Kinetics of Carbon Dioxide Evolution in Agitation System

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Kinetics of carbon dioxide evolution was investigated in agitation system. Reaction steps of carbon dioxide evolution in submerged fermentations may consist of three steps; the first, hydration of carbon dioxide liberated from living cells, the second, dehydration of bicarbonate ions and the third, formation of carbon dioxide bubbles. Taking into account the equilibrium between hydration of carbon dioxide and dehydration of bicarbonate ions at physiological pH value, the followings may be rate-limiting steps in mass transfer of carbon dioxide in submerged fermentations, dehydration of bicarbonate ions and the bubble formation. The overall velocity constant of these two reaction steps was determined in the agitation vessel. This reaction obeyed good first-order kinetics and the term of $k HCO_3^-$ was introduced as a velocity constant. This value was influenced by agitation speed, temperature, viscosity of the fluid and carbonic anhydrase. The value of carbon dioxide coefficient ($Kd CO_2$) was higher than the oxygen absorption coefficient $Kd$. The driving force of mass transfer for carbon dioxide, $DCO_2 - pCO_2$, therefore, was lower than that for oxygen, $PB - PL$. The relationship between the overall coefficient of oxygen transfer across gas-liquid interface $K_{La}$ and the overall velocity constant of carbon dioxide evolution $k HCO_3^-$ was expressed in the formula $k HCO_3^- = 0.40(K_{La})^{0.68}$.

In our previous paper,1) we demonstrated an inhibitory effect of carbon dioxide on product formation and the ventilation effect in inosine fermentation. In these experiment, however, no detail study of the partial pressure difference of carbon dioxide between liquid phase and gas phase was performed. It was due to the reason that the partial pressure of carbon dioxide in culturing liquid was equilibrated at almost the same partial pressure in bubbles of the fermenting broth.2)

In case of the oxygen transfer in L-glutamic acid fermentation, the partial pressure of oxygen in liquid phase was shown to be much lower than that in gas phase.3) It suggests some differences between carbon dioxide and oxygen in their mass transfer mechanisms in submerged fermentations. A comparisons of different patterns of mass transfer may give a key to evaluate the suitable aeration and agitation conditions under the consideration of ventilation effect as well as oxygen transfer.

Number of physiologists have investigated the mass transfer of carbon dioxide in human blood systems and plant tissues.4-7) All of those studies, however, were carried out where the agitation strength was quite different from that in the submerged fermentations. In order to study the kinetics of carbon dioxide evolution in culturing systems, it was necessary to determine the velocity constant of carbon dioxide evolution under the same agitation conditions of submerged fermentations. A few experiments were reported on the kinetics of mass transfer of carbon dioxide in such agitation conditions. Karwat8) studied the effect of stirrer shapes on agitator action in carbonate-bicarbonate solution and Nyiri et al.9) investigated the kinetics of carbon dioxide evolution in a phosphate buffer system by a pH meter. Those studies, however, did not note the mechanisms and reaction steps of mass transfer of carbon dioxide in aeration-agitation systems for submerged culture.

Because of the equilibrium between hydra-
tion of carbon dioxide and dehydration of bicarbonate ions, the gaseous carbon dioxide liberated from living cells into culturing liquid instantly dissociated into ionic forms. The mass transfer of carbon dioxide in submerged fermentations, therefore, is defined by the following two reaction steps: the first, formation of carbon dioxide by dehydration of bicarbonate ions, the second, bubble formation of carbon dioxide in liquid phase. The kinetic study of carbon dioxide evolution can be attained by the determination of the overall velocity constant of the reactions from bicarbonate ions to gaseous carbon dioxide.

From these view-points, the velocity constant of carbon dioxide evolution from bicarbonate ions in an agitation vessel was determined and the factors affecting this reaction system were investigated. On the basis of these results, the comparative studies were made between oxygen transfer and carbon dioxide elimination in aeration-agitation systems.

MATERIALS AND METHODS

1. Reagents

Phosphate buffer. Contaminated carbon dioxide and bicarbonate ions in m/15 phosphate buffer were removed by the gassing-out method with pure nitrogen before use.

Sodium bicarbonate. Sodium bicarbonate crystals of guaranteed reagent grade (Japanese Industrial Standards) were dried over a sulfuric acid desiccator for a few days.

