

Kinetics of CH₄ oxidation in mixed culture

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ABSTRACT

Cultures used for CH₄ oxidation experiments were prepared by enrichment of extract from leachates from two landfill sites and a digested sewage sludge under 20 % of CH₄, but not isolated. Therefore, the cultures were the mixture of CH₄ oxidizing and other aerobic microorganisms. The CH₄ oxidation experiments were conducted with a batch reactor, in which the change in gas volume can be measured. The mixture of air and CH₄ was introduced; initial CH₄ concentrations were ranged from 5 % to 20 %. CH₄ oxidation rate was expressed as a Monod equation for CH₄ and first order reaction for O₂. A determined maximum oxidation rate (V_{max}) was 2.1×10^{-13} to 3.4×10^{-11} hr⁻¹ cell⁻¹, and a half saturation constant (K_M) was 5.1×10^{-5} to 6.5×10^{-4} mol L⁻¹. The mole ratio of consumed O₂ to CH₄ was 1.6-1.8

Keywords - Methane oxidation; kinetics; mixed culture; batch reactor; leachate; digested sewage sludge

1. INTRODUCTION

Methane is known as a 'greenhouse' gas and produced in large quantities from a wide range of sources such as swamps, marshes, and paddy fields. Municipal solid waste landfill sites are one of the methane production sources. The main paths of methane emission from a landfill site are cover soils and gas venting pipes. A lot of research for the potential of cover soils to oxidize methane has been reported (Bogner et al. (1997), Borjesson et al. (1998), Visscher et al. (1999)). Another place where methane can be oxidized is gas venting pipes. To design a device for methane oxidation in gas venting pipes by methane oxidizing bacteria, it is necessary to clarify the methane oxidation rate at various methane and oxygen concentrations.

Although many researches on kinetics of methane oxidation were reported (Bender et al. (1992), Czepiel et al. (1996), and Boeckx et al. (1996)), the effect of oxygen concentration was scarcely discussed (Visscher et al. (1999)). In addition, many experiments for methane oxidation were conducted in batch reactor and there was little consideration of the change in the volume of gas phase, although the methane oxidation reaction accompanies the decrease in the volume of gas phase at constant pressure.

The purpose of this study is to determine the kinetics of methane oxidation and kinetic parameters at various methane and oxygen concentrations in consideration of the change in the volume of gas phase.

2. METHODS

2.1 Culture

Leachates from landfill sites H and T and a digested sewage sludge were used as inocula. Approximately 1 mL was added to 100 mL of mineral salt medium in a 300 mL flask, which was connected to a 1 L bag filled with 20 % of methane and 80% of air. The enrichment was incubated statically at 30 °C for 2 weeks. This procedure was continued through more than 5 transfers. Obtained cultures from the landfill sites H and T and the anaerobic digested sewage sludge were referred to as

Culture H, Culture T, and Culture S, respectively.

When methane oxidation is performed in gas venting pipes, it is expected that aerobic microorganisms exist with methane oxidizing bacteria. Therefore, no isolation was performed, so that the obtained cultures contained aerobic microorganisms in addition to methane oxidizing bacteria.

2.2 Media

The mineral salt medium consisted of the following: distilled water, 1 L; KNO_3 , 1.0 g; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.3 g; KH_2PO_4 , 0.2 g; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.15 g; $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.02 g; $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, 0.001 g; and agar if added, 50 g. The YG culture consisted of the following: distilled water, 1 L; yeast extract, 1.0g; glucose, 1.0 g; K_2HPO_4 , 0.2 g; KH_2PO_4 , 0.2 g; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.2 g; and agar, 15.0 g

2.3 Experimentals

Figure 1 shows experimental apparatus. Five mL of enriched culture and 500 mL of the autoclaved mineral salt medium were placed in a 1 L of flask, which was connected to a 500 mL of cylinder with a connection tube. The gas phase in the flask and cylinder was replaced with a methane-air mixture (20% of methane). The cylinder was connected to saturated NaCl solution reservoir. The change in the volume of the gas phase was equivalent to the volume of saturated NaCl solution dropped into the cylinder from the reservoir. Saturated NaCl solution was used to prevent gas absorption. The culture in the flask was incubated for 2 weeks to grow bacteria.

