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Studies on Dissolved Hydrogen Behavior in Autotrophic Culture of *Alcaligenes eutrophus* ATCC 17697^T

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The partial pressure of dissolved hydrogen was measured to investigate the behavior of dissolved hydrogen during the autotrophic growth of the hydrogen-oxidizing bacterium, *Alcaligenes eutrophus* ATCC 17697^T. A dissolved hydrogen sensor was constructed by modifying a Clark type dissolved oxygen probe. By using this sensor the overall volumetric coefficient of hydrogen mass transfer, $(K_La)_{H2}$, was determined in the culture system and compared with that of oxygen, $(K_La)_{O2bioI}$. Good straight line relationships were demonstrated between (1) $\ln(K_La)_{O2bioI}$. and $\ln(K_La)_{O2sulI}$.(K_La for oxygen determined by sulphite oxidation) and (2) $\ln(K_La)_{O2bioI}$. and $\ln(K_La)_{H2}$. The critical partial pressure for dissolved hydrogen was shown to be 11.6kPa, while that for oxygen was 3.17 kPa. The *Kd* (volumetric absorption coefficient) values for hydrogen and oxygen were determined from the respective K_La's under a range of operating conditions and whereas the K_La values for hydrogen were greater than those for oxygen the *Kd* values were very similar.

INTRODUCTION

Poly-β-hydroxybutyric acid (PHB) is a raw material for the manufacture of biodegradable plastics. The hydrogen-oxidizing bacterium, *Alcaligenes eutrophus*, accumulates various kinds of polyhydroxyalcanoates (Doi *et al.*, *1988;* Kunioka *et al.*, 1989) and is capable of producing PHB from carbon dioxide autotrophically (Ishizaki and Tanaka, 1991). Microbial PHB production from carbon dioxide is an attractive process because it may help to solve two difficult environmental problems: the increase in carbon dioxide concentration in the atmosphere and plastic pollution.

We have developed a recycled-gas closed-circuit culture system for the production of PHB from high cell density cultures of *A. eutrophus* ATCC 17697^T growing on carbon dioxide and hydrogen under aerobic conditions (Ishizaki and Tanaka, 1991; Ishizaki and Tanaka, 1990). However, there is an inherent danger of explosion associated with this process and it is vital that the composition of the gas phase is strictly controlled to ensure that the ratio of oxygen to hydrogen is not within the detonation range. According to the stoichiometry of this system (Ishizaki and Tanaka, 1991; Ishizaki and Tanaka, 1990) the ratio of $H_2 : O_2 : CO_2$ required for cell growth is 21.36: 6.21: 4.09 and that for PHB accumulation is 33: 12: 4. Although this composition is within the detonation range, the composition of the gas phase in a fermentation would be determined by the relative mass transfer capacities of the different gasses, the critical concentration partial pressures of the dissolved gasses and the effect of the partial

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pressures of the individual gasses on the physiology of the organism. Thus, to determine the possible gas composition it is necessary to study the behavior of the substrate gasses in the culture system. The mass transfer kinetics and behavior of oxygen and carbon dioxide have been well documented (Ishizaki and Tanaka, 1991; Ishizaki and Tanaka, 1990; Ishizaki and Hirose, 1973; Ishizaki et al., 1973). However, although there are many reports measuring dissolved hydrogen concentration (Kodama et al., 1976; Shiegel and Ollis, 1984; Heinzle and Laffety, 1980; Pauss et al., 1990; Kuroda et al., 1991; Sweet et al., 1980; Niedrach and Stoddard, 1982; Kurosawa and Hagihara, 1986; Oki et al., 1979) very little data are available on the determination of the mass transfer kinetics of hydrogen and its behavior in a fermentor.

In this paper the development of a dessolved hydrogen sensor is reported and the kinetics of hydrogen mass transfer in the fermentation system compared with that of oxygen. The problem of obtaining high PHB productivity from hydrogen under safe operating conditions is also discussed.