CMC. Carboxymethylcellulose sodium salt (Wako Pure Chemical Co.) was used.

Enzyme. Carbonic anhydrase from bovin erythrocytes (Sigma Chemicals Co.) was used, and its activity was assayed as 3100 Roughton-Booth unit/mg according to the supplier.

Water. Dissolved carbon dioxide and bicarbonate ions were removed, and the treated water was stored with the precautions to prevent contamination from air by the method described in the previous work.

2. Analysis and instruments

Bicarbonate ion concentration was analyzed by the titration method using hydrochloric acid with BPB indicator according to the instruction of Japanese Industrial Standards (JIS).

Partial pressure of carbon dioxide was measured by a mercury manometer directly connected to the gas phase of the reaction vessel or the shaken flask.

Oxygen tension in gas phase was determined by a Beckman’s Fieldlab type oxygen analyzer.

Viscosity of the fluid was measured at the reaction temperatures by a rotational viscometer Type-B (Tokyo Instrument Co. Ltd.) with rotors No. BL or No. 1 at 60 rpm.

Agitation speed was measured and monitored by a portable photoelectric tachometer Type LP-11 (Yokogawa Electric Co.).

3. Equipment

A glass vessel whose total capacity was approximately 1 liter was employed for the reaction. The liquid in the vessel was agitated by a magnet driven agitator at a constant tip velocity. The temperature of the reaction vessel in all experiments was controlled at constant degree within the deviation of 0.1°C using a water chiller Model CTR-1B (Yamato Scientific Co.) and an electric thermoregulator Thermo-Unit (Taiyo Kagaku Sangyo Co.).

In the vessel, 300 ml of phosphate buffer was placed and approximately 20 ml of sodium bicarbonate solution was added to the buffer solution through a burette connected to the top of the reaction vessel after dissolved carbon dioxide and bicarbonate ions were completely expelled by the gassing-out method with nitrogen. A rubber bellows with a carbon dioxide absorber was used to apply enough pressure to pour sodium bicarbonate solution into the reaction vessel instantly. The whole apparatus is presented in Fig. 1.

The determination of the overall velocity constant of carbon dioxide evolution for shaken flasks was carried out by the apparatus shown in Fig. 2. The baffle plates of the flask were designed as in the previous work. The flask was shaken reciprocally at 140 oscillation per minute (70 mm stroke) in a temperature regulated room.

4. Calculations

Sodium bicarbonate in aqueous solution is almost completely dissociated into ionic forms and the initial concentration of bicarbonate ions is assumed to be equal to that of added sodium bicarbonate to the phosphate buffer.

\[ [\text{HCO}_3^-] = [\text{NaHCO}_3] \]

Carbon dioxide evolves from the liquid phase follow-
The overall reaction of carbon dioxide evolution from bicarbonate ions can be written as the following formula.

$$\text{HCO}_3^- \rightarrow \text{CO}_2 + \text{OH}^- \quad (3)$$

The rate of carbon dioxide evolution in this reaction system obeys first-order kinetics and the velocity constant of dehydration of bicarbonate ions ($k_{\text{HCO}_3}$) is shown by the following equation,

$$\frac{d(CO_2)_t}{dt} = k_{\text{HCO}_3}(C_{\text{HCO}_3}_0 - (C_{\text{CO}_2}_t)) \quad (4)$$

where $(C_{\text{HCO}_3})_0$ is the concentration of bicarbonate ions at time 0 and $(C_{\text{CO}_2})_t$ is the concentration of carbon dioxide evolved resulted by dehydration of bicarbonate ions for time $t$. By intergrating equation (4), one can rearrange as follow.

$$k_{\text{HCO}_3} = \frac{1}{t} \ln \frac{(C_{\text{HCO}_3})_0 - (C_{\text{CO}_2})_t}{(C_{\text{HCO}_3})_0} \quad (5)$$