After 5 mL of the culture in the flask was taken to count the number of bacteria, the gas phase was replaced with a methane-air mixture (20% of methane), and then experiment was started. Gas concentrations and volume of solution in the cylinder were measured. Gas of 4 mL was sampled from the sampling port with a syringe. For the different initial methane concentration of 10 % and 5 %, experiments were proceeded as the same manner. As a control, in any methane concentration, 1 mL of toluene was added to the mineral salt medium instead of enrichment culture to prevent biological activity. All experiments were conducted in the temperature controlled room at 25 °C.

2.4 Analysis

Gas samples were analyzed by a gas chromatograph (Shimadzu GC-6A) with TCD. Analytical conditions are as follows: column, Shimadzu GW-100; injection/column temperature, 70 °C; detection temperature, 40 °C; carrier gas and flow rate, helium and 100mL/min; and amplifier, Shimadzu AMP7.

With the spread plate technique, 0.1 mL of aliquots of each of the dilutions were plated in triplicate onto the mineral salts agar plates for methane oxidizing bacteria and the YG culture for aerobic bacteria. The petri dishes were incubated at 30 °C for 2 weeks in a methane-air mixture (20% of methane) and in air, respectively. The number of bacteria per mL of culture was determined by calculating the mean of the numbers of bacterial colonies.

3. ANALYTICAL METHODS

Methane oxidation rate was assumed as follow:

$$r_M = -V_{\max} \frac{C_M}{K_M + C_M} C_O \quad (1)$$

Where:

r_M = methane oxidation rate in mol L⁻¹ hr⁻¹ cell⁻¹

V_{\max} = rate constant in hr⁻¹ cell⁻¹

K_M = half saturation constant in mol L⁻¹

C_M = methane concentration in gas phase in mol L⁻¹

C_O = oxygen concentration in gas phase in mol L⁻¹

For methane, a Monod equation was considered because many researchers used it. To consider the effect of oxygen concentration to methane oxidation rate, Visscher et al. (1999) assumed a Mono equation. In this study, first order equation was assumed to reduce the number of kinetic parameters.

Reaction rate of oxygen and carbon dioxide was expressed as follows;

$$r_O = ar_M \quad (2)$$

$$r_C = -br_M \quad (3)$$

Where:

a = mole ratio of consumed oxygen to consumed methane

b = mole ratio of generated carbon dioxide to consumed methane

Both of a and b were calculated from the difference between initial and final moles of gas component in whole gas phase. The moles of gas component was calculated by gas concentration and volume of gas phase.

Assuming that transfer of gas component occurred by diffusion and the decrease in the volume of gas phase in the flask caused by methane oxidation, the flux of i-th gas component from the cylinder to the flask was described as follows:

$$J_i = -D_i \frac{dC_i}{dz} - \frac{1}{S} \frac{dV_m}{dt} C_{mi} = -D_i \frac{C_{fi} - C_{mi}}{L} - \frac{1}{S} \frac{dV_m}{dt} C_{mi} \quad (4)$$

Where:

D_i = diffusion coefficient of i-th gas component

C_{fi} = concentration of i-th gas component in the flask

C_{mi} = concentration of i-th gas component in the cylinder

L = length of the connection tube

S = cross section area of the connection tube

V_m = volume of gas phase in the cylinder

It was assumed that gradient in concentration was constant at a moment. $\frac{dV_m}{dt}$ was expressed as follow:

$$\frac{dV_m}{dt} = RTN(r_M + r_O + r_C) - \frac{dV_{mCont}}{dt} \quad (5)$$

Where:

R = gas constant

T = temperature

N = Number of methane oxidation bacteria in the flask

V_{mCont} = volume of gas phase in the cylinder of a control

The second term in the right hand in Eq.(5) expressed the effect of factors except biological activity on volume of gas phase in the cylinder.

Fundamental equations were obtained by making mass balances for gas components at the flask and cylinder. Obtained differential equations were solved by a Runge-Kutta method. The kinetic parameters, V_{max} and K_M , were determined to minimize the sum of square of difference between observed and calculated methane concentration in the flask.

