BIOMETHANATION UNDER ELEVATED PRESSURE

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As H_2 and CO_2 can be utilized by methanogenic bacteria, as well as volatile fatty acids such as formic acid, biomethanations of the gaseous substrates and formic acid were carried out under elevated pressure. The pH of the medium was adjusted to the appropriate value for the methanogenic bacteria (pH 6.8) by changing the amount of Na_2CO_3 added to the medium. Biomethanation was not inhibited by elevated partial pressure of the gaseous substrates in the absence of mass transfer limitation of H_2, nor retarded by elevated pressure when formic acid was used as a carbon source. However, the amount of methane evolved from CO_2 and H_2 for 3 days under 101 kPa decreased to 25% compared with that under 355 kPa in the presence of the mass transfer limitation of H_2. This effect is explained by the mass transfer model.

Introduction

Methane fermentation, as well as the activated sludge process, has often been applied to waste water treatment. In the activated sludge process, tower-type fermentors with a height of more than 50 m have been developed as a deep shaft plant.[7] They are used especially in urban districts to minimize the building site for waste water treatment. In methane fermentation, the energy of organic compounds can be recovered as methane gas. Therefore, considerable attention has been focused on bioconversion of organic materials to methane[18,25,26] from the standpoint of energy recovery from wastes. However, methane fermentation usually requires a hydraulic retention time of more than 10 days and a spacious building site. In this context, development of a tower-type fermentor is desirable. For this purpose, it should be confirmed that the elevated pressure does not retard biomethanation rate.

Methane fermentation is a complex ecosystem with the following three steps[22]: 1) hydrolytic step of polymerized substrates such as carbohydrate and protein, 2) acidogenic step where volatile fatty acids are produced, and 3) methanogenic step where methane is produced from the acids. Generally, growth rates of the methanogenic bacteria are far lower than those of the acidogenic bacteria, suggesting that the rate-limiting step of methane fermentation is the methanogenic step.

In the acidogenic step, production of the acids is usually accompanied by evolution of CO_2 and H_2. These gas products can be utilized by the methanogenic bacteria as well as the volatile fatty acids.[1,28]

In the present work, with a view to developing an effective biomethanation system such as a tower-type fermentor, a pressurized methane fermentation was carried out by using formic acid as a representative of the volatile fatty acids and a mixed gas of CO_2 and H_2. In addition, a mass transfer model was developed in order to express the influence of pressure on the production rate of methane.

1. Experimental

1.1 Methane fermentation

A methanogenic bacterial population was obtained from digested sewage sludges in the Yamazaki Waste-Treatment Plant, Nagoya. The population was used as seed culture after adaptation to the following medium by successive subculture for a few months. The basal composition of the medium was yeast extract (Difco), 1 kg; K_2HPO_4, 0.30 kg; KH_2PO_4, 0.18 kg; NH_4Cl, 0.68 kg; $(NH_4)_2SO_4$, 0.15 kg; cysteine·HCl·H_2O, 0.25 kg; resazurin, 1×10^{-3} kg; trace minerals,[1] 0.01 m^3 and trace vitamins,[1] 0.01 m^3 per m^3 of distilled water. Unless otherwise described, Na_2CO_3 was added to the medium for adjustment of pH and its amount was calculated as mentioned below. In the fermentation with gaseous substrates, a mixture of CO_2 and H_2 (1 : 4, v/v) was used as carbon and energy sources for the methanogenic bacteria and atmosphere for anaerobiosis. In the fermentation using formate (1 kg/m^3) as carbon source, the concentration of yeast extract was reduced to 0.1 kg/m^3, and pure H_2 or a mixture of CO_2 and N_2 (1 : 4, v/v)

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was introduced to the fermentor in place of CO_2 and H_2. When pure H_2 was used as gas phase, Na_2CO_3 was omitted from the medium and the concentrations of K_2HPO_4 and KH_2PO_4 were 0.75 kg/m^3 and 0.5 kg/m^3, respectively, to keep the medium pH at 6.8. Throughout the experiments, the fermentation was carried out at 310 K. After preculture in an Erlenmyer flask for two days, the seed culture (10% of total volume) was inoculated in a fermentor.

