Determination of Dissolved Hydrogen Concentration and $[K_La]_{H_2}$ in Submerged Culture Vessels[†]

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The determination of dissolved hydrogen concentration (C_{H_2}) in the culture medium of hydrogen bacteria was performed. Dissolved hydrogen in the medium was stripped by bubbling nitrogen and thus stripped hydrogen was quantitatively determined by a gas chromatograph. C_{H_2} values similar to Bunsen's absorption coefficients were observed by this method. In addition, volumetric hydrogen transfer coefficients, $[K_La]_{H_2}$ were calculated under various conditions based on the observed C_{H_2} values. $[K_La]_{H_2}$ values thus obtained were found to be similar to the estimated $[K_La]_{H_2}$ values according to Wilke's equation and $[K_La]_{O_2}$ values.

Many studies have been performed on the cultivation of hydrogen bacteria.^{1~4)} However, there is no report on the determination of dissolved hydrogen concentration during cultivation of hydrogen bacteria perhaps because no appropriate methods, such as an electrochemical one, has developed. Dissolved gas can be normally determined by combustion method or by measuring the decrease in volume of the absorbed gas. In the case of hydrogen, however, due to the low gas solubility, these methods are judged to be insufficiently accurate. Robra *et al.* calculated $[K_La]_{H_2}$ values on the basis of the utilization of hydrogen and oxygen in different ratios by *Hydrogenomonas eutropha* cells.⁵⁾

$[K_La]_{H_2}$ values thus calculated, however, are not fully applicable to the cultivation of other hydrogen bacteria. Therefore, determination of $[K_La]_{H_2}$ is to be performed in the system without hydrogen bacteria cells. The purpose of this paper is firstly to establish a simple and accurate method for determination of C_{H_2} in cultivation vessels and secondarily, to investigate $[K_La]_{H_2}$ under various gassing conditions.

MATERIALS AND METHODS

Determination of C_{H_2} . Jar fermentor (Labotec Co.

Type LF-20) used throughout this study consisted of 2-liter Pyrex vessel of internal diameter 130 mm, having three baffles and a four-bladed turbine type impeller (Fig. 1). The working volume of the jar was 1 liter. Liquid samples dissolving hydrogen were taken directly from the jar fermentor and dropped into another apparatus filled in advance with hydrogen gas in order to eliminate hydrogen bubbles (Fig. 2). Thus dropped samples were taken again from this apparatus by means of a syringe to examine dissolved hydrogen concentration. These sampling procedures were performed quickly. The stripping of dissolved hydrogen from the samples was done with the aid of the apparatus shown

FIG. 1. Structure of the Jar Fermentor.
The unit of the numbers in the figure represents mm.

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FIG. 2 Sampling Device for the Determination of Dissolved Hydrogen Concentration

The unit of the numbers in the figure represents mm.

FIG. 3 Apparatus for the Stripping of Hydrogen

1. N ₂ Bomb	2. Reducing valve
3. Flow controller	4. Flow meter
5. Stopcock	6. Stripping tube
7. Gas burette	8. Water jacket
9. Injection port	10. Gas-mixing apparatus

in Fig. 3. Samples taken with a syringe were poured into the stripping tube and bubbled by sufficient volume of nitrogen to strip dissolved hydrogen. Thus stripped hydrogen was collected in a gas burette, mixed thoroughly determined with a Shimadzu gas chromatograph (Type GC-3AH) in terms of standard calculation curve for hydrogen (Fig. 4). Analytical conditions were the same as in the previous paper,⁶⁾ with this gas chromatographic method, the determination of as little as 10^{-5} ml (30°C, 1 atm.) of hydrogen was possible.

Determination of $[K_{La}]_{H_2}$. The principle of the determination of $[K_{La}]_{H_2}$ is based on measuring the increase in dissolved hydrogen concentration in jar fermentor. Figure 5 shows the apparatus for dissolving hydrogen gas into water. After the jar fermentor containing 1 liter of deionized water was left

FIG. 4. Standard Curve for C_{H₂} Determination.

Shimadzu gas-chromatograph GC-3AH

Column: Dual column; Molecular sieve 5A, 3 m
Active charcoal, 1 m

Temperature: 35°C Carrier gas rate: N₂ 10 ml/min
Detector: TCD Range: 1 mV

FIG. 5. Apparatus for Dissolving Hydrogen.