4. RESULTS AND DISCUSSION

4.1 Results

Figures 2-4 show the change in gas concentrations and volume of saturated NaCl solution in the cylinder. In any case, the decrease in the volume of gas phase was recognized, 150-250 mL. Because the volume of sampled gas was about 100 mL, the net decrease was estimated to 50-150 mL. Whole volume of gas phase in the flask and cylinder was about 1 L. If the experiments were conducted in a batch reactor with a constant volume, the decrease in total pressure during the experiments was estimated to 0.05-0.15 atm, so that partial pressure of gas component also decreased. This indicates that no consideration of change in the volume of gas phase would make kinetic parameters uncertain.

Obtained a and b are listed in Table 1. a was ranged from 1.5 to 1.8, which was as same as that found by Brown et. al. (1964). For b , at 20 % and 10 % of initial methane concentration, the values of 0.4-0.6 were near to the theoretical value, 0.5 (Brown et. al. (1964)); however, at 5 % of initial methane concentration the values of 0.7-0.9 were higher than the theoretical value. The reason was that the carbon dioxide generated from methane oxidation at 5 % was less than other initial methane concentration and biological reactions in addition to methane oxidation would occur because aerobic bacteria were contained in the cultures and organic materials were detected in the cultures. At 5 % initial methane concentration, therefore, the contribution of aerobic bacteria to carbon dioxide generation was greater than other initial methane concentration experiments.

4.2 Kinetic parameters

Obtained kinetic parameters are listed in Table 2 and the comparisons between calculation and observation are shown in Figures 5-7. At 20 % of initial methane concentration, a several set of parameters were obtained, so that the optimum parameters could not be determined.

The figures show that for all of gas concentration and volume of dropped solution, calculations (solid lines) were in good agreement with observations. Therefore, the methane oxidation rate equation and analytical methods used in this study were adequate. However, at the last parts in experiments, calculated methane and oxygen concentrations in the cylinder were higher than observed. This suggests that the expression for gas transfer might be a little insufficient. Because the difference between the calculation and observation was within 2 %, it seems that the difference had little effect on the determination of kinetic parameters.

In the same culture, different initial methane concentrations led different V_{max} . V_{max} at 10 % of initial methane concentration was higher than that at 5 % of initial methane concentration. There were no references to be compared with the values in this study because many researches were performed using soil and oxidation rate was expressed in per weigh of soil.

In any case except Culture H at 10 %, K_M had the same order, 10^{-4} mol L⁻¹. The range of K_M , 5.1×10^{-5} - 6.5×10^{-4} (1200 - 16000 ppmv) is within the range found by Czepiel et al. (1996), Bogner et al. (1997), and Visscher et al. (1999); 5847 ppmv, 25380 ppmv, and 1450-5000 ppmv, respectively.

5. CONCLUSIONS

The methane oxidation experiments were conducted with a batch reactor, in which the change in gas volume can be measured.

Obtained results are as follows:

1. The decrease in total pressure in a constant volume batch reactor was estimated to 0.05-0.1 atm.
2. The effect of the change in oxygen concentration on methane oxidation kinetics was expressed as first order reaction.
3. V_{max} was ranged from 2.1×10^{-13} to 3.4×10^{-11} $\text{hr}^{-1} \text{ cell}^{-1}$; in the same culture, V_{max} increased with initial methane concentration.
4. K_M was ranged from 5.1×10^{-5} to 6.5×10^{-4} mol L^{-1} .

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Captions

Table 1 Obtained a and b

Table 2 Obtained kinetic parameters

Figure 1 Experimental apparatus

Figure 2 Time course of CH₄ oxidation (Culture H)

Figure 3 Time course of CH₄ oxidation (Culture T)

Figure 4 Time course of CH₄ oxidation (Culture S)

Figure 5 Comparison between calculation and observation (Culture H)

Figure 6 Comparison between calculation and observation (Culture T)

Figure 7 Comparison between calculation and observation (Culture S)

Table 1 Obtained a and b

Culture	H			T			S		
CH ₄ (%)	20	10	5	20	10	5	20	10	5
a	1.5	1.8	1.8	1.5	1.7	1.8	1.6	1.7	1.7
b	0.44	0.6	0.82	0.41	0.53	0.93	0.57	0.56	0.73

Table 2 Obtained kinetic parameters

Culture	H		T		S	
CH ₄ (%)	10	5	10	5	10	5
V_{max} (hr ⁻¹ cell ⁻¹)	3.4×10^{-11}	4.6×10^{-12}	8.4×10^{-13}	3.9×10^{-13}	3.2×10^{-12}	2.1×10^{-13}
K_M (mol L ⁻¹)	5.1×10^{-5}	6.5×10^{-4}	2.0×10^{-4}	6.5×10^{-4}	2.0×10^{-4}	2.4×10^{-4}

