Enumeration and Activity of Methanogenic Microorganisms on the Anaerobic Digestion Process

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ABSTRACT/ The anaerobic digester with sludge from sewage treatment plant was operated in the laboratory for two years to investigate the enumeration and activity of methanogenic microorganisms.

In this experimental study, the effects of HRT on the degradation characteristics of organic materials and on the number of methanogenic bacteria produced were investigated. By making the media with the repeated experiment, the number and activity of methanogenic bacteria were measured. The increase of the removal rate of organic materials with respect to HRT was found. And the maximum production rate of organic acid in the digester was observed at HRT of 3 days. The total number of methane forming bacteria estimated by the MPN method showed 2.3×10^7 at HRT of 3 days, 7×10^7 of 5 days and 7.9×10^7 MPN/mℓ of 10 days. The optimum incubation time for measuring the number of methanogenic bacteria was found as more than four weeks. The PMA revealed 161mℓ CH_4/ℓ·day at HRT of 10 days and the PUA 290mg·COD/ℓ day. At the incubation time of 4.3 days, the maximum value of CH_4 (59.1%) was found. At this time, N_2 was found as 15.3% and CO_2 25.6%.

1. Introduction

A microorganism in the anaerobic digestion process can be considered in four groups, i.e. hydrolytic, hydrogen-producing acetogenic, homoacetogenic and methanogenic bacterias. These coacting microorganisms degraded the complex organic materials to CH_4 and CO_2 through the multi-step reactions including hydrolytic, acetogenic and methanogenic reactions. The anaerobic digestion process is commonly classified as acetogenic phase and methanogenic phase.[1][2][3]

These microorganisms coexist in the digester and the reaction rate of it affects the degree of digestion. Of these microorganisms, the methanogenic bacteria exists only in the entirely anaerobic conditions. The number of methanogenic bacteria plays an significant role in the anaerobic digestion process.[4][5] In the kinetic study of anaerobic process, the number of

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methanogenic bacteria has been estimated from the MLSS concentration in the digester due to the inherent characteristics of it.[6][7]

The purpose of the study is, therefore, to separate the methanogenic bacteria from the digester and estimate the number of it and to measure the activity of it.

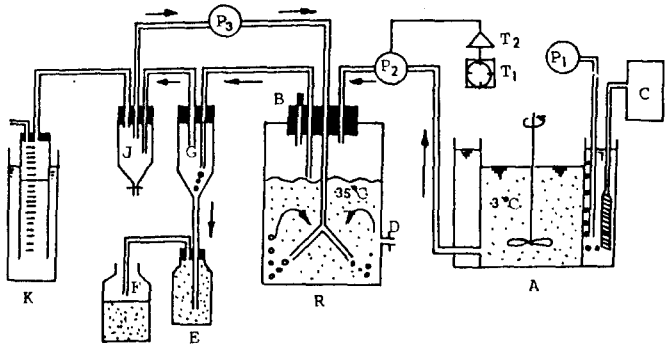
The anaerobic digester was operated in the three different HRTs through two years. The media with the components required to microorganisms was made, seeded and incubated in the anaerobic condition. The number of methanogenic bacteria was estimated by the MPN method through whether methane gas was produced or not.

2. Experiment and Procedure

2-1. Digester and substrate

The continuous type of anaerobic digester with the volume of 2ℓ was used as shown in Fig. 1.

The digester was operated with HRT of 5 days in the first year(1989), and with HRTs of 3 and 10 days in the second year(1990).



C, P_1 :Cool dip system
A :Substrate tank
T_1, T_2 :Time control system
P_2 :Feed pump
R :Reactor
D :Mixed liquor sampling port
B :Gas sampling port
G, E, F :Mixed liquor overflow system
J, K :Gas collection system
P_3 :Gas recirculation pump

Fig. 1 Experimental apparatus of continuous type

The settled sludge of secondary sedimentation tank in the sewage treatment plant was used as a substrate. The composition of substrate used here is shown in Table 1.