MATERIALS AND METHODS

Cultivation and analyses

The strain, medium preparation and analytical methods were as previously reported (Ishizaki and Tanaka 1991; Ishizaki and Tanaka, 1990).

Dissolved hydrogen sensor

The dissolved hydrogen sensor is shown in Fig. 1 and was developed by modifying a

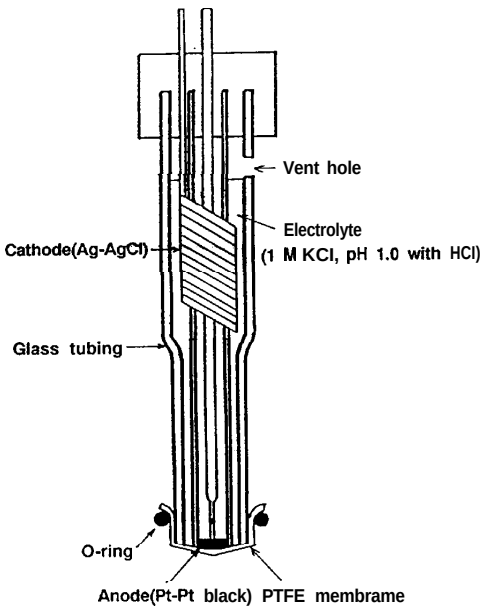

Fig. 1. Cross section view of the dissolved hydrogen sensor.

Clark type oxygen sensor, DG-5P, 134 mm long and 10 mm diameter (Biott Co., Ltd., Tokyo), using a very similar method to that reported by previous workers (Niedrach and Stoddard, 1982; Kurosawa and Hagihara 1986). The sensor consisted of a silver/silver chloride cathode and a platinum/platinum black anode with 1 M KCl (pH 1.0 with HCl) as the internal electrolyte. To coat the spiral Ag electrode with AgCl, the probe was filled with a 1 M KC1 solution and electrolyzed at DC 10 mA for 1 d using a platinum plate as a counter electrode. The solution was then discarded and the inside of the probe rinsed with 1 M KCl. Similarly, to coat the plane disc Pt electrode with platinum black (Pt black) the tip of the probe was dipped into a 3% hydrogen hexachloroplatinate (IV) solution without lead acetate and was electrolyzed at DC 5.0 V for 1.5 min using a platinum plate as a counter electrode. The probe was then rinsed with 1 M KCl. The tip of the sensor was covered with a 2.54 μm thick polytetrafluoroethylene membrane which was fixed to the sensor with 3 O-rings (i.d. 3.7 mm and o.d. 7.5 mm) and silicone lubricant SH-103 (TORAY DOW CORNING, Tokyo) as a sealing material. Finally, the sensor was filled with 1 M KC1 electrolyte, the pH of which had been adjusted to 1.0 with HCl to ensure that the sensor would not be affected by pH changes.

Instrumentation for the hydrogen sensor

The electrical circuit (Fig. 2) was designed to generate a polarizing potential of DC 550 mV between the Pt-Pt black anode (sensing electrode) and the Ag-AgCl cathode (counter electrode) to achieve hydrogen oxidation (Kuroda et al., 1991; Niedrach and Stoddaed, 1982). The polarizing potential was continuously monitored by a digital multi tester, model 7532-01 (YOKOGAWA Instruments Co., Ltd., Tokyo) with a high input impedance of more than 10 MΩ. The output current from the sensor was converted into a voltage signal by a 101.3 Ω resistor and the voltage value recorded on a recorder, PRP-5021 (TOA Electronics Ltd., Tokyo) with a high input impedance of more than 1 MΩ.

