MECHANISM OF VOLATILE FATTY ACID REMOVAL IN A FIXED BIOFILM METHANE FERMENTATION REACTOR

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ABSTRACT

It was found that hydrogen plays a most important role as an acceleration factor of acetate decomposition to methane; this was investigated based on the theory of catabolized reactions of acetate, propionate, and butyrate. To demonstrate the hydrogen effect, several batch experiments were carried out with the fixed biofilm methane fermentation reactor (FBMFR) using a sole substrate of acetate, propionate and butyrate or a mixture substrate(acetate-propionate, acetate-butyrate). While using each volatile fatty acid and the mixture, the experiment of continuous incubation was carried out to investigate biofilm biota and structure through a scanning electron microscope. From these experimental results, the authors present a new reaction rate equation of acetate decomposition.

KEYWORDS

Anaerobic biological process; Fixed biofilm methane fermentation reactor; Hydrogen effect; Acceleration factor; Reaction kinetics of acetate decomposition

INTRODUCTION

The authors have carried out the investigation to elucidate the removal mechanism of volatile fatty acids (VFA) (INOUÉ, 1985a), in proposing a two phase anaerobic biological system to treat wastewaters. From the results of simulation for substrate removal based on the above mentioned model, it was revealed that in the case of a mixture substrate of VFA, the rate of acetate decomposition in the FBMFR was accelerated when compared with the case of a sole substrate of acetate. When methanogenesis limits the whole process, the acetate decomposition is often an essential limiting reaction. Therefore to make clear the acceleration mechanism of acetate decomposition is important in practice and in microbiology.

In this paper from the theory of methanogenic and acetogenic reaction of three VFA substrates and experimental approaches, the acceleration factors of acetate decomposition will be explored and the kinetic equation will be proposed and evaluated based on the experimental data.

SELECTION OF THE ACCELERATION FACTORS

In general, pH and temperature have an influence on the maximum rate of acetate decomposition. But it was found that acetate decomposing rate increased even under the same pH and temperature. This phenomenon can be clearly seen when butyrate coexists. According to Bryant's hypothesis (BRYANT, 1976), propionate and butyrate are decomposed by acetogenic bacteria as shown in the following equations;

\[
\text{CH}_3\text{CH}_2\text{COO}^- + 2 \text{H}_2\text{O} \leftrightarrow \text{CH}_3\text{COO}^- + 3 \text{H}_2 + \text{CO}_2 \quad (1)
\]

\[
\text{CH}_3(\text{CH}_2)_2\text{COO}^- + 2 \text{H}_2\text{O} \leftrightarrow 2 \text{CH}_3\text{COO}^- + 2 \text{H}_2 + \text{H}^+ \quad (2)
\]

In these reactions hydrogen and proton could play the most important roles. The concentrations of hydrogen and proton directly relate to redox potential (ORP) and pH which are very important in the methane fermentation process. On the other hand, acetate decomposition by the aceticlastic methanogenes is considered to be as follows;

\[
\text{CH}_3\text{COO}^- + \text{H}_2\text{O} + \text{XH} \leftrightarrow \text{CH}_3 + \text{H}_2 + \text{HCO}_3^- \quad (3)
\]

\[
\text{XCH}_3 + \text{H}_2 \leftrightarrow \text{XH} + \text{CH}_4 \quad (4)
\]
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XH is a carrier of a methyl group called coenzyme M. These two reactions show that hydrogen concentration is very important. If the rate coefficient \( k_{+1} \) is extremely greater than \( k_{-1} \), the concentration of the XH is very small and almost all of XH is considered to become the coenzyme-substrate complex XCH₃. Eq.(4) is an enzyme reaction which generates methane through methylreductase. Therefore it is obvious that the concentration of hydrogen has an influence on the methane production rate, i.e. acetate decomposing rate. But if the concentration of hydrogen becomes much stronger, as shown in Eq(3) the following phenomena will occur; a state of equilibrium will move the right, then the concentration of XCH₃ will become weak, as a result the acetate decomposing rate will decrease.