Table 1. Composition of substrate

	1st year(1989)	2nd year(1990)
pH	5.24	5.25
SS	18,987	19,023
VSS	15,238	15,070
T – C COD	29,255	29,550
S – COD	6,361	6,920
T – Protein	6,467	6,280
S – Protein	1,188	–
T – Carbohydrate	6,482	6,537
S – Carbohydrate	207	137
T – Lipid	3,053	3,188
S – Lipid	530	–
Acetic acid	892	986
Propionic acid	634	665
iso – Butyric acid	32	36
n – Butyric acid	363	365
iso – Valeric acid	72	79
n – Valeric acid	116	113

2-2. Estimation of number of methanogenic bacteria

The number of the methanogenic bacteria was estimated by the MPN method. The bacteria was incubated in the selected media with the anaerobic condition.

2-2-1. Handling

The gas injection method due to Hungate[8][9][16] was introduced for maintaining the anaerobic condition. The deoxygenated CO_2 by passing the copper column at 350 ℃ was used as injection gas at the time of making the media, diluting the sample and seeding.

2-2-2. Dilution water[10][11][12]

The composition of dilution water for diluting the sample used in the anaerobic condition as required diluting ratio is shown in Table 2.

Table 2. Composition of dilution water

Solution components	(g/ℓ)
K_2HPO_4	0.4
KH_2PO_4	0.4
NH_4Cl	1.0
$MgCl.6H_2O$	0.1
CCysteine. HCl	0.1
Yeast extract	0.01

Table 3. Composition of the basic media used for enumeration of methanogenic bacteria

Components	Total methanogenic bacteria	H_2 degraders bacteria	Acetate degraders bacteria
Carbon sources	2.0g	–	Sodium acetate 3.0
H_2(80%) +++ CO_2 (20%))	2atm	2atm	
KH_2PO_4	0.4g		
K_2HPO_4	0.4g		
NH_4Cl	1.0g	same as left	
$MgCl_2$	0.1g		
Mineral solution	10ml		
Vitamin solution	10ml		
Yeast extract	2.0g	500mg	
Digester supernant liquor	150ml	50ml	
$NaHCO_3$	6.0g		
Cysteine hydrochloride	0.5g		
Cysteine hydrochloride	0.5g		
Na_2S $9H_2O$	0.25g	same as left	
Resazurine	0.002g		
pH	7.0–7.2		

2-2-3. Media composition

The composition of media is an important factor for measuring the number of methanogenic bacteria. The optimum component of media was selected from the literature survey[13][14][15][16][17][18] and by seeding and incubating the methanogenic bacteria. The media is fundamentally composed of carbon source as an energy source, mineral, vitamin and additives. The basic component of media is shown in Table 3.

2-2-4. Seeding and incubation

The digested liquid was taken from the digester operated with steady state. The liquid of 10 mℓ was injected into the bial bottle (90ml) and mixed well. Those sample were diluted to 10^{-1}-10^{-10} dilution ratio step by step.

The seeded sample was injected to test tube containing the media of 9 mℓ. The five test tubes were prepared for five steps. Those tubes with the seeded sample were incubated at 35 ℃ through over four weeks.

2-2-5. MPN estimation[19][20]

Those incubated samples were checked whether the methane gas was produced or not. The number of methanogenic bacteria was estimated by using the MPN table.

2-3. Measurement of activity[21][22]

2.3.1 Basic concept.

The rate of substrate removal and methane forming velocity can be obtained from Eq. (1) and Eq. (2)

$$-\frac{dS}{dt} = \frac{K_{max} \cdot S}{K_s + S} \cdot X \qquad (1)$$

$$\frac{dM}{dt} = Y \cdot \left(-\frac{ds}{dt}\right) = Y \cdot \frac{K_{max} \cdot S}{K_s + S} \cdot X \qquad (2)$$

where S : substrate concentration
K_{max}: maximum specific substrate utilization rate
K_s : half velocity coefficient
X : concentration of methanogenic bacteria
M : methane gas produced
Y : yield coefficient of methane

If the substrate concentration is high enough, Eqs. (1) and (2) can be expressed as

$$-\frac{ds}{dt} = K_{max} \cdot X \tag{3}$$

$$\frac{dM}{dt} = Y \cdot K_{max} \cdot X \tag{4}$$

In this case, it is found that the maximum value of the substrate removal rate and of the methane forming velocity depend on the concentration of microorganism and the methabolic capacity.