The experimental apparatus is shown in **Fig. 1**. A beer bottle made of polyethylene terephthalate (2×10^{-3} m^3, Sapporo Breweries Co.) was used as a fermentor. The pressure of the atmosphere in the fermentor, which was monitored by a pressure gauge (E in Fig. 1), was kept approximately constant by frequent feeding of the gas. Sampling of gas was carried out by insertion of a gas-tight syringe into the fermentor (at atmospheric pressure C in Fig. 1) or collection with a gas sampler (at elevated pressure D in Fig. 1) after the bottle was vigorously shaken for a few minutes. The fermentor was usually set in a vertical arrangement as shown in Fig. 1, resulting in larger depth of fermentation broth, 0.075 m (broth volume: 0.001 m^3). In another experiment, the fermentor was set horizontally (turning the bottle through 90°) and the broth volume was reduced to 2×10^{-4} m^3. In this case, mean depth of the broth was 0.013 m.

1.2 Analysis

Methane concentration was determined by a gas chromatograph (Shimadzu, model GC-7AG) equipped with a flame ionization detector (FID) for analysis of methane, a thermal conductivity detector (TCD) for analysis of other gases and a 1 m glass column packed with Active Carbon 30/60 mesh (Gasukuro Kogyo Inc.). Helium was used as carrier gas (column temperature: 353 K, injection temperature: 373 K, and TCD temperature: 373 K). Concentration of formate was colorimetrically measured.[13] The actual pH of the medium under a given pressure was measured with a pH electrode introduced into the fermentation system after achieving gas-liquid equilibrium.

2. Results

2.1 pH estimation of medium under various pressures

In methane fermentation with an atmosphere of CO_2 and H_2 (or CO_2 and N_2), it should be considered that pressurization of the gas phase results in the increase of solubility of CO_2 in the medium. This leads to pH lowering of the medium, which is undesirable for the methanogenic bacteria. Thus, the medium pH as a function of the partial pressure of CO_2 and Na_2CO_3 concentration was predicted as follows.

Hydrogen ion donors or acceptors were considered to be major medium components such as K_2HPO_4, KH_2PO_4, Na_2CO_3, NH_4Cl and $(NH_4)_2SO_4$. Though dissolved CO_2 forms H_2CO_3, which dissociates into HCO_3^- and CO_3^{2-}, the concentration of H_2CO_3 is negligible in the vicinity of pH 7. Similarly, phosphate mainly exists as $H_2PO_4^-$ and HPO_4^{2-}. For convenience, only the following association/dissociation reactions were considered:

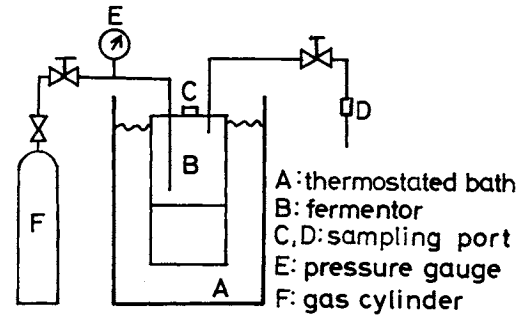

Fig. 1. Schematic diagram of methane fermentation.

$$CO_2\ (\text{dissolved}) + H_2O = HCO_3^- + H^+ \quad (1)$$

$$HCO_3^- = CO_3^{2-} + H^+ \quad (2)$$

$$H_2PO_4^- = HPO_4^{2-} + H^+ \quad (3)$$

$$NH_4^+ = NH_3 + H^+ \quad (4)$$

$$H_2O = H^+ + OH^- \quad (5)$$

Here, the equilibrium constants were defined as follows:

$$K_1 = [HCO_3^-][H^+]/[CO_2] \quad (6)$$

$$K_2 = [CO_3^{2-}][H^+]/[HCO_3^-] \quad (7)$$

$$K_3 = [HPO_4^{2-}][H^+]/[H_2PO_4^-] \quad (8)$$

$$K_4 = [H^+][NH_3]/[NH_4^+] \quad (9)$$

$$K_w = [H^+][OH^-] \quad (10)$$

By employing Eqs. (6)–(10) as well as electrostatic and mass balances, the hydrogen ion concentration is calculated from the root of the following implicit equation,

$$C_1 + C_2 + \left([H^+] - \frac{K_w}{[H^+]}\right) = \frac{K_1[CO_2]}{[H^+]}\left(1 + \frac{2K_2}{[H^+]}\right) + C_3\frac{2K_3 + [H^+]}{K_3 + [H^+]} + \frac{C_4K_4}{[H^+] + K_4} \quad (11)$$