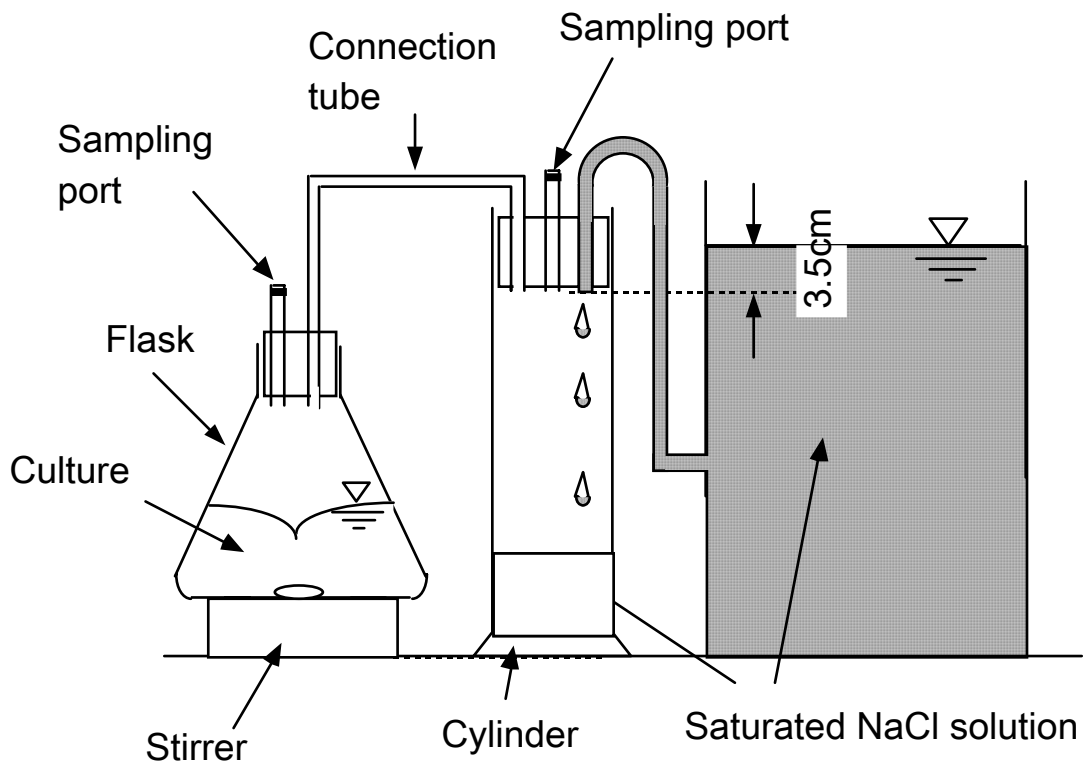
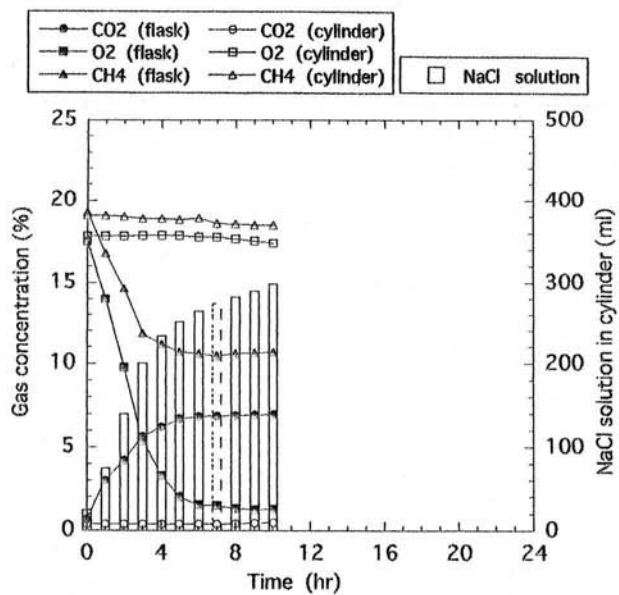
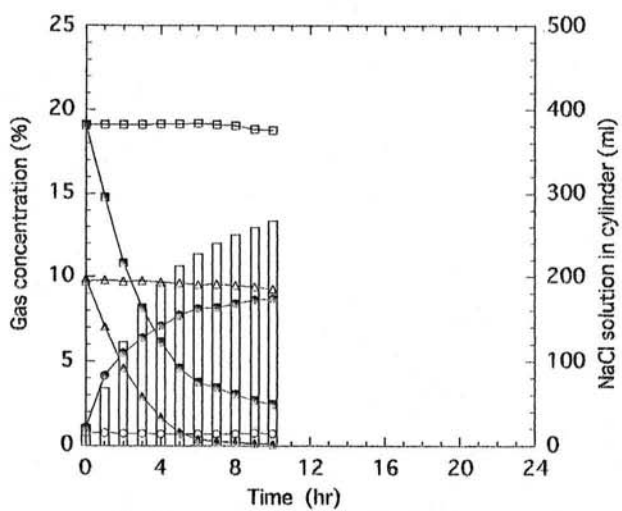