Accordingly, the maximum rate of substrate removal and the maximum methane forming velocity can be defined by the activity of microorganism such as PUA (Potential Substrate Utilizing Activity) and PMA (Potential Methanogenic Activity).

2-3-2. Measurement method

The sample obtained from the digester was moved to bial bottle (120mℓ) and added the required substrate and incubated with vibration at 35℃, pH 7.20. The quantity of gas formed, gas composition and VFA were measured.

3. Experimental Results and Discussion

3-1. Removal rate of organic materials.

The variation of organic components according to the HRT is found from Table 4. In addition, the removal rate of organic components with respect to the HRT is shown in Fig. 2

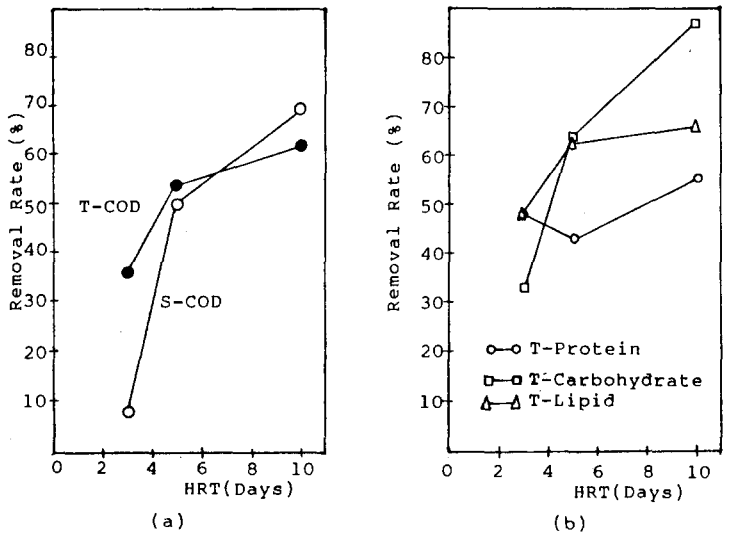


Fig. 2 Effect of HRT on removal rate of organic materials

Table 4. Variation of organic components according to the HRT.

	Inflow	HRT 3 days	HRT 5 days	HRT 10 days
TCOD	29,255(29,550)	18,670	13,150	11,080
SCOD	6,361(6,920)	5,880	3,445	1,949
T-Protein	6,467(6,280)	3,389	3,561	2,909
S-Protein	1,188	1,226	-	792
T-Carbohydrate	6,482(6,537)	4,330	2,343	826
S-Carbohydrate	207	137	-	103
T-Lipid	3,053(3,188)	1,587	1,190	1,046
S-Lipid	530	-	-	123

From Fig. 2(a), the removal rates for the TCOD show 36% for HRT of 3 days, 54% for 5 days, and 62% for 10 days. This reveals that the removal rate is gradually increased with the increase of HRT.

In case of SCOD, the removal rates reveal 8% for HRT of 3 days, 50% for 5 days and 69% for 10 days. In this case, it is evident that the removal rate for SCOD is considerably increased comparing with that for TCOD.

The effect of T-Protein, T-Carbohydrate and T-Lipid on the removal rate as a function of HRT is shown in Fig. 2(b).

It can be seen from Fig. 2(b) that the removal rates for T-Carbohydrate and for T-Lipid are increased with increasing the HRT. It is particularly noticed that the removal rate for T-Carbohydrate is remarkably increased with respect to HRT.

3-2. Acid and gas production

The organic materials such as protein, carbohydrate and lipid within the sample continuously induced into the digester are degraded to organic acids. The concentration of organic acids produced and the gas production rate with respect to HRT are shown in Table 5.

The trend for the production rate of organic acid shows acetic acid > propionic > valeric acid > butyric acid.