**MATERIALS AND METHODS**

Experimental set-up

The schematic diagram used in this study is shown in Fig.1. The equipment was composed of the methane fermentation reactor, back mixing tank and circulation pump and has a 5 l liquid volume (reactor; 2.4 l, backmixing tank; 2.1 l, others; 0.5 l). One component of reactor is a cylinder with a height of 26 cm and a diameter of 10 cm. Acclimation was taken for about 6 months under the following conditions: incubation temperature; 35±2 °C, hydraulic detention time; 1-5 days. The composition of the substrate is shown in Table 1. In all experiments pH in the reactor was kept constant within the 6.8-7.3 without being control.

Experiment A. In order to investigate the acceleration effect of acetate decomposition using a sole and a mixture substrate of VFA, substrate removal experiments were conducted by batch process using acetate only or a mixture of acetate and propionate or butyrate. Sodium salts were used in these acids. Experimental conditions are shown in Table 2.

Experiment B. In order to study the influences of the concentration of hydrogen and redox potential on acetate decomposing rate. In this experiment hydrogen gas was injected from an inlet pipe fitted in the pipe on suction side of the circulation pump. The experimental conditions are also shown in Table 2.

Experiment C. In order to investigate the differences of biota and structure of the attached biofilm incubated with different substrates. This experiment was conducted using a small apparatus like Fig.1. Reactors are plastic cylinders having about 500 ml volume (diameter; 27 cm, height; 27 cm). Honeycomb tubes cut in disk-like shapes with a height of 10 cm, were put one on top of another sequentially in the reactor. Circulation flow rate was about 1.5 l/min and upflow rate was about 0.8 cm/s. In this experiment of incubation, 4 kinds of substrates were used; 1)acetate, 2)propionate, 3)butyrate and 4)their mixture having 6,000 mg/l concentration as acid. Methane forming bacteria obtained from the fixed biofilm reactor which had incubated for over 2 years in our laboratory was used as the seed. The seed sludge dosed in reactors was 0.5 g-VS per reactor. After incubation for 53 days, packed media forming bacterial film was brought out and observed through an electron microscope.

Analytical methods

Liquid sample to determine VFA was filtered by a 0.45 µm membrane filter. Volatile fatty acids and gas compositions were determined by gaschromatograph operated on the following conditions: Chromosorb 101,3% FFAP, glass, Temp., Inlet 230 °C, column 175 °C, H₂ 45 ml/min; HITACHI 163FID: W-100, SUS, Temp.; 50 °C, Ar 30 ml/min; JEOL JGC-20K TCD or Activated charcoal, SUS, Temp. 75 °C, Ar 30 ml/min; HITACHI l5 4TCD respectively. In experiments A and B, the backmixing tank was fitted with an electrode for continuous measurements of pH and redox potential. For scanning electron microscopy, the biofilm pieces with attached media were fixed in 3 % glutaraldehyde in cacodylate buffer before postfixation in 1 % OsO₄. After dehydration in alcohol, dried by critical point method and coated with gold-palladium, the biofilm samples were observed through a HITACHI S-450 scanning microscope.

Table 1 Composition of substrate for acclimation

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetate</td>
<td>3000</td>
</tr>
<tr>
<td>propionate</td>
<td>800</td>
</tr>
<tr>
<td>butyrate</td>
<td>1200</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>460</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>100</td>
</tr>
<tr>
<td>yeast extract</td>
<td>1 ml/l (v/w 10%)</td>
</tr>
</tbody>
</table>