where C_1, C_2, C_3 and C_4 are $[K^+]$, $[Na^+]$, $[H_2PO_4^-]+[HPO_4^{2-}]$ and $2[SO_4^{2-}]+[Cl^-]$, respectively. The concentration of dissolved CO_2 can be related to the partial pressure of CO_2 in the gas phase by Henry's law. Taking the major salts into account, a modified Henry's law constant H^* is obtained from the following equation[3]:

$$\log(H/H^*) = \sum(h_iI_i) \quad (12)$$

where the parameter h_i can be obtained from the literatures.[3,19] By employing the following constants,[8-10]

$$K_1 = 4.88 \times 10^{-7}\ \text{kmol/m}^3,$$

$$K_2 = 6.03 \times 10^{-11}\ \text{kmol/m}^3,$$

$$K_3 = 6.31 \times 10^{-8}\ \text{kmol/m}^3,$$

$$K_4 = 5.62 \times 10^{-10}\ \text{kmol/m}^3,$$

$$K_w = 2.09 \times 10^{-14}\ \text{kmol}^2/\text{m}^6$$

and

$$H^* = 2.48 \times 10^{-7}\ \text{kmol/(m}^3 \cdot \text{Pa)},$$

pH is calculated from Eq. (11).

As shown in **Fig. 2**, the results calculated as functions of Na_2CO_3 concentration and total pressure (lines in Fig. 2) were in approximate agreement with the experimental measurements. Thus, the pH of the medium could be evaluated as above.

2.2 Optimal pH of methane production

The effect of pH on bioconversion of CO_2 and H_2 to methane was examined for establishment of the optimal condition. **Figure 3** represents the effects of pH on methane production at 72 h under various pressures in gas phase. The optimal pH was 6.5 to 7.2, independent of the pressure. This agrees with observation in many methanogenic processes.[11,24] In subsequent experiments, the pH of the medium was adjusted to 6.8.

2.3 Effect of pressure on methane production from formate

To investigate the effect of pressure on methane production, methane fermentations under various pressures were carried out using formate as representative carbon and energy source for the methanogenic bacteria. In this case, pure H_2 or CO_2–N_2 mixture (1 : 4, v/v) was used as gas phase so that methane is produced only from formate.

As shown in **Table 1**, no difference in methane production rate under 101–355 kPa was observed in the atmosphere of CO_2–N_2 or H_2. Thus, it was found that the methane fermentation was not reduced by pressure of at least 355 kPa.

2.4 Effect of pressure on methane production from CO_2 and H_2

Figure 4 shows the fermentation results under various pressures when the fermentor was set horizontally. Methane was evolved logarithmically (shown in the inset of Fig. 4) at almost the same rates regardless of pressure, and it was also confirmed that the biomethanation rate was not retarded by pressures up to 355 kPa.

When the fermentor was set vertically, methane production rate under 355 kPa was almost comparable with that for the horizontal arrangement of the fermentor, as shown in **Fig. 5**. On the other hand, the amounts of methane evolved for 3 days under 203 and 101 kPa decreased to 50% and 25%, respectively, compared with that under 355 kPa.

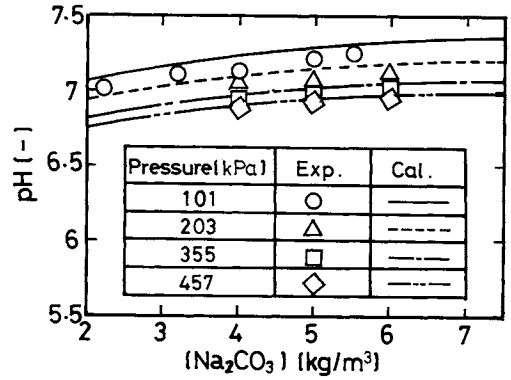

Fig. 2. Relationships between pH and Na_2CO_3 concentration under various pressures. The lines were calculated from Eq. (11).

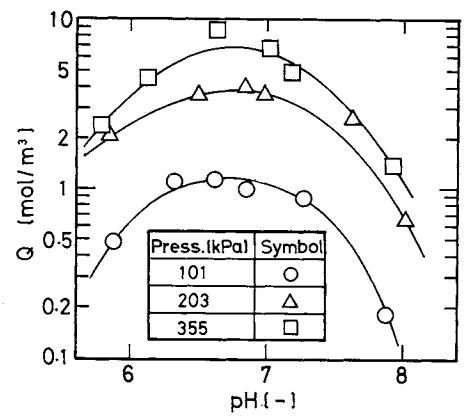

Fig. 3. Effect of pH on methane fermentation under various pressures. CO_2 and H_2 were used as carbon and energy sources. The amount of Na_2CO_3 to be added was estimated from the relations as shown in Fig. 2.