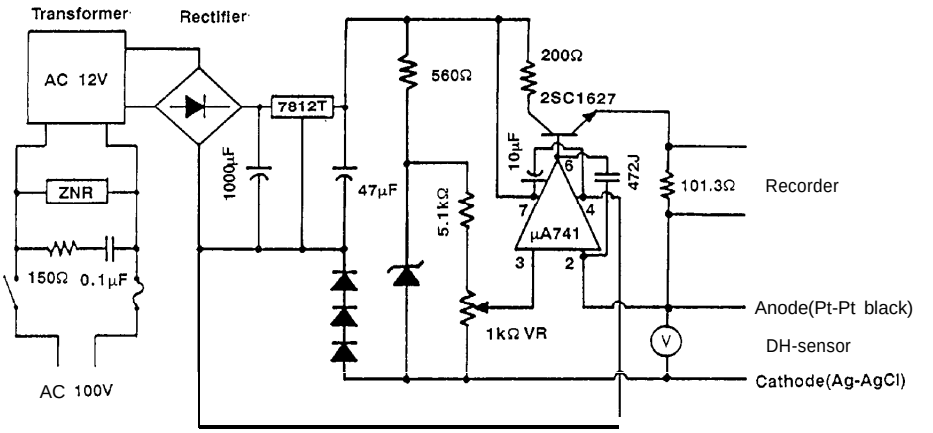

Fig. 2. Schematic diagram of the circuit for the dissolved hydrogen sensor.

Dissolved oxygen sensor

The partial pressure of dissolved oxygen was measured with a galvanic oxygen sensor, 10AN-S, and a dissolved oxygen meter, Al-1007 (Biott Co. Ltd., Tokyo). The output voltage from the meter was recorded on the same PRP-5021 recorder used for the hydrogen sensor.

Determination of $(K_La)_{H2}$ by dynamic gassing out and steady state method

The overall volumetric coefficient of hydrogen mass transfer was determined during the autotrophic batch culture of A. *eutrophus* ATCC 17697^T. When the dry cell weight reached 8.0 g/l the gas supply was suspended and the agitation speed lowered to 250 rpm-indicated as 'gas off' in Fig. 3. The gas supply was resumed and the agitation speed returned to normal before the dissolved hydrogen partial pressure reached the critical (limiting hydrogen uptake) value - indicated as 'gas on' in Fig. 3. The change in the dissolved hydrogen partial pressure was recorded by the dissolved hydrogen sensor. Because the hydrogen consumption rate was constant throughout the gassing out stage the following relationship describes the hydrogen consumption rate during this stage:-

$$R_{H2}=H_{H2}\cdot(P_{LB}-P_L)/t \qquad (1)$$

At steady state dissolved hydrogen concentration, the hydrogen consumption rate equals the hydrogen transfer rate into solution, thus:-

$$\& = (K_La)_{H2}\cdot H_{H2}\cdot(P_G-P_{LB}) \qquad (2)$$

Thus, the overall volumetric coefficient of hydrogen mass transfer in the biological system, $(K_La)_{H2}$, can be calculated from the following equation:-

$$(K_La)_{H2}=(P_{LB}-P_L)/\{(P_G-P_{LB}) \bullet t\} \qquad (3)$$

Determination of $(K_La)_{O2}$

The overall volumetric coefficient of oxygen mass transfer in the fermentor was determined by using the sulphite oxidation method $[(K_La)_{O2sul.}]$ and by the dynamic gassing out and steady state method $[(K_La)_{O2biol.}]$.

Determination of the critical partial pressure of dissolved hydrogen and oxygen

The critical partial pressure of dissolved hydrogen and oxygen for A. *eutrophus*

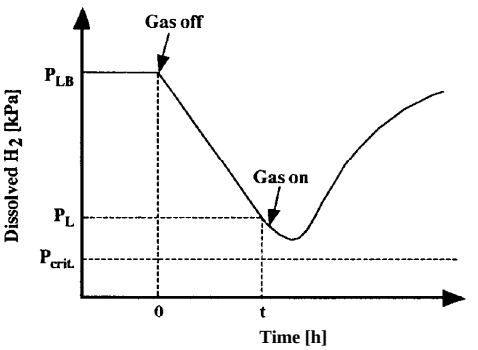

Fig. 3. Change in partial pressure of dissolved hydrogen during the determination of the hydrogen consumption rate.