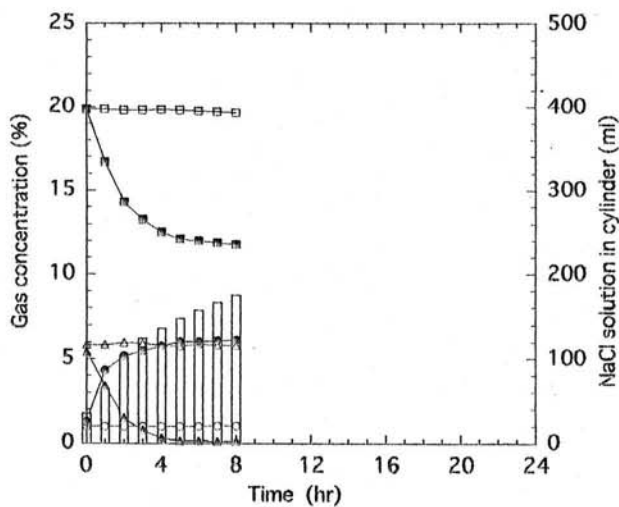
Figure 1 Experimental apparatus



a) Culture H (Initial methane, 20%)

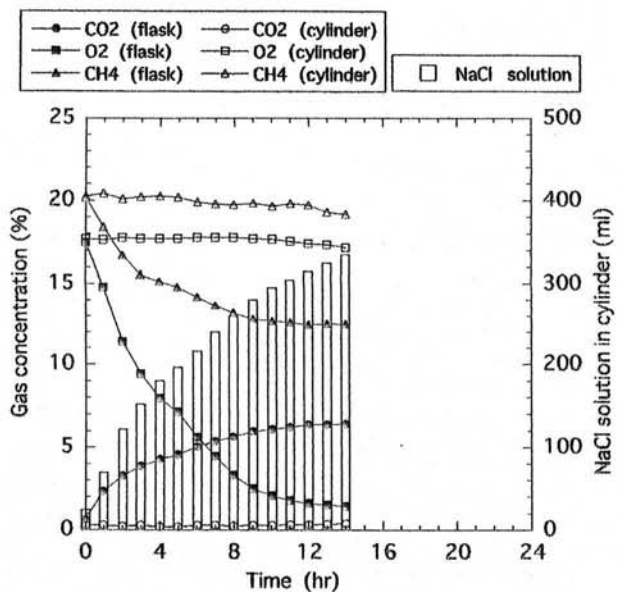


b) Culture H (Initial methane, 10%)

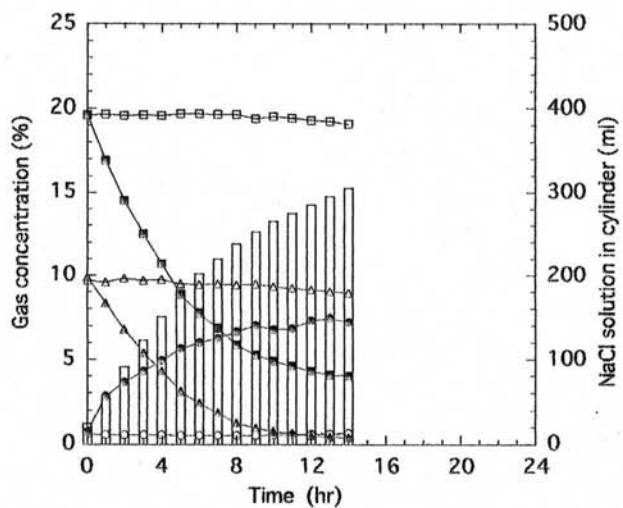


c) Culture H (Initial methane, 5%)