Table 1. Effect of pressure on methane fermentation, in which formate was used as carbon and energy source

Pressure [kPa]		Q^a [mol/m^3]		Q^b [mol/m^3]
	Fermentation time	21 h	66 h	48 h
101		1.1	2.0	1.3
203		0.82	1.5	1.1
355		1.1	1.9	1.5

[a] Gas phase was a mixture of N_2 and CO_2. The following amount of Na_2CO_3 was added: 0.80 kg (101 kPa), 1.63 kg (203 kPa) and 2.85 kg (355 kPa) per m^3 of the medium.
[b] Gas phase was pure H_2 and Na_2CO_3 was not added.

3. Discussion

Recently, attempts to enhance methane production rate have been extensively studied, including immobilization of methanogenic bacteria,[11] pretreatments of

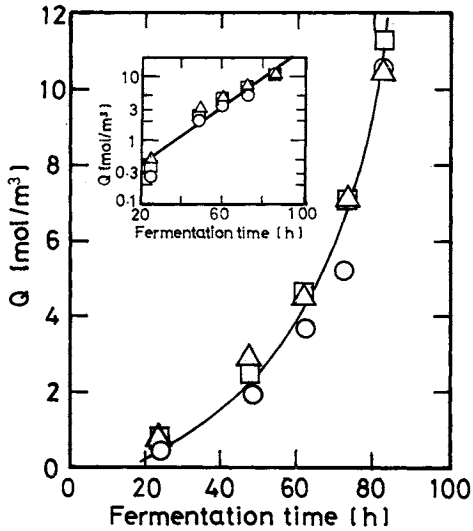

Fig. 4. Effect of pressure on methane production, in which the fermentor was set horizontally (turn the fermentor shown in Fig. 1 through 90°). CO_2 and H_2 were used as carbon and energy sources. The symbols are the same as those in Fig. 3. The inset represents the plot of Q against fermentation time on semilogarithmic scale.

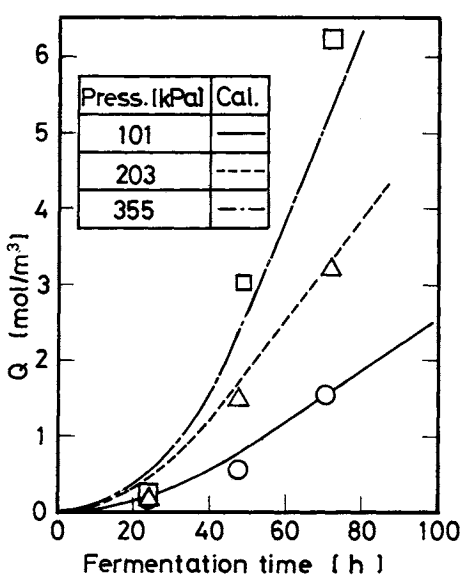

Fig. 5. Effect of pressure on methane production, in which the fermentor was set vertically as shown in Fig. 1. CO_2 and H_2 were used as carbon and energy sources. The symbols are the same as those in Fig. 3. The lines were calculated from Eqs. (14)–(17), employing $k=7.3\times10^{-5}$ mol/m^3 and $\mu=0.079\,h^{-1}$.

raw materials such as soybean coat[17] and separation of the methane fermentation process into acidogenic and methanogenic phases.[4,15] All methanogenic bacteria are able to utilize H_2 and CO_2 as carbon and energy sources.[1,28] Effective biomethanation of these gases to methane will be of great advantage to enhancement of overall methane production rate and final methane concentration in gas phase.

Mangel *et al.*[14] carried out methane production from waste materials under elevated pressure, and noticed a decrease of CO_2 concentration in final gas phase by pressurization. Wise *et al.*[27] investigated methane fermentation under high-pressure conditions for the purpose of utilizing CO_2, H_2 and CO evolved from the coal gasification process, but did not discuss the pH lowering of fermentation broth due to increased partial pressure of CO_2. We could evaluate the pH for given Na_2CO_3 concentration and pressure from the electrostatic and mass balances of the major ions in the medium. Thus, the pH could be adjusted to the appropriate value (pH 6.8) for the methanogenic bacteria by adding various amounts of Na_2CO_3 into the medium (Fig. 2).