1. H ₂ Bomb	2. Reducing valve
3. Flow controller	4. Flow meter
5. Stopcock	6. Jar fermentor
7. Sampling tube	8. Gas inlet
9. Vacuum pump	10. Manometer
11. Gas outlet	

under reduced pressure (at 30 mm Hg abs.) for 30 min to ensure the removal of dissolved gases, hydrogen was introduced to the gaseous phase till an inside gas phase pressure of 1 atm. abs. was reached. Agitation and bubbling by hydrogen were immediately initiated and C_{H₂} was determined at regular intervals. Absorption of hydrogen was carried out at 30°C. Agitation speeds were 200~1000 rpm and the flow rate of hydrogen was 1 liter/min, measured by calibrated flow meters. $[K_{La}]_{H_2}$ was calculated according to the following equation.

$$K_{La}(t-t_0) = \ln \left(\frac{(C_{H_2})_1 - (C_{H_2})_0}{(C_{H_2})_1 - C_{H_2}} \right)$$

Where

K_{La} : volumetric mass transfer coefficient (hr⁻¹)
t : time (hr)

- t_0 : time at which hydrogen supply was initiated (hr)
 C_{H_2} : dissolved hydrogen concentration (ml H_2 /ml H_2O)
 $(C_{H_2})_i$: saturated dissolved hydrogen concentration (ml H_2 /ml H_2O)
 $(C_{H_2})_0$: C_{H_2} at time t_0

Determination of volumetric oxygen transfer coefficient, $[K_{La}]_{O_2}$. $[K_{La}]_{O_2}$ values were calculated by sodium sulfite oxidation method and dissolved oxygen analysing method in terms of oxygen analyser (Beckman Type 778). When the dissolved oxygen in the jar fermentor reached zero by bubbling of nitrogen, aeration of air instead of nitrogen and agitation were initiated. $[K_{La}]_{O_2}$ values by dissolved oxygen analysing method were calculated based on dissolved oxygen concentration. Operation conditions of the jar fermentor were the same as those for the determination of $[K_{La}]_{H_2}$ except for the agitation speeds were 400, 600 and 800 rpm in dissolved oxygen analysing method. Approximately the same $[K_{La}]_{O_2}$ values were obtained by either method.

RESULTS

Measurement of stripping efficiency

Five milliliter aliquots of deionized water saturated with hydrogen at 30°C were bubbled by nitrogen gas at the flow rate of 100 ml/min. The dependence of the data of dissolved hydrogen concentration on the volume of nitrogen used for stripping is shown in Table I together with stripping efficiency. Stripping efficiency $(= (\text{Observed } C_{H_2} / \text{Bunsen's absorption coefficient}) \times 100)$ reached was almost maximum by the stripping with 200 ml nitrogen.

TABLE I. MEASUREMENT OF STRIPPING EFFICIENCY

Total volume of N_2 used for stripping (ml)	Dissolved hydrogen concentration (ml H_2 /ml solvent)	Stripping efficiency (%)
100	0.017	90
200	0.018	95
300	0.018	96
400	0.018	96

Stripping efficiency $= (\text{Observed } C_{H_2} / \text{Bunsen's absorption coefficient}) \times 100$

N_2 gas flow rate : 100 ml/min

Sample : pure water saturated with hydrogen at 30°C
 (0.019 ml H_2 /ml solvent)

Volume : 5 ml

Total volume of hydrogen in the sample: 0.095 ml

Measurement of the dissolved hydrogen concentration

Dissolved hydrogen concentrations of deionized water and 5 M aqueous solution of sodium chloride both saturated with hydrogen were measured by the above-mentioned method. Shaking flasks and a jar fermentor were used as reaction vessels in which hydrogen absorption occurred. Shaking flasks containing sample liquid were degassed at 30 mm Hg abs. for 30 min and then filled with hydrogen at a total pressure of 1 atm. abs. After the sufficient shaking for hydrogen absorption on a reciprocal shaker, dissolved hydrogen concentration was measured. In the case of the jar fermentor, sample liquid was bubbled by hydrogen and agitated at the agitation speed of 200, 600 and 1000 rpm. After the sufficient bubbling and agitation for hydrogen absorption, C_{H_2} was measured. Recovery $(= (\text{Observed } C_{H_2} / \text{Bunsen's absorption coefficient}) \times 100)$ was higher than 95% under any absorption conditions (Table II).

TABLE II. MEASUREMENT OF THE DISSOLVED HYDROGEN CONCENTRATION

Absorption conditions	Reaction vessels and agitation	Dissolved hydrogen concentration (C_{H_2}) (ml· H_2 /ml·solvent)	Recovery (%)
Solvent: pure water	A	0.18	96
Temperature: 30°C	B	0.18	96
P_{H_2} : 1 atm.	C	0.18	96
	D	0.18	96
Solvent: aqueous solution of NaCl (5 M)	A	0.065	98
	B	0.066	99
Temperature: 15°C	C	0.067	101
P_{H_2} : 1 atm.	D	0.067	101

Recovery $= (\text{Observed } C_{H_2} / \text{Bunsen's absorption coefficient}) \times 100$

A : Shaking flask

B : Jar fermentor 200 rpm

C : Jar fermentor 600 rpm

D : Jar fermentor 1000 rpm

Correlation between $[K_{La}]_{H_2}$ and agitation speed

As a result of the experiments performed at various agitation speeds, an approximately linear relationship was obtained between the agitation speed and observed $[K_{La}]_{H_2}$ on log-log