Table 2 Experimental condition of batch removal process of substrates

<table>
<thead>
<tr>
<th>Item</th>
<th>Experiment A</th>
<th>Experiment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulation flow rate</td>
<td>( 20 \times 10^{-3} ) m³/min</td>
<td>( 20 \times 10^{-3} ) m³/min</td>
</tr>
<tr>
<td>Shape of packed media</td>
<td>Honey comb</td>
<td>Honey comb</td>
</tr>
<tr>
<td>Specific surface area</td>
<td>308 m²/m³</td>
<td>500 m²/m³</td>
</tr>
<tr>
<td>Temperature</td>
<td>35 °C</td>
<td>37 °C</td>
</tr>
<tr>
<td>( \text{H}_2 ) dosage rates</td>
<td>0 ml/min</td>
<td>250 ml/min</td>
</tr>
</tbody>
</table>

Fig.1. Schematic diagram of FBMFR
RESULTS AND DISCUSSION

Acceleration effect on acetate decomposition by propionate and butyrate

Several examples of substrate removal experiments by batch method with acetate and mixtures of acetate and propionate or butyrate are shown in Figs. 2, 3 and 4 respectively. Acetate decomposing rate in the mixture of substrate was obtained through a converted concentration of acetate (T-C2) which was determined by adding the acetate concentration to its equivalent concentration of propionate and butyrate by using Eqs. (1) and (2). As shown in a previous report (INOUE, 1985b), a sole substrate of acetate or propionate is decomposed in accordance with 0-order reaction, but butyrate is not simple like these. The butyrate decomposing rate is determined by taking into account a conversion of butyrate into acetate and an isomerization of butyrate into iso-butyrate. The net decomposing rate of acetate is determined by taking a gradient of the curve of the concentration of total acetate (T-C2). In the case of the acetate-butyrate mixture, the net decomposing rate of acetate greatly increased early in the experiment (Fig. 4), but in the case of the acetate-propionate mixture this tendency was not as strong (Fig. 3). Subsequently the decomposing rate also decreases gradually with the consumption of butyrate. Fig. 5 shows the ratios of the net decomposing rate in the case of the mixture to 0 order one in the case of acetate alone. The net decomposing rate increased to 1.4 times in the case of propionate coexisting, and to the extent of 1.8 times in the case of butyrate coexisting. These results clearly show that the decomposing rate of acetate is accelerated by the existence of propionate and butyrate.

Redox potential changed in general in the following ways: it was suddenly raised by a dosage of substrate, afterwards it recovered gradually to the original level and raised again when the substrate was consumed. In the case of the acetate-butyrate mixture, the degree of change was distinguished from the other two cases. In this case, the amount of recovery of redox potential was much greater; 10 mV at its maximum level. It was suggested that the amount of change of redox potential is much greater in biofilm than that in bulk liquid. The same phenomenon also occurred in hydrogen partial pressure. In the cases of a sole substrate of acetate and acetate-propionate mixture, the concentration of hydrogen increased only a little compared with the initial values. But in the case of the acetate-butyrate mixture it increased from $10^{-6}$ to $10^{-5}$ atm. It seemed that such a change of hydrogen partial pressure during experiments is linked to a change of redox potential. Then, it was found that the acceleration effect of acetate decomposition by propionate was much smaller in spite of having generated about the same amount of hydrogen by the decomposition of propionate and butyrate. Hydrogen should scarcely diffuse from biofilm to bulk liquid because it should be consumed more rapidly in the case of propionate than in the case of butyrate in biofilm. The redox potential also should barely decrease. We shall discuss these later.

Fig. 2. Removal experiment of a sole substrate in batch process in FBMFR

Fig. 3. Removal experiment of acetate-propionate mixture in batch process in FBMFR

Fig. 4. Removal experiment of acetate-butyrate mixture in batch process in FBMFR

Fig. 5. Acceleration of acetate decomposition under coexistence of propionate or butyrate

Fig. 6. Influence on acetate decomposition by hydrogen hydrogen: injection; ○ w/o injection: ●
Fig. 7. Scanning electron micrographs of the biofilm surface in FBMFR substrate: a,b; acetate, c,d; propionate, e,f; butyrate, g,h; mixture of acetate, propionate and butyrate.