Microorganisms are often inhibited by high concentration of substrate such as methanol, phenol and so on. The same phenomena are observed for gaseous substrate. Sato *et al.*[20] reported that high dissolved oxygen concentration decreased the growth rate and cellular yield in aerobic cultivation of *Pseudomonas aeruginosa*. King *et al.*[12] reported that dissolved CO_2 had an inhibitory effect on the growth of *P. aeruginosa*.

In methane fermentation under elevated pressure, the toxicities of CO_2 and H_2 should be investigated.[5,6] As shown in the experiments using formate and a mixture of CO_2 and N_2 (Table 1), methane production was not inhibited under 71 kPa partial pressure of CO_2. Similarly, other experiments shown in Table 1 indicated that 355 kPa partial pressure of H_2 did not exhibit toxicity to the methanogenic bacteria. Thus, methane production rate in the tower-type fermentor may not be decreased by elevated pressure.

In general, CO_2 and H_2 are metabolized at higher rate than formate and acetate by methanogens and about 30% of methane is produced from CO_2 and H_2 in methane fermentation.[21,23] Thus, mass transfer of CO_2 or H_2 may decrease methane production rate. In the present study, decrease of methane production rate was observed, as shown in Fig. 5. To explain these results, a model for mass transfer of the gaseous substrate was established.[3] For simplification of the model, the following was postulated. 1) Methane is produced according to the following reaction.[16]

$$H_2+1/4CO_2 = 0.22CH_4+P\ (\text{cell mass}, H_2O\ \text{etc.}) \quad (13)$$

2) Since excess Na_2CO_3 is added to the medium and the solubility of H_2 is much lower than that of CO_2, the mass transfer of H_2 is the rate-limiting step. 3) The reaction order of Eq. (13) is assumed to be zero-order with respect to H_2, based upon the results shown in Fig. 4. 4) The methanogenic bacteria are assumed to be in logarithmic growth phase, which is supported by

the logarithmic evolution of methane shown in the inset of Fig. 4. 5) Under initial condition, the medium is saturated with H_2 by full bubbling of the CO_2–H_2 mixture prior to fermentation, and by vigorous shaking of the fermentor at sampling time.

The mass balance for H_2 in the liquid phase gives the following equation,

$$\frac{\partial C_H}{\partial t}=D_H\frac{\partial^2 C_H}{\partial x^2}-ke^{\mu t} \tag{14}$$

with the initial and boundary conditions:

$$t=0,\quad x>0;\qquad C_H=C_{H0} \tag{15}$$

$$t>0,\quad x=0;\qquad C_H=C_{H0} \tag{16}$$

$$t>0,\quad x=\infty;\qquad C_H=C_{H0}-k(e^{\mu t}-1)/\mu \tag{17}$$

The solution for Eq. (14) is given in **Appendix**. The parameters of this model, k and μ, were obtained from the fermentation results, in which the mass transfer of H_2 was considered not to be limited (Fig. 4). The lines in Fig. 5 are calculated from the model and are in fair agreement with the experimental results. The dependence of pressure on methane evolution was successfully evaluated by the model.

Conclusion

1) The pH of the medium under various pressures of CO_2 could be adjusted by adding various amounts of Na_2CO_3 to the medium, as estimated from the mass and electrostatic balances of the major ions in the medium.

2) Optimal pH for methanogenic activity was between 6.5 and 7.2 under 101–355 kPa.

3) Methane production rate was not reduced by pressure of at least 355 kPa in the absence of the mass transfer limitation.

4) Methane production rate was decreased in the presence of the mass transfer limitation and the effect was elucidated by the mass transfer model.