FIG. 6. Correlation between $[K_L a]_{H_2}$ and Agitation Speed.

graph paper (Fig. 6). Uemura *et al.*⁷⁾ reported that the transfer rate of gaseous hydrocarbon was able to be estimated from that of oxygen. Inferring their data, mass transfer coefficients for gaseous substrates can be estimated from $[K_L a]_{O_2}$ based on the assumptions that (1) the gas composition does not affect "a" value under the same agitation speed and gas flow rate, (2) K_L value is proportional to D or $D^{1/2}$ (D : diffusion coefficient). Diffusion coefficients are available in the literature,⁸⁾ or they can also be calculated according to the following equation presented by Wilke *et al.*⁹⁾:

$$D = 7.4 \times 10^{-8} (T(2.6M)^{0.5} / \eta V^{0.6})$$

Where

D = diffusion coefficient, cm^2/sec

T = temperature, $^{\circ}\text{K}$

M = molecular weight of solvent

V = molal volume of solute at normal boiling point, $\text{ml/g} \cdot \text{mole}$

η = viscosity of solution, centipoise

$[K_L a]_{H_2}$ values observed and estimated from $[K_L a]_{H_2}$ values under various conditions are shown in Table III. Observed $[K_L a]_{H_2}$ values were similar to estimated ones from the assumption that K_L value is proportional to $D^{1/2}$ in which D is calculated according to Wilke's equation.

DISCUSSION

The great difficulty to be overcome in measuring dissolved gas concentration is to eliminate the influence of gas bubbles. In the

presence of bubbles, dissolved gas concentration is unsuccessfully measured by stripping method. Especially, under various conditions of agitation and aeration, measurement of dissolved gas concentration is very difficult due to the presence of various levels of bubbles in the sample liquids. By the method using the sampling device shown in Fig. 2, most of the bubbles, large bubbles in particular, could be eliminated from sample liquids. Prior to employing the sampling device, the apparatus receiving sample liquids from the jar fermentor should be sufficiently cleaned and the sampling procedures must be performed very quickly. In stripping process, dissolved hydrogen stripped by nitrogen was collected in the gas burette containing a certain amount of water. However, the loss of stripped hydrogen arising from dissolving into water seems to be negligible due to the low solubility of hydrogen. In analytical procedures by means of gas chromatographic technique, detectors were operated at high sensitivity. Standard calibration curves for hydrogen, therefore, were to be obtained each time analyses were performed. A higher-efficient gas-chromatogram is preferably used for such an analysis like this. Initial concentration of dissolved hydrogen, $(C_{H_2})_0$ was 2×10^{-4} ($\text{ml} \cdot \text{H}_2 / \text{ml} \cdot \text{solvent}$) under the operation conditions employed. $[K_L a]_{H_2}$ values were calculated only in such cases that plots of $\log C_{H_2}$ vs. $\log t$ gave a straight line. From the comparison of observed and estimated $[K_L a]_{H_2}$ values listed in Table III, it seems reasonable to assume K_L value to be proportional to $D^{1/2}$ rather than to D itself as previously described concerning K_L of propane and oxygen,¹⁰⁾ for $[K_L a]_{H_2}$ values estimated from the former assumption were more similar to observed values than those from the latter case. It may suggest that Higbie's "penetration theory" or "surface renewal theory" of Danckwerts is valid with respect to K_L . As to the values of diffusion coefficient used for the estimation of $[K_L a]_{H_2}$, those calculated according to Wilke's equation generally gave $[K_L a]_{H_2}$ more similar to observed values than those being referred to the literature. As a

TABLE III. COMPARISON BETWEEN OBSERVED AND ESTIMATED $[K_L a]_{H_2}$

A 2 liter-jar fermentor containing 1 liter of water was used. Gas flow rate was 1 vvm. for both hydrogen and air.

rpm	Observed [K _{La}] _{O₂} (hr ⁻¹)	Estimated [K _{La}] _{H₂} (hr ⁻¹)				Observed [K _{La}] _{H₂} (hr ⁻¹)
		A		B		
		a	b	a	b	
400	105	245	155	161	128	104
600	230	538	340	352	280	250
800	400	936	592	612	488	460
1000	620	1450	918	949	756	869

A, Estimated assuming that K_L is proportional to D ; B, Estimated assuming that K_L is proportional to $D^{1/2}$; a, Estimated from D of the literature; b, Estimated from D calculated according to Wilke's equation.

result, the estimation with the assumption that K_L is proportional to root of D calculated according to Wilke's equation gave the most similar $[K_L a]_{H_2}$ values to observed ones, although the former values were a little higher at low agitation while a little lower at vigorous agitation than the latter values.

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