ATCC 17697^T were determined by terminating the gas supply to the culture and recording the decline in the concentrations of the dissolved gasses. The gas composition fed to the reactor was adjusted to achieve either hydrogen or oxygen limitation after termination of the gas supply. Oxygen limitation was achieved by using a gas composition of H_2 : O_2 : CO_2 : N_2 of 8:1:1:0 whilst hydrogen limitation was achieved using a composition of 4:2:1:3, respectively.

RESULTS AND DISCUSSION

Characteristics of the hydrogen sensor

The hydrogen sensor was calibrated using hydrogen dissolved in distilled water. Output currents from the hydrogen sensor at various partial pressure of hydrogen are shown in Fig. 4. When dissolved hydrogen was removed from distilled water by sparging with nitrogen, the output current from the sensor was 205 nA. The output current generated by the sensor under hydrogen saturated conditions was 28.4 μA. The correlation coefficient of the linear relationship in Fig. 4 is 0.999.

Although sulphite compounds are known to affect the current output of hydrogen sensors (Kuroda et al., 1991) it was confirmed that none of the ingredients used in the culture medium interfered with the probe response.

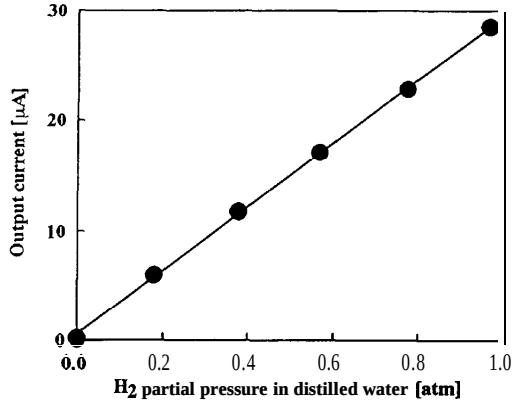

Fig. 4. Calibration of the assembled dissolved hydrogen sensor.

Table 1. The volumetric mass transfer coefficients (h^{-1}).

	Agitation (rpm)		
	1100	1400	1700
$(K_La)_{O_2sul.}$	257	319	417
$(K_La)_{O_2biol.}$	385	558	716
$(K_La)_{H_2}$	628	847	1430

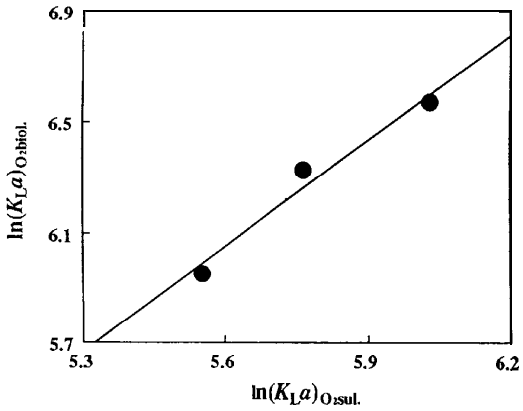

Fig. 5. Relationship between $(K_La)_{O2bio1.}$ and $(K_La)_{O2bio1.}$

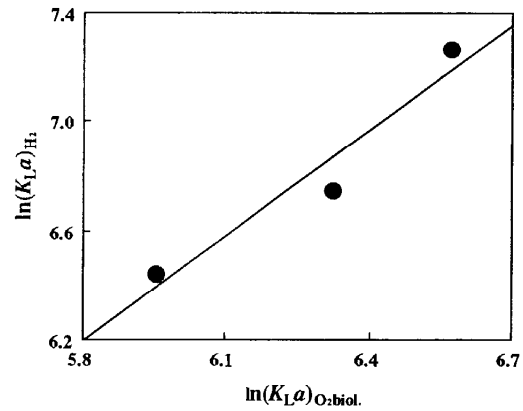

Fig. 6. Relationship between $(K_La)_{H2}$ and $(K_La)_{O2bio1.}$

Overall volumetric coefficients of hydrogen and oxygen mass transfer

$(K_La)_{H2}$, $(K_La)_{O2sul}$ and $(K_La)_{O2biol}$ were determined at agitation speeds of 1100, 1400 and 1700 rpm. The results of these determinations were shown in Table 1. For oxygen, the K_La value determined using the dynamic gassing out and steady state method, $(K_La)_{O2biol}$, was larger than the value obtained using the sulphite oxidation method, $(K_La)_{O2sul}$, a phenomenon also reported (Hsieh et *al.*, 1969). The $(K_La)_{H2}$ value was higher than that of the $(K_La)_{O2biol}$, supporting similar date (Kodama et *al.*, 1976) generated from microorganism-free media.