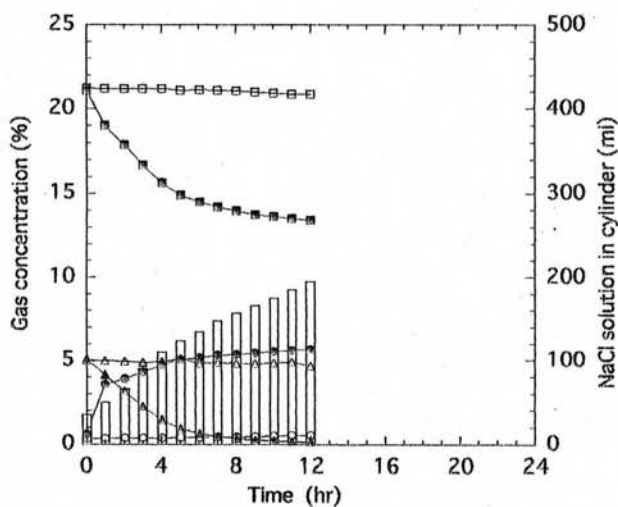
Figure 2 Time course of CH₄ oxidation (Culture H)



a) Culture T (Initial methane, 20%)



b) Culture T (Initial methane, 10%)



c) Culture T (Initial methane, 5%)

Figure 3 Time course of CH₄ oxidation (Culture T)

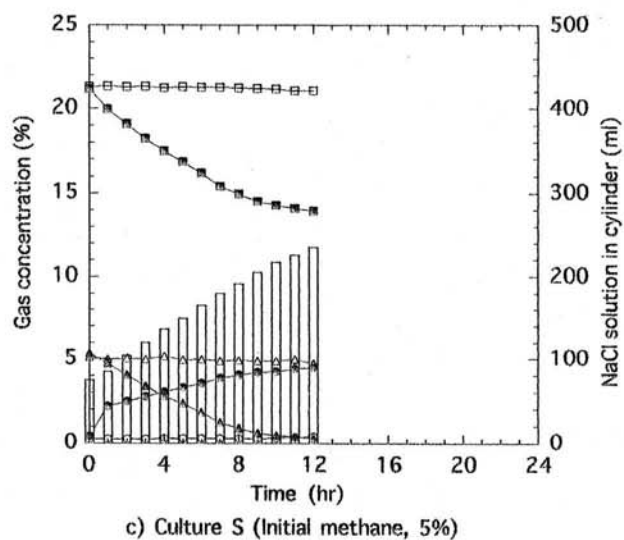
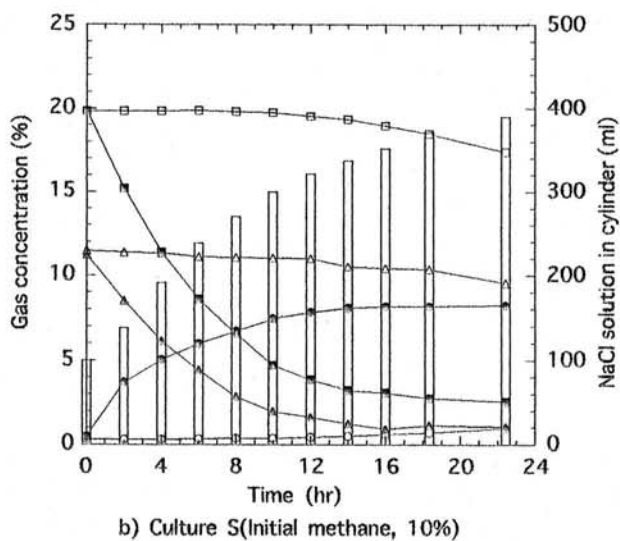
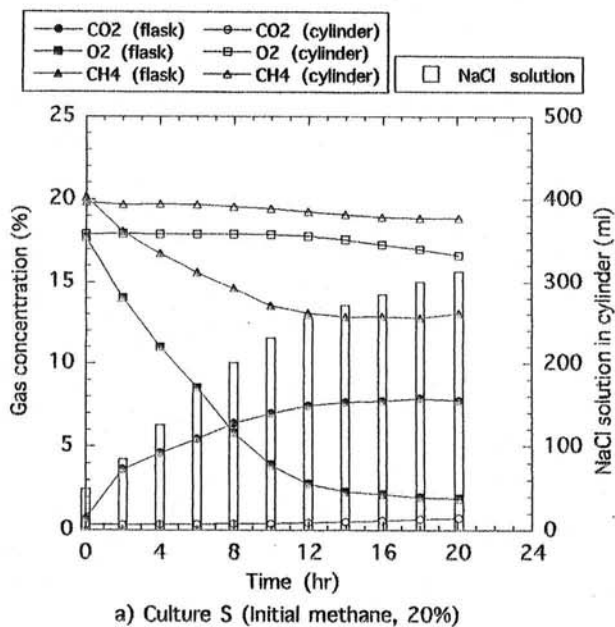