Effect on acetate decomposition by hydrogen

Experimental results are shown in Fig. 6. We have made it clear that the reactor exhibits an initial lag in acetate decomposition when batch removal experiments are carried out after the acetate in the reactor has been completely consumed. Such a phenomenon of initial lag clearly appears in this experiment. On the other hand, as shown in the figure, this phenomenon did not entirely appear when hydrogen was injected into the reactor. As soon as the acetate is added, it is removed with a 0-order reaction, but the increment of the decomposition rate is small compared with no injection of hydrogen. It was not possible to judge if the increment would be significant, namely the acceleration effect by hydrogen should exist. But we should suggest that it is significant from following reasons: When propionate and butyrate are decomposed, hydrogen is generated at a rate of 350 ml/h and 230 ml/h in biofilm respectively; while hydrogen was injected at a rate of 250 ml/h. Judging that hydrogen scarcely diffused to the bulk liquid by a decomposition of them, it was suggested that in this extent of the dosing rate, hydrogen barely penetrated to the inner part of biofilm. Zeikus et al. (1975) demonstrated that acetate decomposition was accelerated under hydrogen coexists in pure culture of Methanosarcina barkeri as shown below. Biofilm in our experiments was predominantly composed of methanogen similar to Methanobacterium soehngenii though substantial differences may not exist between these methanogens.
Biota and structure of biofilm

After a 53 day incubation period of acetate, propionate, butyrate, and their mixture, the attached biofilm was observed through a scanning microscope. The photomicrographs are shown in Figs. 7-(a,b),(c,d),(e,f), and (g,h) respectively. These figures show that a biota and structure of the attached biofilm are very different from each other with each of the substrates. Biofilm formed by acetate entirely consists of the bacteria, M. soehngenii-like (Bryant, 1974), which were intertwined with each other and constructed a homogeneous interstructure (Figs. 7-(a,b)). Biofilm formed by propionate was similar to that formed by acetate, but simultaneously fine grained colonies were created and scattered through (Figs. 7-(c,d)). In the case of butyrate the biofilm also had fine grained colonies as in the one formed by propionate, although in detailed observation the plexus of both biofilms clearly differed with each other (Figs. 7-(e,f)). The latter mainly consisted of curled bacilli", relatively large (10-20 μm). On the other hand, the former consisted of cocc"., fairly small, and the curled one almost cannot be seen in the biofilm. In the mixture of them, as shown in Figs. 7-(g,h), the biota and structure of the biofilm show a medium feature, between the case of propionate and butyrate. From these results we assumed that the structure of fine grained colonies in biofilms generated by the mixed substrates are composed of two layers. Organisms living in outer layer may be the aceticogenic bacteria which decompose butyrate. Organisms in inner layer may be the acetogenic bacteria which decompose propionate and may be hydrogen consuming methanogens. This assumption is thermodynamically very rational, because the decomposing reaction of propionate Eq(1) is more sensitive to hydrogen than that of butyrate Eq(2), i.e. the change of free energy in this reaction is likely to be positive. Hydrogen generated by propionate decomposing bacteria is consumed rapidly in the colonies by syntrophic bacteria adjacent to them and therefore the concentration of hydrogen in the biofilm does not increase. On the other hand, the hydrogen generated by butyrate decomposing bacteria is mainly released out of the colonies, the amount of hydrogen consumption decreases and a much greater amount of hydrogen diffuses into bulk liquid.

Reaction kinetics

The results obtained in these experiments and the previous investigation (Tomiyama, 1985b) are brought together as follows:

Acetate: Decomposition in biofilm progresses as 0-order reaction. Hydrogen partial pressure in gas phase increases in sole substrate. Initial lag phenomenon in batch process of acetate removal occurs when the acetate in the reactor is completely consumed. Acetate decomposition is accelerated when propionate or butyrate coexists.

Propionate: Decomposition in biofilm progresses as 0-order reaction. Decomposing reaction follows Eq(1). The increment of hydrogen partial pressure in propionate decomposing is small and the effect of acceleration appears slight.