The relationship between the overall volumetric coefficients for oxygen detemined by the sulphite method and the dynamic gassing out and steady state method is shown in the natural logarithm plot of the two factors (Fig. 5). This graph indicates a linear relationship between the natural logarithms of the differently determined K_La's, expressed in equation (4) with a correlation coefficient of 0.970. The relationship between $(K_La)_{H2}$ and $(K_La)_{O2biol}$ is shown in Fig. 6, the linear relationship between the natural logarithms of the K_La values being expressed by equation (5) at a correlation coefficient of 0.930.

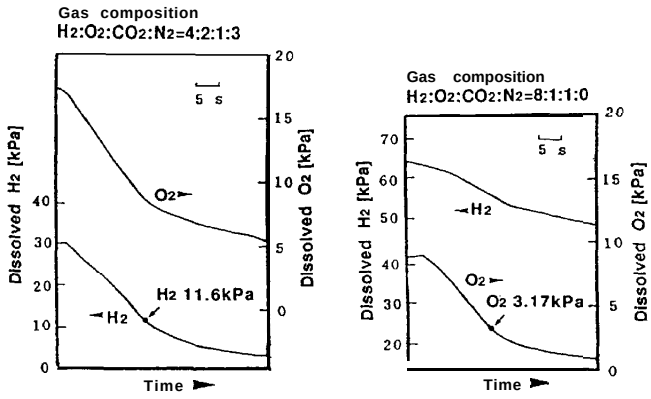

Fig. *7.* Determination of the critical partial pressure for dissolved hydrogen (left) and dissolved oxygen (right) in autotrophic batch *A. eutrophus.*

Table 2. The volumetric absorption coefficients.

	Agitation (rpm)		
	1100	*1400*	1700
$(Kd)_{H2}$	$4.70 \cdot 10^{-3}$	$6.34 \cdot 10^{-3}$	$1.07 \cdot 10^{-3}$
$(Kd)_{O2}$	$4.43 \cdot 10^{-3}$	$6.42 \cdot 10^{-3}$	$8.23 \cdot 10^{-3}$

$$(K_La)_{O2biol} = 0.347\,(K_La)_{O2sul}{}^{1.27} \qquad (4)$$

$$(K_La)_{H2} = 0.280\,(K_La)_{O2biol}{}^{1.29} \qquad (5)$$

Critical partial pressure of dissolved hydrogen and oxygen

The uptake of dissolved oxygen and hydrogen by cultures of *A. eutrophus* after termination of the gas supply is shown in Fig. 7. Either oxygen or hydrogen limitation was induced after gas termination by the composition of the gas supply, as indicated in the methods section. From Fig. 7 it may be seen that the critical partial pressure of dissolved hydrogen was much greater than that for oxygen - 11.6 kPa for hydrogen compared with 3.17 kPa for oxygen. The ratio of the critical partial pressure of dissolved hydrogen to that of oxygen is 3.66. It is interesting to note that this ratio agrees well with the molar ratio of hydrogen to oxygen, 3.44, calculated from the stoichiometric requirements of the organism.

The volumetric absorption coefficients of the two gasses $(Kd)_{H2}$ and $(Kd)_{O2}$ were calculated as the product of the relevant K_La and Henry's constant. From Table 2 it may be seen that the Kd values for hydrogen and oxygen are very similar despite the fact that the K_La values for hydrogen were greater than those for oxygen. This may be explained by the Henry's constant for hydrogen being lower than that for oxygen.