The overall velocity constant of carbon dioxide evolution in agitation system from bicarbonate ions to gaseous carbon dioxide was defined as $k'_{\text{HCO}_3}$, and it can be assumed that the value of $k'_{\text{HCO}_3}$ may be the same as $k_{\text{HCO}_3}$ determined in the above reaction system. By determination of $(C_{\text{HCO}_3})_0$ and $(C_{\text{CO}_2})_t$, $k'_{\text{HCO}_3}$ can be evaluated as follows.
The concentration of carbon dioxide evolved for reaction time $t$ can be estimated by the partial pressure increase in the closed reaction system,

$$\left( C_{\text{CO}_2} \right)_t = \frac{(p_{\text{CO}_2})_t}{22.4 \times 760} \left[ V_G \left( \frac{273}{273 + T} \right) + a V_S \right]$$

where $(p_{\text{CO}_2})_t$ is a reading of the manometer in mmHg at the time $t$, $V_G$ is the total volume of gas phase of the reaction vessel, $V_S$ is the total volume of the liquid in the reaction system, $T$ is temperature in centigrade degree and $a$ is the Bunsen's absorption coefficient of carbon dioxide. The initial concentration of bicarbonate ions can also be expressed in the function of the partial pressure of carbon dioxide,

$$\left( C_{\text{CO}_2} \right)_0 = \frac{(p_{\text{CO}_2})_0}{22.4 \times 760} \left[ V_G \left( \frac{273}{273 + T} \right) + a V_S \right]$$

where $(p_{\text{CO}_2})_0$ is the concentration of carbon dioxide evolved for infinite time of the reaction and $(p_{\text{CO}_2})_t$ is the partial pressure observed at infinite time of the reaction. The overall velocity constant of carbon dioxide evolution from bicarbonate ions in agitation system can therefore be determined by modifying the equation (6) as follows.

$$k'_{\text{HCO}_3} = -\frac{2.303}{t} \log \left( \frac{(C_{\text{HCO}_3})_t - (C_{\text{CO}_2})_t}{(C_{\text{HCO}_3})_0} \right)$$

Total volume of the reaction vessel was measured by the weight of the vessel in which water was entirely filled up and all accessories for measurement such as electrodes and a manometer were installed at the proper positions. The volume of gas phase in the vessel, $V_G$, was obtained by subtracting the liquid volume $V_S$ (the sum of the volume of phosphate buffer and bicarbonate solution added) from the total volume of the reaction vessel.

The overall velocity constant for carbon dioxide evolution in each shaken flask was evaluated in the same procedure.

The overall coefficient of oxygen transfer across gas-liquid interface $K_{L,a}$ was determined by the sulfite oxidation method. The value of $\log (P_{r}), (P_{r})_0$ was plotted against $t$ and $K_{L,a}$ was calculated by the following equation,

$$K_{L,a} = \frac{1}{22400} \frac{V_G}{V_S} \frac{273}{273 + T} \frac{H_{O_2}}{t} \log \left( \frac{P_{r}}{(P_{r})_0} \right)$$

where $H_{O_2}$ is the Henry's constant for oxygen, $1.47 \times 10^8$ atm·ml/mole, $(P_{r})_0$ is the partial pressure of oxygen in gas phase of the reaction vessel and $(P_{r})_0$ is the initial partial pressure of oxygen in gas phase 0.21 atm.

### RESULTS

1) **Effect of temperature on the overall velocity constant of carbon dioxide evolution $k'_{\text{HCO}_3}$**

The effect of temperature on the overall velocity constant of dehydration of bicarbonate ions was investigated at the revolution 2000 rpm at temperatures, 0, 10, 20, 30 and 40°C. The time courses of carbon dioxide evolution at various temperatures are presented in Fig. 3. The values of $\log \left( (p_{\text{CO}_2})_0 - (p_{\text{CO}_2})_t \right)/(p_{\text{CO}_2})_0$ are plotted against $t$ for a parameter temperature in Fig. 4.

2) **Effect of agitation on the overall velocity constant**

The effect of agitation speed on $k'_{\text{HCO}_3}$ was investigated at temperatures 0 to 30°C. The carbon dioxide evolution at these temperatures was remarkably influenced by agitation speed. The results are presented in Table I.

3) **Influence of viscosity on the overall velocity constant**

Fluid viscosity was varied by the concentration of carboxymethylcellulose (CMC). Each experiment was carried out at 30°C under agitation speed 1000 rpm. Viscosity of the fluid influenced carbon dioxide evolution as shown in Table II.
In mass transfer of carbon dioxide between gas phase and liquid phase in agitation systems, the liquid film resistance was assumed to be a controlling factor. The viscosity of the fluid might affect the mass transfer of carbon dioxide across gas-liquid interface rather than dehydration of bicarbonate ions.