From the Table 5., it is clear that the short HRT gives the more gas production rate. The composition rate of CH_4 maintains the normal condition with above 61 % regardless of the variation of HRT.

3-3. Distribution of methane forming bacteria

Table 5. Concentration of organic acids and gas production rate

	Inflow	HRT 3 days	HRT 5 days	HRT 10 days
Acetic acid	982 (986)	447	85	28
Propionic acid	634 (665)	709	41	7
Butyric acid	395 (401)	40	15	0
Valeric acid	188 (192)	273	17	8
Gas production rate (mg/day ℓ)		1,950	1,860	995
Gas composition (%)				
N_2		2.7	2.65	2.7
CH_4		61.0	63.25	63.0
CO_2		36.3	34.10	34.3
H_2		–	–	–

Table 6. Effect of incubation time on number of methane forming bacteria.

Species	Total	H_2 degraders	Acetate degraders
Dilution rate			
Incubation time (days)	10^6 10^7 10^7 10^9 10^{10}	10^6 10^7 10^8 10^9	10^5 10^6 10^7 10^8 10^9
7	4 5 1 0 0	4 2 1 0	5 3 0 0 0
16	5 5 2 1 0	5 5 1 0	5 5 3 0 0
28	5 5 2 1 0	5 5 2 0	5 5 4 0 0
37	5 5 2 1 0	5 5 2 0	5 5 4 0 0
82	5 5 2 1 0	5 5 2 0	5 5 4 0 0
Number of bacteria (MPN/mℓ)	7.0×10^7	5.2×10^7	1.5×10^7

3-3-1. Effect of incubation time on number of methane forming bacteria

The medias used according to the required substrate here were Total, H_2 degraders and Acetate degraders. To obtain the optimum incubation time, the incubation experiment was performed. The experimental results are shown in Table 6.

It is found from Table 6 that the normal condition for the Total methanogenic bacteria reveals from the incubation time of 16 days.

The normal condition for the H_2 degraders, however, shows from the incubaton time of 28 days. It is evident from those results that the optimum incubation time for measuring the number of methanogenic bacteria is more than 4 weeks. This result shows good agreement with those of Mackie and Bryand[12] and of Qian.[23]

3-3-2. Number of methanogenic bacteria produced.

The number of methanogenic bacteria produced from the digester with normal condition for HRTs of 3, 5 and 10 days is shown in Table 7. As mentioned earlier, the number with respect to substrate was estimated by the MPN method.

It is found from Table 7 that the bacteria number for Total shows 2.3×10^7 at HRT of 3 days, 7.0×10^7 of 5 days, and 7.9×10^7 MPN/$m\ell$ of 10 days. This reveals that it reaches the stable condition after HRT of 5 days. For both H_2 degrading and Acetate degrading bacteria, the highest number of bacteria was found at HRT of 5 days.

3-4. Methane gas forming activity.

To measure the activity of methane gas forming, the sample was obtained from the digester with HRT of 10 days. The activity of methane gas forming was measured from 10:45 a. m., July 13, 1990 to 7. 30 p. m., July 18, 1990 for two times per day. The methane gas produced was obtained from the following equation taking into account the sample injected to the bial bottle and volume of incubation water

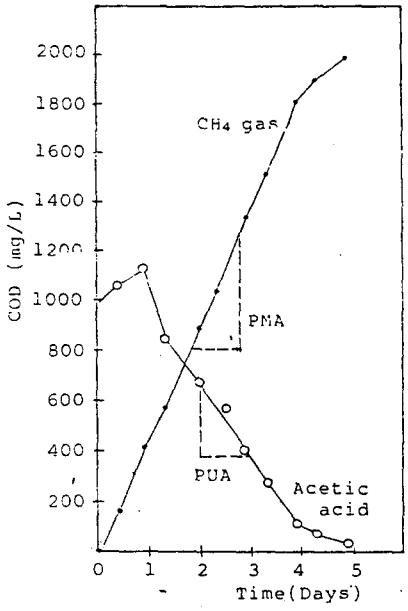
Table 7. The number of methanogenic bacteria produced with respect to HRT.