Butyrate: Decomposition follows Eq(2), but with isomerized reaction. A scheme of the reaction is as follows:

\[ \text{E} + \text{S} \not\rightarrow \text{E.S} \not\rightarrow \text{E} + \text{P}, \quad \text{E}^\prime + \text{S} \not\rightarrow \text{E}^\prime \text{S} \not\rightarrow \text{E}^\prime + \text{S}' \quad (5) \]

Decomposition from n-butyrate to acetate progresses 0-order reaction. The increment of hydrogen partial pressure in butyrate decomposing is large and the effect of acceleration appears clearly.

Hydrogen: In constant decomposition of acetate the concentration of hydrogen can keep constant according to Eqs(3) and (4). Methanogenesis from hydrogen follows a type of michaelis-menten kinetics.

Proposal of reaction kinetics of acetate decomposition

Acetate decomposes according to 0-order reaction, but the rate changes as the concentration of hydrogen changes in the bacterial cell. Such a fact has been reported by Zeikus et al.,(1975). On the other hand, as mentioned above, aceticlastic methanogens can keep the concentration of hydrogen constant in the cell, the biofilm also could maintain a constant decomposing rate of acetate. On the basis of this discussion, the reaction kinetics of acetate is as follows:

\[ R_2 = \frac{R_{c1} \cdot RC_{\text{C2}}}{1 + RC_{\text{C2}} + KH} \quad (6) \]

Reaction kinetics of propionate, butyrate and hydrogen

propiionate: \( \frac{dRC_{\text{C3}}}{dt} = -kOC3 \quad (7) \quad RC3_{\text{C2}} = RC3_{\text{H}} = RC3 \quad (8) \)

n-butyrate: \( \frac{dRC_{\text{C4}}}{dt} = -RC4_{\text{C2}} + RC4' \quad (9) \quad RC4_{\text{C2}} = RC4_{\text{H}} = kOC4 \quad (10) \)

iso-butyrate: \( \frac{dRC4'}{dt} = \frac{RM \cdot C4' \cdot Kc}{1 + C4/Kc + C4' / Kc'} \quad (11) \)

hydrogen: \( \frac{dRC}{dt} = \alpha(k2_{2} \cdot RC_{\text{C1}, H}) - \frac{RM \cdot C4'H}{KH} \quad (12) \)

\( \alpha \) has a range of 0 to 1 and is a coefficient that changes by a difference of distribution in habitat between the propionate and the butyrate decomposing bacteria. In the case of butyrate, \( \alpha \) has a large value, but in the case of propionate it is small.
Mathematical model of batch process

In the development of basic equations we express fundamental handling on a fixed biofilm reactor. Media packed into the reactor has a honey comb shape, but can be approximately regarded as a plate in a 8 mm diameter of a cell or more (INOUE,1980). The reactor assumes a completely mixing tank with circulation apparatus. Batch process of substrate removal in fixed biofilm reactor is a heterogeneous reaction and is expressed as follows:

1. Diffusion process of substrates from bulk liquid to biofilm surface
2. Diffusion process of them in biofilm
3. Ingestion process of them to the cell and catabolism process of them in the cell

(2) and (3) occur simultaneously in biofilm.

In such a case, heterogeneous reaction process is divided into three major domain as shown in Fig.8, which consists of the bulk liquid, diffusion boundary layer and biofilm. Basic equations are led from the law of conservation of mass in each domain. Thus in bulk liquid:

\[ \frac{\partial C_L}{\partial t} = \frac{V}{A} N_L \]  

In diffusion boundary layer:

\[ -N_L = K_L (C_L - C_s) = -N_s \]  

In biological film:

\[ \frac{\partial C}{\partial t} = \frac{De}{\partial x^2} + U_r \]  

(15)

\[ U_r = \left( \frac{V/A}{X_a} \right) R \]  

(16)

In biofilm the following boundary conditions are given:

\[ \frac{\partial C}{\partial x} = 0 \text{ at } x = x_a \]  

\[ C = C_s, N_s = De(\frac{\partial C}{\partial x}) \text{ at } x = 0 \]  

(17)

These equations show the transport phenomena in a heterogeneous system with an interior reaction term \( U_r \) under unsteady state, i.e. batch reaction of substrate removal in the anaerobic biofilm reactor. In this paper these equations were solved by using a numerical method (finite-difference solution: explicit method).

