Note

A Typical Methane Fermentation of Glucose

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Methane fermentation of cellulosic materials proceeds by the sequential actions of acidogenic and methanogenic bacteria. The formation of volatile fatty acids and gas evolution depend on the activities of both kinds of bacteria. The course of the fermentation may be represented by a set of modified Michaelis-Menten equations in which the accumulation of volatile fatty acids is represented by the amounts of volatile fatty acids remaining in the solution during fermentation. In this paper, the velocities of acid and methane formation were calculated from the data obtained from the methane fermentation of glucose. The velocity equation of Michaelis-Menten type of enzymatic reaction was applied to the methane fermentation of glucose. The velocity of substrate utilization is expressed by differential equation (1),

$$-\frac{d(S)}{dt} = \frac{C_1 X(S)}{K_A + (S)} \quad (1)$$

The velocity of gas evolution is expressed by differential equation (2),

$$\frac{d(G)}{dt} = \frac{C_2 X(A)}{K_G + (A)} \quad (2)$$

The velocity of volatile fatty acid accumulation is expressed in terms of the following differential equation (3),

$$\frac{d(A)}{dt} = \frac{C_1 X(S)}{K_A + (S)} - \frac{C_2 X(A)}{K_G + (A)} - f(A) \quad (3)$$

where

S is the concentration of substrate in $\text{g} \cdot \text{l}^{-1}$,

A is the concentration of volatile fatty acids in $\text{g} \cdot \text{l}^{-1}$,

G is the concentration of gas evolved in $\text{g} \cdot \text{l}^{-1}$,

X is the microbial concentration in $\text{g} \cdot \text{l}^{-1}$,

$f(A)$ represents the acid concentration, adjusting differences between theoretical and experimental data in $\text{g} \cdot \text{l}^{-1} \cdot \text{day}^{-1}$,

C_1, C_2 are the experimental kinetic constants for substrate consumption and gas evolution in day^{-1} , respectively,

K_A and K_G are the Michaelis constants for acid formation and gas evolution in $\text{g} \cdot \text{l}^{-1}$, respectively.

The rate of microbial growth in the mash is expressed using the maximum growth terms (4),

$$\frac{d(X)}{dt} = U_m X \quad (4)$$

U_m is the maximum specific growth rate, day^{-1} . After the microbial growth reached the maximum value, the microbial concentration decreased gradually. The microbial concentrations at t days of incubation, X_t , are represented by cell weight in the mash, and these values are calculated by Eq. (5),

$$\frac{d(X)}{dt} = D(X_1) \quad (5)$$

X_1 is the microbial concentration at the maximum growth in $\text{g} \cdot \text{l}^{-1}$, and D is the rate of decreasing microbial concentration in day^{-1} .

Methane fermentation was carried out in a 200 ml syringe containing 20 ml of acclimatized sludge which had been used for agro-wastes methane fermentation,^{1~4)} and 40 ml of substrate solution in the total volume of 60 ml. The fermentation was performed at 37°C for 5 days. The volume of gas evolved was measured in a gas burette using saturated sodium chloride solution to equilibrate the pressure. Gas composition and the amounts of volatile fatty acids in the sample extracted by acidic ethyl ether were determined by gas chromatography.²⁾ Reducing sugar was determined by the Schaffer-Somogyi method.⁵⁾ The biomass content, total organic carbon (TOC) and DNA in the sludge, was determined according to the quick, novel procedure reported by Hashimoto *et al.*⁶⁾ In our study, the biomass content in the sludge is conveniently represented by the cell weights per liter.

All chemicals were of commercial grade.

Various amounts of acids, 20 to 200 mg, were added to the reaction mixture, and gas evolution was examined for 24 hr at 37°C. Gas evolution from formic and acetic acids increased proportionally to the added amounts of acids. However, the addition of more than 100 mg of propionic or butyric acid inhibited gas evolution. The gas evolved, in all cases, was 70% methane by volume. Double reciprocal plots of these results are shown in Fig. 1. The values of K_G and C_2 were calculated from the intercept values on the abscissa and vertical line, and C_2 was 0.797 day^{-1} and K_G was 3.57 $\text{g} \cdot \text{l}^{-1}$ against acetate. The C_2 values for formic, propionic and butyric acids were 0.381, 0.138 and 0.284 day^{-1} , respectively. The K_G values for formic, propionic and butyric acids were 5.00, 0.556 and 0.188 $\text{g} \cdot \text{l}^{-1}$, respectively.

Amounts of glucose added to the reaction mixture were varied from 50 mg to 2 g. After 24 hr of incubation, the

FIG. 1. Lineweaver-Burk Plot of Gas Evolution from Volatile Acids by Acclimatized Sludge.

—○—, acetic acid; —●—, propionic acid; —■—, formic acid; —□—, butyric acid.