HRT (days)	3	5	10
Methanogens (MPN/mℓ)			
Total	2.3×10^7	7.0×10^7	7.9×10^7
H_2 degraders	1.1×10^7	5.2×10^7	1.7×10^7
Acetate degraders	1.1×10^7	1.5×10^7	1.3×10^7

$$G_M = 45\ m\ell \times (M\%_2 - M\%_1) + G_{T1} \times M\%_2$$

where GM : methane gas produced.
45$m\ell$: initial volume of bial bottle.
M%1 : CH_4% just before measuring time.
M%2 : CH_4% at measuring time.
G_{T1} : CH_4($m\ell$) at measuring time.

The effect of incubation time on the production of CH_4 gas and acetic acid is shown in Fig. 3. The quantity of methane gas and of acetic acid was converted to COD. It is found from Fig. 3 that PMA shows 460 mg·COD/ℓ day (=161mℓ CH4/ℓ day) and PUA 290 mg ·COD/ℓ day.



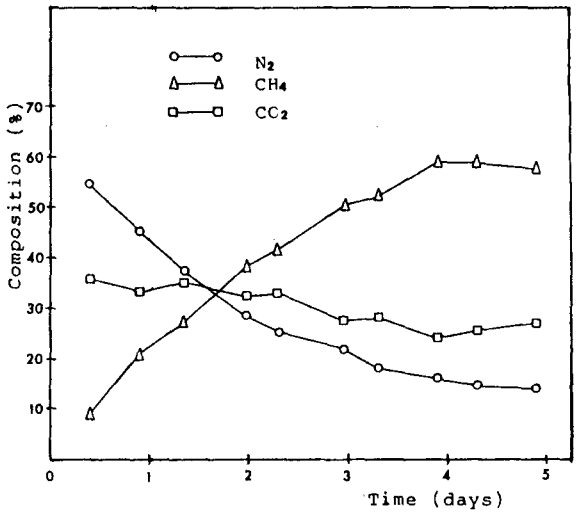


Fig. 4 Effect of incubation time on composition of gas produced

Fig. 3. Effect of incubation time on the production of CH_4 gas(PMA) and acetic acid(PUA)

3-5. Effect of incubation time on composition of gas produced

The influence of incubation time on the composition of gas produced during the incubation period is shown in Fig. 4. It is evident from the figure that N_2 gas is remarkably decreased with the increase of time, while CH_4 is rapidly increased with respect to time. CO_2 gas is, however, nearly constant regardless of incubation time.

The maximum value of CH_4 (59.1%) can be observed at the incubation time of 4.3day

4. Conclusions.

To separate the methanogenic bacteria from the digester and estimate the number of it, and to measure the activity of it, the anaerobic digester was operated in the different HRTs through two years. The MPN method was introduced to estimate the number of methanogenic bacterias. Important conclusions to emerge from this experimental study on the enumeration and activity of microorganisms in the anaerobic digestion process are:

1. The removal rate of organic materials such as COD, protein, carbohydrate, and lipid was increased with increasing the HRT.
2. The maximum production rate of organic acid in the digester reveals at HRT of 3 days. After HRT of 5 days, the production rate of organic acid was rapidly decreased.
3. The effect of HRT on the gas procuced was clealy found, while the composition ratio of CH_4 was not effected by the HRT.
4. The total number of methane forming bacteria estimated by the MPN method showed

2.3 x 10^7 at HRT of 3 days, 7 x 10^7 of 5 days and 7.9x10^7 MPN/$m\ell$ of 10 days. In addition, the number of H_2 degrading methane bacteria and of acetic degrading methane bacteria were also obtained.

5. The optimum incubation time for measuring the number of methanogenic bacteria was found as more than 4 weeks.
6. The PMA revealed 460 mg COD/ℓ·day(=161 $m\ell \cdot CH_4/\ell \cdot$ day) and the PUA 290 mg COD/$\ell \cdot$ day.
7. The maximum value of CH_4(=59.1%) was found at the incubation time of 4.3 day. In this case, N_2 revealed 15.3% and CO_2 25.6%.

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