Calculation results and evaluations

Numerical calculations were carried out under the conditions shown in Tables 3 and 4. The parameters of Table 3 were obtained from our experiments and were considered fairly accurate. But some parameters of Table 4, which were not previously determined, were obtained by comparison between the experimental data and the calculation curve. Fig.9 shows a calculation curve and the experimental results in batch removal process of n-butyrate. It seems that the calculation curve nearly explains the experimental results. In order to make clear the hydrogen effect, several numerical calculations were carried out in two cases, in...
existence or nonexistence of its effect. The calculated curves are shown in Fig.10. When the hydrogen effect was not considered, the calculated curve did not agree with the experimental results in any way. On the other hand, considering its effect, the calculated one easily agreed with the experimental curve. This result reveals qualitatively that acetate decomposing is affected by concentration of hydrogen in the cell.

SUMMARY AND CONCLUSIONS

A biological conversion of acetate to methane occurs through a reducing reaction of the methyl group, in which hydrogen in the cell plays an important role. This shows that it can be explained based on the theory that the decomposition of acetate under the coexistence of propionate or butyrate is accelerated. This was demonstrated by our experiments. However the acceleration effect of propionate was much smaller than that of butyrate. The similar phenomenon also appeared in the amount of change of hydrogen partial pressure and redox potential.

Through the observation of biofilm with a scanning microscope, the biota and the structure of the biofilm formed by a mixed substrate of acetate, propionate and butyrate were made clear, i.e. the biofilm was mainly composed of filamentous methanogens, M. soehngenii-like in which fine grained colonies were scattered. Regarding the difference of acceleration effect between propionate and butyrate as the difference between the living organisms in interior and outer layer of colonies, we proposed the kinetic model of acetate decomposition.

Batch removal kinetics of substrates with the fixed biofilm methane fermentation reactor was developed by using a heterogeneous model and numerical analysis was carried out. As a result it was shown qualitatively that the proposed kinetic equation may be correct.

NOMENCLATURE

A : surface area of packed media [m²]  \( R \) : reaction rate of a specific substance [mg/l/h]  
C : conc. of a specific substance in biofilm [mg/l]  
C_{1} \text{ acetate, C}_{2} \text{ propionate, C}_{3} \text{ n-butyrate, C}_{4} \text{ iso-butyrate, C}_{5} \text{ hydrogen}  
C_{p} : conc. of a specific sub. in bulk liquid [mg/l]  
C_{s} : conc. of a specific sub. in biofilm surface [mg/l]  
D_{e} : effective diffusion coeff. of a specific substance in biofilm [m²/h]  
inbiofilm:  
\( k_{1}, k_{2}, k_{3} \) : reaction rate constants [h⁻¹]  
\( k_{1,i} \) : conversion coeff. from propionate and butyrate to acetate [-]  
\( k_{2,i} \) : conversion coeff. from propionate and butyrate to hydrogen [-]  
\( K_{01} \) : decomposition rate of propionate to acetate [mg/l/h]  
\( K_{02} \) : decomposition rate of n-butyrate to propionate [mg/l/h]  
\( k_{c} \) : saturation const. of isobutyrate [mg/l]  
\( k_{m} \) : saturation const. of hydrogen to acetate [-]  
\( k_{p} \) : saturation const. of hydrogen to acetate [mg/l]  
\( k_{d} \) : mass transfer coeff. of a specific substance in diffusion layer [m/h]  
\( N_{f} \) : mass flux of a specific substance from bulk liquid to boundary layer [g/m²/h]  
\( N_{s} \) : mass flux of a specific substance from boundary layer to biofilm surface [g/m²/h]

REFERENCES