4) **Effect of carbonic Anhydrase on the overall velocity constant**

The influence of carbonic anhydrase activity on the kinetics of mass transfer of carbon dioxide in agitation system was also investi-
TABLE III. VALUE OF \( K_L^a \) OF THE VESSEL USED FOR \( K_{HCO_3} \) DETERMINATION

<table>
<thead>
<tr>
<th>Temperature at 30°C.</th>
<th>( K_L^a ) value (min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>2.61</td>
</tr>
<tr>
<td>1500</td>
<td>7.06</td>
</tr>
<tr>
<td>2000</td>
<td>14.31</td>
</tr>
</tbody>
</table>

An experiment was carried out at 0°C with agitation speed 1000 rpm. Overall velocity constant of carbon dioxide evolution \( k_{HCO_3} \) with 1 unit/ml of carbonic anhydrase showed 0.411 min\(^{-1}\) at this condition. This value was almost double of the velocity constant without enzyme.

5) Effect of baffle plates in a shaken flask on the overall velocity constant.

The experiment was made at 30°C in a shaken flask. The results are shown in Fig. 5. A flask with three buffer plates gave a constant 3.77 min\(^{-1}\) and one buffer plate gave 2.83 min\(^{-1}\). Thus, buffer plates also promoted carbon dioxide evolution in the same way as in oxygen transfer.

6) Determination of \( K_L^a \) of the apparatus

Values of \( K_L^a \) of the reaction vessel were measured by the sulfite oxidation method\(^{16}\) at 30°C. Agitation speed was maintained at 1000 rpm, 1500 rpm and 2000 rpm with surface aeration. In Fig. 6, the value of \( (P_f)/P_o \) vs \( t \) is plotted on a semilog graph for a parameter agitation speed. The values of \( K_L^a \) calculated are presented in Table III.

TABLE IV. CALCULATED \( k_{HCO_3} \) AND DETERMINED \( k_{HCO_3} \)

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>( k_{CO_2-OH} \times 10^{-5} ) mole(^{-1})min(^{-1})</th>
<th>( pK_w )</th>
<th>( pK_{HCO_3} )</th>
<th>( pK_{OH^-} )</th>
<th>( K_{OH^-} \times 10^{-8} )</th>
<th>( k_{HCO_3} ) min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.65</td>
<td>14.944</td>
<td>6.552</td>
<td>8.392</td>
<td>0.78</td>
<td>0.0005</td>
</tr>
<tr>
<td>10</td>
<td>1.53</td>
<td>14.535</td>
<td>6.452</td>
<td>8.083</td>
<td>0.96</td>
<td>0.0014</td>
</tr>
<tr>
<td>20</td>
<td>3.54</td>
<td>14.167</td>
<td>6.392</td>
<td>7.775</td>
<td>3.52</td>
<td>0.0125</td>
</tr>
<tr>
<td>30</td>
<td>7.44</td>
<td>13.833</td>
<td>6.348</td>
<td>7.485</td>
<td>7.12</td>
<td>0.053</td>
</tr>
<tr>
<td>40</td>
<td>14.4</td>
<td>13.535</td>
<td>6.312</td>
<td>7.223</td>
<td>8.90</td>
<td>0.128</td>
</tr>
</tbody>
</table>

While equation (11) is rearranged as,

\[ k_{HCO_3} = k_{CO_2-OH} \times pK_{HCO_3} \]

the value of velocity constant of dehydration

\[ k_{HCO_3} = k_{CO_2-OH} \times pK_{HCO_3} \]

Table IV. Calculated \( k_{HCO_3} \) and Determined \( k_{HCO_3} \)

* These were determined by Pinsent et al.

