560	316 - 1306	ND	ND

ND: not detectable.

Methanogenic activity

Table 2 summarizes the variation and average of SMA degrading various substrate measured in February, June, August, October and December, 1995. The activity measurement has been used as a tool to characterize the bacterial trophic composition in anaerobic system (Dolfing and Bloemen, 1985) and compare the capacities of digester content for degrading different substrates. Based on the data shown in Table 1 and Fig.3, the average of specific methane production rate (SMPR) in the full-scale digester was

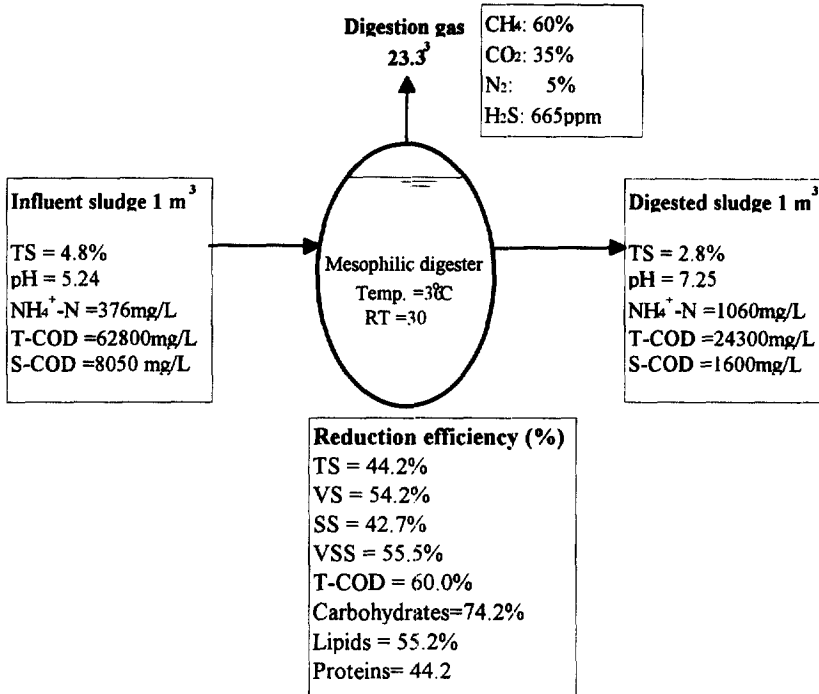


Figure 3 Reduction efficiencies of various components and digestion gas in the full-scale egg-shaped

calculated to be 29.3 ml-CH₄/g-VSS.day. Except for formate-degrading SMA, all SMAs summarized in Table 2 were higher than the SMPR, indicating that the methane production rate in the full-scale digester was limited by the feeding rate and the bacterial system in the digester has a more ability for methane production. This suggests that the retention time of the digester could be reduced. In addition, the maximum SMA was high as 60.5 ml-CH₄/g-VSS.day which is two times higher than the SMPR of the full-scale digester, indicating that the bacterial system in the full-scale digester had a high potential of methane production and the influent loading rate could be doubled. It means that the retention time of the full-scale digester could be reduced from 30 days to 15 days without affecting their performance.

Table 2 The variation of SMA degrading various substrate measured at different time

Substrate	MPA of digester sludge (ml-CH ₄ /g-VSS.day)					
	February	June	August	October	December	Average
Formate	17.0	21.5	21.1	22.9	30.0	21.6
Acetate	29.0	29.9	30.8	37.2	32.4	32.4
Propionate	34.8	30.8	31.1	25.2	33.6	31.2
Butyrate	61.8	70.8	66.0	67.2	58.8	60.5
Glucose	41.5	45.5	54.2	45.6	40.8	46.4
Peptone	45.8	47.4	38.4	49.2	52.8	46.8
Lipid	41.6	40.8	36.7	43.2	34.8	39.5

Bacterial population

Table 3 summarizes the population of each trophic group of methanogenic bacteria measured in February, April, June, October and December, 1995. Both the hydrogen-consuming methanogens and acetate-consuming methanogens were enumerated on the order of 10^7 MPN/ml or 10^9 MPN/g-VSS which are comparable to those of bench-scale experiments using sewage sludge (Li and Noike, 1990) and using cattle waste (Mackie and Bryant, 1981).