FIG. 2. Lineweaver-Burk Plot of Consumption of Glucose by Acclimatized Sludge.

residual amounts of glucose in the mash were measured, and the reciprocal values of glucose consumed were plotted against reciprocal values of glucose added at first, as shown in Fig. 2. The values of C_1 and K_A were 6.75 day^{-1} and $6.67 \text{ g} \cdot \text{l}^{-1}$ from the values of the vertical intercept and abscissa intercept, respectively.

The gas evolution and accumulation of volatile fatty acids were measured and analyzed for 5 days. When 1 to 2 g of glucose was added to the mash, the pH of the mash dropped to 5.4 after 24 hr of incubation and the volume percentage of methane in the gas evolved became lower than 50%. Therefore we chose, for a typical fermentation, to use 200 mg of glucose. In the experiment, 20 mg L-

FIG. 3. Course of Gas Evolution and Accumulation of Volatile Acids in the Glucose Medium.

(200 mg glucose, 20 mg L-glutamic acid, and 20 ml acclimatized sludge, in 60 ml of the mash in 200 ml of syringe at 37°C .)

A: DNA and TOC; —○—, DNA; —●—, TOC.

B: CH_4 and pH; —○—, CH_4 ; —●—, pH.

C: Gas evolution, accumulation of volatile fatty acids and consumption of glucose.

—△—, sugar content; —■—, total volatile fatty acids; —□—, acetic acid; —○—, butyric acid; —●—, gas evolution.

glutamate was added to the reaction mixture to prevent a drop of pH. Also, no gas evolution occurred from 20 mg L-glutamate. As shown in Fig. 3, about 80% of 200 mg of glucose was consumed in 24 hr of incubation, and the total amount of fatty acids accumulated was $1.75 \text{ g} \cdot \text{l}^{-1}$. The volume percentage of methane in the gas was 53~58% in the whole course of incubation. TOC biomass value was $0.523 \text{ g} \cdot \text{l}^{-1}$ at first, and increased to $0.670 \text{ g} \cdot \text{l}^{-1}$ after 24 hr, and then it decreased slowly for 2 to 5 days

incubation.

The initial values of (S) , (A) and (X) fitted to Eqs. (1), (2) and (3) were 3.32, 0.32 and $1.14 \text{ g} \cdot \text{l}^{-1}$, respectively. The weight of gas evolved was calculated conveniently from the volume percentage of gas which contained 50% CH_4 and 50% CO_2 . The cell weight in the mash was calculated from TOC biomass values. The DNA biomass value was $0.0279 \text{ g} \cdot \text{l}^{-1}$, the TOC biomass, $0.523 \text{ g} \cdot \text{l}^{-1}$, and the cell concentration, $1.14 \text{ g} \cdot \text{l}^{-1}$ at first. After one day of incubation, (X) value reached the maximum, $1.46 \text{ g} \cdot \text{l}^{-1}$. The value of U_m was 0.23 day^{-1} . After the microbial growth reached the maximum value, the microbial concentration in the mash decreased gradually during the fermentation. In Eq. (5), X_t is the cell concentration at t days incubation in the mash, and X_1 is the cell concentration at the maximum. D is the rate of decreasing cell concentration after 2 days incubation. The value of D was -0.025 day^{-1} . Equation (5) was applied to the mash after 2 to 5 days incubation. The term (A) in Eq. (3) was expressed by the experimental values, i.e., at 1 and 2 days incubation, $f(A_1)$,

$$f(A_1) = 0.2t + 0.30$$

and after 3 days incubation, $f(A_2)$ was fitted to Eq. (3),

$$f(A_2) = 0.2t - 0.40$$

In the above equations, the unit of time was t , days. From the preceding considerations, we derived the following equations:

$$-\frac{d(S)}{dt} = \frac{6.75X(S)}{6.67 + (S)} \quad (1')$$

$$\frac{d(G)}{dt} = \frac{0.797X(A)}{3.57 + (A)} \quad (2')$$

$$\frac{d(A)}{dt} = \frac{6.75X(S)}{6.67 + (S)} - \frac{0.797X(A)}{3.57 + (A)} - f(A) \quad (3')$$

The calculated data match the experimental data, and

hence, allow some confidence in the set of kinetic equations.

The accumulated volatile fatty acids were acetic and butyric acids, and the relative amounts of these acids were about 1 to 0.5 by weight. When 100 mg of butyric acid or propionic acid were used as the substrate, the formation and accumulation of acetic acid were observed continuously for 2 or 3 days with gas evolution. The conversion of butyric or propionic acid to methane gas *via* acetic acid was apparent. Butyric acid was utilized faster than acetic acid at lower concentrations, as seen in Fig. 1. Total amounts of methane formed from 200 mg glucose during 5 days incubation corresponded to about 42% of the total amounts of acids formed in this time. More study of the intermediate metabolism of anaerobic microbes and checking of the appropriateness of the model by computer simulation techniques are necessary to elucidate the overall reactions concerning methane fermentation.

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