Figure 4 Time course of CH₄ oxidation (Culture S)

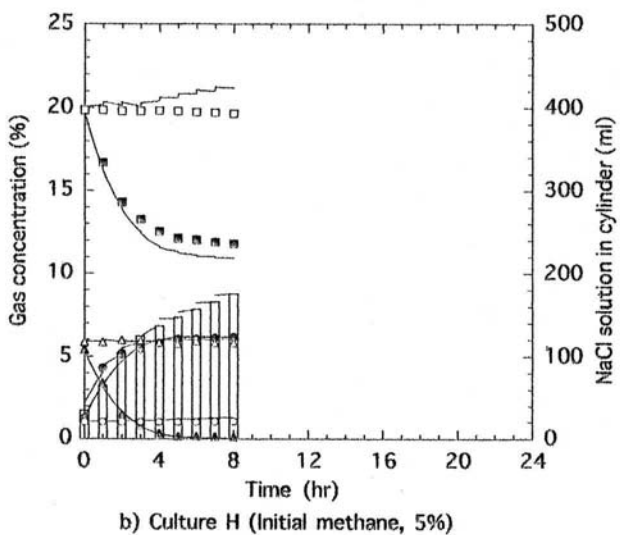
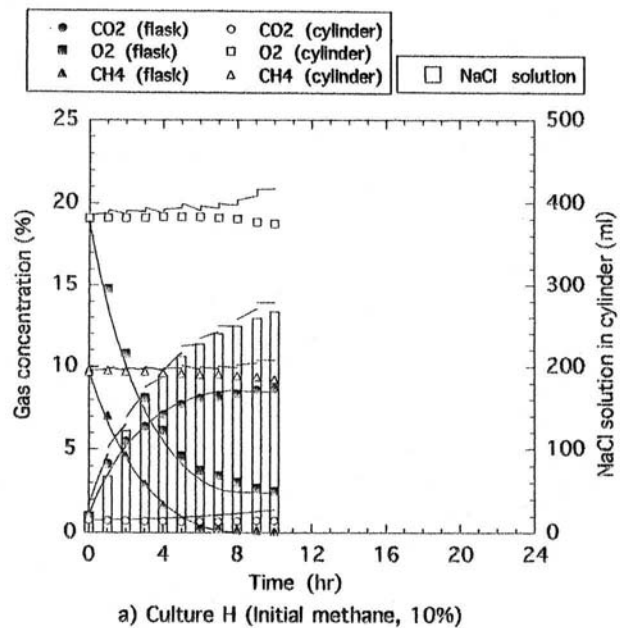


Figure 5 Comparison between calculation and observation (Culture H)