To ensure the safe operation of the process it is essential that the composition of the gas phase is strictly controlled to ensure that the ratio of oxygen to hydrogen is not within the detonation range. Using a modified Hempel's method we have demonstrated

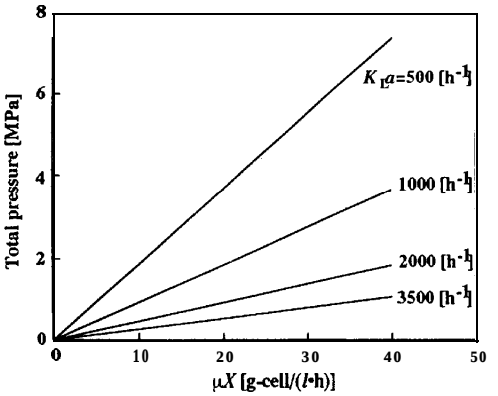

Fig. 8. Relationship between productivity, gas pressure and mass transfer capacity.

that the highest safe concentration of oxygen in a gas mixture of hydrogen, oxygen, carbon dioxide and water was 6.9%. Thus, to allow a reasonable safety margin, the oxygen concentration in the gas mixture should not exceed 6%. Since the critical partial pressure of dissolved oxygen for the organism is 3.17 kPa the maximum transfer rate attainable can be represented by the equation:-

$$(K_La)_{O2bio} \cdot H_{O2}(0.06P - 3.17) = \mu X / Y_{O2}$$

where Y_{O2} is 15.7 calculated from the stoichiometry previously published (Ishizaki and Tanaka, 1990). The relationship between $(K_La)_{O2bio}$, P and μX is shown in Fig. 8. As may be seen from this figure high cell productivity may be achieved by either increasing the $(K_La)_{O2bio}$ .or increasing the head pressure in the vessel, P. We constructed a high K_La, explosion-proof bench fermentor equipped with a specially designed agitator and, using this vessel, we obtained high productivity under safe operating conditions for the full duration of the fermentation (Chem. Eng. in press). However, it should be remembered that PHB accumulation occurs under oxygen limitation and, therefore, the oxygen supply must be controlled to achieve a zero dissolved oxygen concentration during the production phase. Thus, whilst it is difficult to obtain high biomass productivity under safe conditions there is very little danger of detonation during the PHB production phase of the fermentation. We are now investigating the feasibility of a two-stage fermentation employing heterotrophic growth using carbohydrate and air for biomass production followed by autotrophic conditions under oxygen limitation for PHB synthesis therefore eliminating the risk associated with a hydrogen process. We are also investigating the behavior of the organism under hydrogen limitation. These results will be published in the near future.

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NOMENCLATURE

H_{H2}: Henry's constant for hydrogen at 30°C, $(7.49 \cdot 10^{-6})$ mol/l·kPa
H_{O2}: Henry's constant for oxygen at 30°C, $(1.15 \cdot 10^{-5})$ mol/l·kPa
$(Kd)_{H2}$: Volumetric absorption coefficient for hydrogen, mol/l·kPa·h
$(Kd)_{O2}$: Volumetric absorption coefficient for oxygen, mol/l·kPa·h
$(K_La)_{H2}$: Overallvolumetric coefficient of hydrogen mass transfer in biological system, 1/h
$(K_La)_{O2biol.}$: Overall volumetric coefficient of hydrogen mass transfer in biological system, l/h
$(K_La)_{O2sul.}$: Overall volumetric coefficient of oxygen mass transfer in sulfite oxidation system, l/h
P: Head pressure in a fermentor, kPa
$P_{crit.}$: Critical partial pressure of dissolved hydrogen, kPa
P_G: Partial pressure of hydrogen in exhaust gas, kPa
P_L: Partial pressure of dissolved hydrogen at gas on, kPa
P_{LB}: Partial pressure of dissolved hydrogen at gas off, kPa
P_{H2}: Hydrogen consumption rate,
t: Time, h
X: Dry cell weight, g-cell
Y_{O2}: Growth yield factor based on oxygen, g-cell
μ: Specific growth rate, l/h

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