DISCUSSION

As stated in the introduction, the mass transfer of carbon dioxide in submerged fermentation at physiological pH value is seemed to be a sort of the dehydration of bicarbonate ions. The kinetics of carbon dioxide evolution in submerged fermentations is therefore defined by the velocity constant of dehydration of bicarbonate ions. This reaction is written as following to the formula (3),

\[
\text{HCO}_3^- \xrightarrow{k_{CO_2-OH}} \text{CO}_2 + \text{OH}^- \quad (3)
\]

where \( k_{CO_2-OH} \) is the velocity constant of hydration of carbon dioxide. The equilibrium constant of the above reaction is given by the following equation,

\[
k_{OH^-} = \frac{k_{HCO_3}}{k_{CO_2-OH}} = \frac{C_{CO_2} \cdot C_{OH^-} \cdot \gamma_{HCO_3}}{C_{HCO_3} \cdot \gamma_{HCO_3}} \quad (11)
\]

where \( \gamma_{HCO_3} \) is the activity coefficient of bicarbonate ions. Since \( K_{HCO_3} = C_{H^+} \cdot C_{HCO_3} \), \( \gamma_{HCO_3} / C_{CO_2} \) and \( K_w = C_{H^+} \cdot C_{OH^-} / C_{H_2O} \), the equation (11) yields,

\[
k_{OH^-} = \frac{K_w \cdot C_{H_2O}}{K_{HCO_3}} \quad (12)
\]

where \( K_{HCO_3} \) is the first stage dissociation constant for carbon dioxide and \( K_w \) is dissociation constant of water. By rewritten in logarithum, equation (12) can be modified as below.

\[
pK_{OH^-} = pK_w - pK_{HCO_3} \quad (13)
\]

While equation (11) is rearranged as,

\[
k_{HCO_3} = k_{CO_2-OH} \cdot K_{OH^-} \quad (14)
\]
of bicarbonate ions is evaluated by the following procedure. As the value of $pK_{OH}$ is obtained by substituting $pK_w$ and $pK_{HCO_3}$ into equation (13), $k_{HCO_3}$ is estimated by using $k_{CO_2,OH}$. The value of $k_{CO_2,OH}$ was determined by the Roughton’s continuous flow thermal method. The results of this calculation are shown in Table IV in contrast with the overall velocity constant of carbon dioxide evolution in agitation systems obtained in this work. The Roughton’s thermal method did, however, not apply agitation in the reaction system, the difference of agitation strength may result the immense gap between $k_{HCO_3}$ and $k_{HCO_3}$.

The rate of carbon dioxide evolution in submerged fermentations may be expressed in the following formula,

$$r_e = K'_{HCO_3}(C_{CO_2}^* - C_{CO_2})$$  \hspace{1cm} (15)

where $C_{CO_2}^*$ is the concentration of carbon dioxide in liquid phase and $C_{CO_2}$ is the concentration of carbon dioxide in bubbles of the fermenting broth. Since the concentration of gas is expressed in the functions of partial pressure and Henry’s constant, equation (15) can be rearranged as follows,

$$r_e = \frac{k'_{HCO_3}}{H_{CO_2}}(DCO_2 - pCO_2)$$  \hspace{1cm} (16)

where $H_{CO_2}$ is Henry’s constant for carbon dioxide, $DCO_2$ is carbon dioxide tension in liquid phase and $pCO_2$ is the partial pressure of carbon dioxide in bubbles. The carbon dioxide evolution coefficient ($Kd_{CO_2}$) was introduced to express the mass transfer of carbon dioxide in biological systems analogously to oxygen transfer. The oxygen absorption rate in biological systems is indicated by the following equations,

$$r_{ab} = Kd(P_B - P_L)$$  \hspace{1cm} (17)

$$Kd = \frac{K_{L,a}}{H_{O_2}}$$  \hspace{1cm} (18)

where $Kd$ is the oxygen absorption coefficient, $K_{L,a}$ is the overall coefficient of mass transfer of oxygen across gas-liquid interface and $H_{O_2}$ is Henry’s constant for oxygen. The carbon dioxide evolution coefficient ($Kd_{CO_2}$) is written in the same manner.

$$Kd_{CO_2} = \frac{k'_{HCO_3}}{H_{CO_2}}$$  \hspace{1cm} (19)

In the culturing systems whose pH value is maintained at a constant level, the carbon dioxide evolution rate $r_e$ is approximately equal to the oxygen absorption rate $r_{ab}$ as far as the respiratory quotient of microorganisms held at unity. Thus, the relationship of mass transfer between carbon dioxide and oxygen in biological systems is expressed as follows.