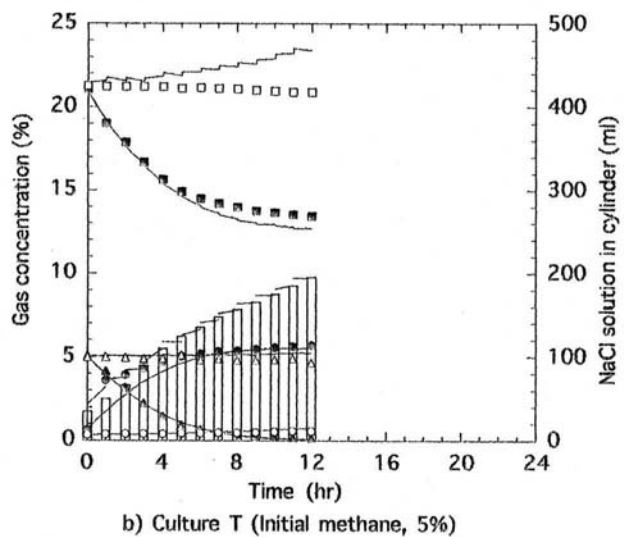
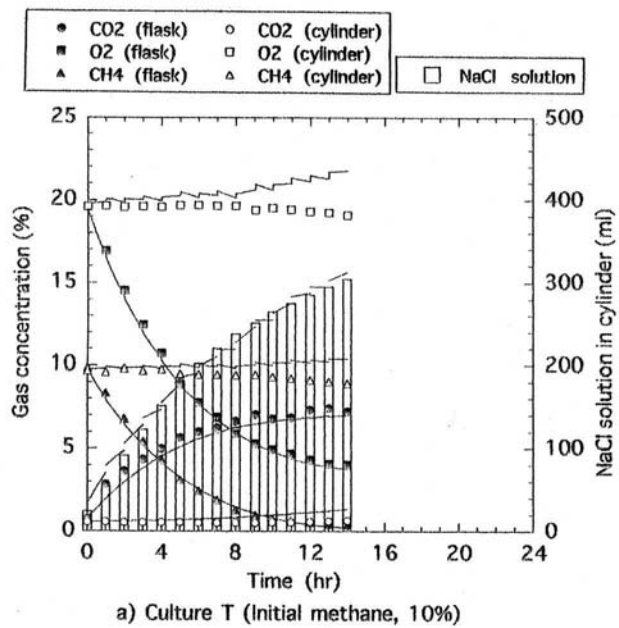
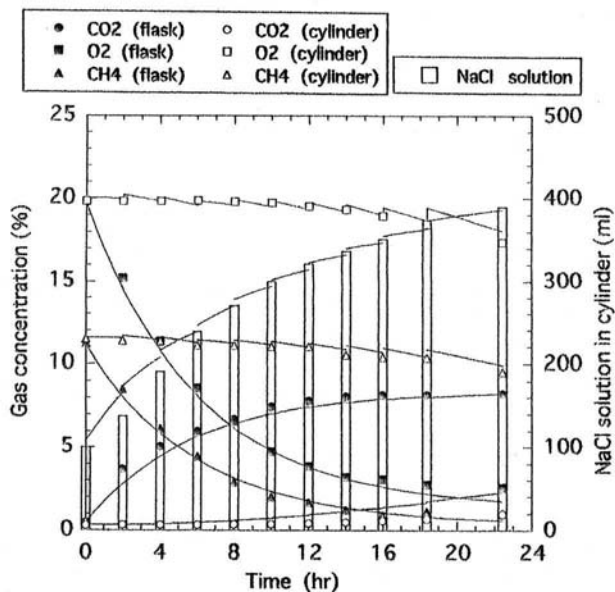
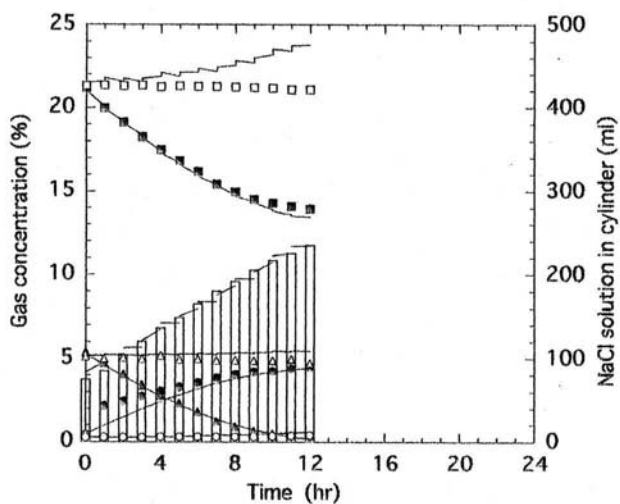


Figure 6 Comparison between calculation and observation (Culture T)



a) Culture S (Initial methane, 10%)



b) Culture S (Initial methane, 5%)

Figure 7 Comparison between calculation and observation (Culture S)