Appendix

The partial differential equation to be solved is

$$\frac{\partial C_H}{\partial t}=D_H\frac{\partial^2 C_H}{\partial x^2}-f(t) \tag{A-1}$$

The initial and boundary conditions are

$$t=0,\quad x\geqq 0;\qquad C_H=C_{H0} \tag{A-2}$$

$$t>0,\quad x=0;\qquad C_H=C_{H0} \tag{A-3}$$

$$t\geqq 0,\quad x=\infty;\qquad C_H=C_{H0}-\int_0^t f(t)dt \tag{A-4}$$

By the variable-transformation

$$u=C_H-C_{H0}+\int_0^t f(t)dt \tag{A-5}$$

Eq. (A-1) is converted to the conventional diffusion-type equation without chemical reaction:

$$\frac{\partial u}{\partial t}=D_H\frac{\partial^2 u}{\partial x^2} \tag{A-6}$$

with the following conditions:

$$t=0,\quad x>0;\qquad u=0 \tag{A-7}$$

$$t>0,\quad x=0;\qquad u=\int_0^t f(t)dt \tag{A-8}$$

$$t\geqq 0,\quad x=\infty;\qquad u=0 \tag{A-9}$$

The Laplace transformation is used to solve Eq. (A-6) with Eqs. (A-7)–(A-9). Transforming and rearranging, the following equation is obtained:

$$\mathscr{L}\{u\}=\mathscr{L}\left\{\int_0^t f(t)dt\right\}\cdot\exp\left(x\sqrt{\frac{S}{D_H}}\right) \tag{A-10}$$

The variable S is the imaginary for the Laplace transformation and $\mathscr{L}\{\ \}$ denotes "to be transformed." In the present study, $f(t)$ is equal to $ke^{\mu t}$, and $\mathscr{L}\{u\}$ is represented by the following equation:

$$\mathscr{L}\{u\}=\left(\frac{1}{S-\mu}-\frac{1}{S}\right)\frac{k}{\mu}\exp\left(-x\sqrt{\frac{S}{D_H}}\right) \tag{A-11}$$

By the inverse transformation

$$u=\frac{k}{\mu}\left[\frac{1}{2}e^{\mu t}\left\{\exp\left(-x\sqrt{\frac{\mu}{D_H}}\right)\operatorname{erfc}\left(\frac{x}{2\sqrt{D_H t}}-\sqrt{\mu t}\right)+\exp\left(x\sqrt{\frac{\mu}{D_H}}\right)\operatorname{erfc}\left(\frac{x}{2\sqrt{D_H t}}+\sqrt{\mu t}\right)\right\}-\operatorname{erfc}\frac{x}{2\sqrt{D_H t}}\right] \tag{A-12}$$

Subsequently, the variable C_H is solved as follows:

$$\frac{C_H}{C_{H0}}=1-\frac{k}{C_{H0}\mu}\left[e^{\mu t}\left[1-\frac{1}{2}e^{\mu t}\left\{\exp\left(-x\sqrt{\frac{\mu}{D_H}}\right)\operatorname{erfc}\left(\frac{x}{2\sqrt{D_H t}}-\sqrt{\mu t}\right)+\exp\left(x\sqrt{\frac{\mu}{D_H}}\right)\operatorname{erfc}\left(\frac{x}{2\sqrt{D_H t}}+\sqrt{\mu t}\right)\right\}\right]-\operatorname{erfc}\frac{x}{2\sqrt{D_H t}}\right] \tag{A-13}$$

Thus, q is calculated from the following equation at a given sampling interval:

$$q=0.22\int_0^t ke^{\mu t}l(t)dt \tag{A-14}$$

where $l(t)$ is represented as follows:

$$l(t)=\begin{cases}1 & (h\geqq L)\\ h/L & (h<L)\end{cases} \tag{A-15}$$

and h is the root for $C_H=0$ at a given time, calculated from Eq. (A-13).

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Nomenclature

C_H	=	concentration of H_2 in liquid phase	[kmol/m^3]
C_{H0}	=	interfacial concentration of H_2	[kmol/m^3]
D_H	=	diffusivity of H_2 in liquid phase	[m^2/h]

H	=	Henry's law constant of CO_2	[kmol/(m^3·Pa)]
H^*	=	modified Henry's law constant of CO_2	[kmol/(m^3·Pa)]
h_i	=	contributions due to cation, anion and gas for individual electrolytes	[m^3/mol]
I_i	=	ionic strength for individual electrolytes	[mol/m^3]
k	=	empirical constant	[kmol/(m^3·h)]
L	=	broth depth	[m]
Q	=	total amount of methane evolved	[mol/m^3]
q	=	amount of methane evolved after shaking the bottle	[mol/m^3]
t	=	time after shaking the bottle	[h]
x	=	distance from gas-liquid interface	[m]
μ	=	specific growth rate	[h^{-1}]
[ ]	=	concentration of species in bracket	[kmol/m^3]

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