$$Kd(P_B - P_L) = (Kd_{CO_2})(DCO_2 - pCO_2)$$  \hspace{1cm} (20)

$$\frac{K_{L,a}}{H_{O_2}}(P_B - P_L) = \frac{k'_{HCO_3}}{H_{CO_2}}(DCO_2 - pCO_2)$$  \hspace{1cm} (21)
The ratio of the driving force for mass transfer of carbon dioxide to that of oxygen is therefore evaluated by the following equation.

\[
\frac{\Delta CO_2 - \rho CO_2}{P_B - P_L} = \frac{Kd}{(Kd)CO_2} = \frac{K_L^{CO_2} - HCO_3^-}{K_L^{HCO_3^-} - H_2O} \quad (22)
\]

The driving force of carbon dioxide evolution in biological systems can be estimated by substituting the values obtained in this work into equation (22). The results in Table V show that the partial pressure difference of carbon dioxide between gas phase and liquid phase is almost one-tenth of that of oxygen. This fact supports our previous experimental results that the partial pressure of carbon dioxide in liquid phase was approximated to that in gas phase. It might be impossible to detect precisely a small difference of the partial pressures by the employed instruments.

The overall velocity constant of carbon dioxide evolution \( k_{HCO_3^-} \) was proportional to agitation strength in analogy with the overall coefficient of oxygen transfer across gas-liquid interface \( K_{L^*} \). The following relationship was obtained from the slope of the graph in Fig. 7.

\[
k_{HCO_3^-} = 0.40(K_{L^*})^{0.68} \quad (23)
\]

This function indicates the relationships of mass transfer between oxygen absorption and carbon dioxide evolution in submerged fermentations.

Analysis was also made for the evaluation of the kinetics of carbon dioxide absorption. The kinetics of carbon dioxide absorption in agitation system was studied by Karwat,\(^9\) Yoshida et al.,\(^22\) Gibbs et al.,\(^20\) and Aiba et al.\(^23\)

According to the formula (3), hydration of carbon dioxide (carbon dioxide absorption) may follow to the second-order kinetics equation below,

\[
\frac{d(C_{HCO_3^-})}{dt} = k_{CO_2,OB}[(C_{CO_2})_0 - (C_{HCO_3^-})_t][C_{OH^-}_0 - C_{HCO_3^-}_t] \quad (24)
\]

where \((C_{CO_2})_0\) is the concentration of carbon dioxide at time 0, \((C_{HCO_3^-})_t\), is the concentration of bicarbonate ion formed for time t and \((C_{OH^-})_0\) is the initial concentration of hydroxide ions. This was confirmed by Kiese et al.,\(^24\) Sirs\(^25\) and Pinsent et al.\(^21\) Hatta also proposed that the chemical absorption of carbon dioxide followed second-order kinetics\(^26\) and Gibbs et al. confirmed that the velocity constant of carbon dioxide absorption in turbulent system was concentration dependent.\(^29\) Hence, molarity of formed bicarbonate ion concentration resulted by hydration of carbon dioxide is equal to the variation of hydrogen ion concentration, equation (24) can be modified,

\[
\frac{d(C_{HCO_3^-})}{dt} = k_{CO_2,OH}[(C_{CO_2})_0 - (C_{HCO_3^-})_t][C_{OH^-}_0 - (C_{H^+})_t] \quad (25)
\]

where \((C_{H^+})_t\) is the concentration of hydrogen ion formed for time t resulted by hydration of carbon dioxide. Assuming that carbon dioxide absorption is carried out in pH-stat, \((C_{H^+})_t\), can be neglected against \((C_{OH^-})_0\) and equation (25) may be approximated in the following formula,

\[
\frac{d(C_{HCO_3^-})}{dt} = k_{CO_2,OH}(C_{OH^-})_0[(C_{CO_2})_0 - (C_{HCO_3^-})_t] = k_{CO_2,OH}[(C_{CO_2})_0 - (C_{HCO_3^-})_t] \quad (26)
\]
where $k'_{\text{CO}_2\text{OH}}$ is the apparent velocity constant of carbon dioxide absorption including the concentration of hydroxide ions in the reaction system. One shows that carbon dioxide absorption follows pseudo-first-order kinetics in a pH-stat reaction system or in a buffer system. Thus, the overall velocity constant of carbon dioxide absorption in an agitated pH-stat can be indicated by substituting equations (11) and (12) into equation (26).