Table 3 Populations of hydrogen- and acetate- consuming methanogens

	Hydrogen-consuming methanogens		Acetate-consuming methanogens	
	MPN/ml	MPN/g-VSS	MPN/ml	MPN/g-VSS
This study				
February	3.7×10^7	2.6×10^9	5.3×10^7	1.7×10^9
April	7.5×10^7	5.2×10^9	8.6×10^7	6.0×10^9
June	5.5×10^7	3.7×10^9	5.4×10^7	3.3×10^9
August	4.5×10^7	2.8×10^9	4.6×10^7	2.8×10^9
October	6.4×10^7	3.7×10^9	4.7×10^7	2.5×10^9
December	4.5×10^7	2.8×10^9	6.6×10^7	4.1×10^9
Average	5.4×10^7	3.5×10^9	5.9×10^7	3.4×10^9
Reference data				
Sewage sludge ^{a)}	7.9×10^7	-	2.7×10^7	-
Sludge ^{b)}	$0.31-2.8 \times 10^7$	-	$0.82-1.1 \times 10^7$	-
Cattle waste ^{c)}	-	-	4.6×10^7	-

a) Li and Noike (1990), b) Li and Noike (1992), c) Mackie and Bryant (1981)

CONCLUSIONS

1. The removal efficiencies of TS, VS, SS, VSS and total COD in the mesophilic egg-shaped sludge digester at a retention time of 30 days were 44.2%, 54.2%, 42.7%, 55.5% and 60.0%, respectively. 1 m³ of influent concentrated sludge produced 23.3 m³ of digestion gas containing 60% CH₄, 35% CO₂ and 665ppm H₂S.
2. The average reduction efficiencies of carbohydrates, proteins and lipids, which are the main components in raw sludge, were 74.2%, 55.2% and 42.2%, respectively.
3. Both the hydrogen-consuming methanogens and acetate-consuming methanogens were enumerated on the order of 10^7 MPN/ml or 10^9 MPN/g-VSS.
4. The methanogenic activities of the digester sludge for degrading the individual substrates of formate, acetate, propionate, butyrate, glucose, peptone and lipid were 21.6, 32.4, 31.2, 60.5, 46.4, 46.8 and 39 ml-CH₄/g-vss.day. The methanogenic activity degrading raw sludge was 771 ml-CH₄/L.day, which was higher than the actual methane production rate of 466 ml-CH₄/L.day in the digester.

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REFERENCE

- APHA (1992). *Standard Methods for the Examination of Water and Wastewater*. 18th Ed. Washington, DC.
- Balch, W. E., Fox, G. E., Magrum, L. J. and Wolfe, R. S. (1979). Methanogens: Reevaluation of unique biological group. *Microbiol. Rev.*, **43**, 260-296.
- Chartrain, M. and Zeikus, J. G. (1986). Microbial ecophysiology of whey biomethanation: characterization of bacterial trophic populations and prevalent species in continuous culture. *Appl. Environ. Microbiol.*, **51**, 188-196.
- Dolfing, J. and Bloemen, W. G. B. M. (1985). Activity measurements as a tool to characterize the microbial composition of methanogenic environment. *J. Microbiol. Methods*, **4**, 1-12.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination sugars and related substances. *Anal. Chem.*, **28**, 350-356.
- Ghosh, S. (1990). Pilot-scale demonstration of two-phase anaerobic digestion of activated sludge. *Wat. Sci. Tech.*, **23** (7-9), 1179-1188.
- Li, Y. Y. and Noike, T. (1992). Upgrading of anaerobic digestion of waste activated sludge by thermal pretreatment. *Wat. Sci. Tech.*, **26** (3-4), 857-866.
- Li, Y. Y. and Noike, T. (1989). Characteristics of bacterial population and organic matter degradation in anaerobic sludge digestion: on methanogenic bacteria and homoacetogenic bacteria. *Japan J. Wat. Pollut. Res.*, **12**, 771-780.
- Li, Y. Y. and Noike, T. (1990). Behavior of acidogenic and methanogenic bacteria in the anaerobic digestion of primary sludge. *Proceedings of the 24th Annual Conference of Japan Society on Water Environment*, 639-640.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265-275.
- Mackie, R. I. and Bryant, M. P. (1981). Metabolic activity of fatty acid oxidizing and the contribution of acetate, propionate, butyrate and CO₂ to methanogenesis in cattle waste at 40 and 60 °C. *Appl. Environ. Microbiol.*, **41**, 1363-1373.
- Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y. and McCarty, P. L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Wat. Res.*, **13**, 485-492.
- SGRMM (Specialist Group on Research Method in Microbiology) (1979). *Research Method in Microbiology*. Dankousha, Tokyo.