$$
k'_{\text{CO}_2\text{OH}} = k'_{\text{HCO}_3} \cdot K_{\text{HCO}_3} \cdot C_{\text{OH}^-} K_{\text{H}_2\text{O}} \tag{27}
$$

Since $K_w$ is dissociation constant of water, equation (27) can be modified,

$$
k'_{\text{CO}_2\text{OH}} = k'_{\text{HCO}_3} \cdot \frac{K_{\text{HCO}_3}}{(C_{\text{H}^+})_0} = k'_{\text{HCO}_3} \cdot \text{antilog} (\text{pH} - pK_{\text{HCO}_3}) \tag{28}
$$

where $(C_{\text{H}^+})_0$ is the hydrogen ion concentration of the reaction system. The relationship between the kinetics of oxygen absorption and that of carbon dioxide absorption in pH-stat agitation system can be expressed in the following equation by substituting equation (23) into equation (28).

$$
k'_{\text{CO}_2\text{OH}} = 0.40(K_{L\text{CO}_2})^{0.68} \cdot \text{antilog} (\text{pH} - pK_{\text{HCO}_3}) \tag{29}
$$

Determination of the velocity constant of carbon dioxide absorption in pH-stat agitation system has not been reported except Karwat's KHCO$_3$-K$_2$CO$_3$ buffer system.$^6$ Karwat, however, presented his results without the information of pH values and temperature of his work. Velocity constant of carbon dioxide absorption of his system was $3 \sim 6 \text{Kmole/m}^3\cdot\text{hr} \cdot \text{atm}$ at aeration rate of 2.5 m$^3$/hr, 4 $\sim$ 10 K mole/m$^3\cdot\text{hr} \cdot \text{atm}$ at 5 m$^3$/hr and 7 $\sim$ 12 K mole/m$^3\cdot\text{hr} \cdot \text{atm}$ at 10 m$^3$/hr, respectively, under various agitation strength. According to the estimation of $k'_{\text{HCO}_3}$ of Karwat's system by equation (28), his results were almost good approximation with our results without detail information on his experimental conditions. As shown in Table VI, the relationship between oxygen absorption and carbon dioxide absorption for Karwat's system was estimated as $k'_{\text{CO}_2\text{OH}} = 0.40(K_{L\text{CO}_2})^{0.75}$ at aeration rate 2.5 m$^3$/hr. This was also fairly good approximation with our results.

Mass transfer of carbon dioxide in blood system is performed by the action of carbonic anhydrase. It was also reported that this enzyme was distributed in a few bacteria.$^{27-29}$ If the microorganisms employed for fermentation process possess carbonic anhydrase, mass transfer of carbon dioxide in culturing systems may effect with this enzyme. Our experimental results confirmed that this enzyme remained its activity in agitation system. The effect of carbonic anhydrase, however, was seemed to be not very important at the agitation strength and temperatures of usual culturing conditions for submerged fermentations.

**NOMENCLATURE**

- $C_{\text{CO}_2} = \text{concentration of carbon dioxide in bubbles of the culturing liquid, mole/ml}$
- $C_{\text{CO}_2}^+ = \text{concentration of carbon dioxide in the culturing liquid, mole/ml}$
- $C_{\text{H}^+} = \text{hydrogen ion concentration, mole/liter}$
- $C_{\text{HCO}_3^-} = \text{bicarbonate ion concentration, mole/liter}$
\begin{align*}
C_{\text{H}_2\text{O}} &= \text{molarity of water, mole/liter} \\
C_{\text{OH}^-} &= \text{hydroxide ion concentration, mole/liter} \\
(C_{\text{CO}_2})_0 &= \text{concentration of carbon dioxide for hydration at time 0, mole/liter} \\
(C_{\text{CO}_2})_t &= \text{concentration of carbon dioxide evolved for time } t \text{ by hydration of bicarbonate ions, mole/liter} \\
(C_{\text{CO}_2})_\infty &= \text{concentration of carbon dioxide evolved for infinite time of hydration of bicarbonate ions, mole/liter} \\
(C_{\text{H}^+})_0 &= \text{hydrogen ion concentration of a pH-stat reaction system for carbon dioxide absorption, mole/liter} \\
(C_{\text{H}^+})_t &= \text{concentration of hydrogen ions formed by hydration of carbon dioxide for time } t, \text{ mole/liter} \\
(C_{\text{HCO}_3}^-)_0 &= \text{concentration of bicarbonate ions for hydration at time 0, mole/liter} \\
(C_{\text{HCO}_3}^-)_t &= \text{concentration of bicarbonate ions formed by hydration of carbon dioxide for time } t, \text{ mole/liter} \\
(C_{\text{OH}^-})_0 &= \text{hydroxide ion concentration of the reaction system for carbon dioxide absorption, mole/liter} \\
\Delta C_{\text{CO}_2} &= \text{carbon dioxide tension in culturing liquid, atm} \\
H_{\text{CO}_2} &= \text{Henry's constant of carbon dioxide, ml-atm/mole} \\
H_{\text{O}_2} &= \text{Henry's constant of oxygen, ml-atm/mole} \\
[H_{\text{CO}_3}^-] &= \text{molarity of bicarbonate ions, mole/liter} \\
k_{\text{CO}_2,\text{OH}} &= \text{velocity constant for hydration of carbon dioxide } 1/\text{mole-min} \\
k'_{\text{CO}_2,\text{OH}} &= \text{apparent velocity constant of carbon dioxide absorption in pH-stat agitation system, 1/min} \\
k_{\text{HCO}_3} &= \text{velocity constant for dehyration of bicarbonate ions, 1/min} \\
k'_{\text{HCO}_3} &= \text{overall velocity constant of carbon dioxide evolution from bicarbonate ions in agitation system, 1/min} \\
K_d &= \text{oxygen absorption coefficient, mole/ml-min-atm} \\
K_{\text{HCO}_3} &= \text{first stage dissociation constant of carbon dioxide.} \\
K_{\text{L},\text{O}} &= \text{overall coefficient of oxygen transfer across gas-liquid interface, 1/min} \\
K_{\text{OH}} &= \text{equilibrium constant of dehyration of bicarbonate ions.} \\
K_w &= \text{dissociation constant of water.} \\
(K_d)_{\text{CO}_2} &= \text{carbon dioxide evolution coefficient, mole/ml-min-atm} \\
[\text{NaHCO}_3] &= \text{molarity of sodium bicarbonate, mole/liter} \\
pC_{\text{O}_2} &= \text{partial pressure of carbon dioxide in bubbles of the culturing liquid, atm} \\
pH &= \text{index of hydrogen ion concentration} \\
pK_{\text{HCO}_3} &= \text{index of first stage dissociation constant of carbon dioxide} \\
pK_{\text{OH}} &= \text{index of equilibrium constant of dehyration of bicarbonate ions} \\
pK_w &= \text{index of ionic product of water.} \\
P_{\text{ab}} &= \text{partial pressure of oxygen in bubbles of the culturing liquid, atm} \\
P_{\text{L}} &= \text{partial pressure of oxygen in the culturing liquid, atm} \\
(pC_{\text{O}_2})_t &= \text{partial pressure of carbon dioxide evolved at time } t, \text{ mmHg} \\
(pC_{\text{O}_2})_\infty &= \text{partial pressure of carbon dioxide evolved at infinite time, mmHg} \\
(P_{\text{t}})_0 &= \text{partial pressure of oxygen at time 0 in sulfite oxidation, atm} \\
(P_{\text{t}})_t &= \text{partial pressure of oxygen at time } t, \text{ atm} \\
r_{\text{ab}} &= \text{oxygen absorption rate, mole/ml-min} \\
r_e &= \text{carbon dioxide evolution rate, mole/ml-min} \\
t &= \text{reaction time, min} \\
T &= \text{temperature in centigrade degree, } ^\circ \text{C} \\
V_o &= \text{total volume of gas phase of the reaction system, ml} \\
V_s &= \text{total volume of liquid phase of the reaction system, ml} \\
\alpha &= \text{Bunsen's absorption coefficient of carbon dioxide ml/ml} \\
\gamma_{\text{HCO}_3} &= \text{activity coefficient of bicarbonate ions} \\
\end{align*}

\textbf{REFERENCES}