Studies on Oxygen and Carbon Source Consumption by Aerobacter aerogenes

Fujio, Yusaku
Laboratory of Biochemical Engineering, Faculty of Agriculture, Kyushu University

Sambuichi, Masao
Laboratory of Biochemical Engineering, Faculty of Agriculture, Kyushu University

Ueda, Seinosuke
Laboratory of Biochemical Engineering, Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/22835

出版情報：九州大学大学院農学研究院紀要. 17 (3/4), pp.263-273, 1973-10. Kyushu University
バージョン：
権利関係：

J. Fac. Apr., Kyushu Univ., 17, 263-273 (1973)

Studies on Oxygen and Carbon Source Consumption by *Aerobacter aerogenes*

Yusaku Fujio, Masao **Sambuichi** and Seinosuke Ueda

Laboratory of Biochemical Engineering, Faculty of Agriculture,
Kyushu University, Fukuoka

(Received April 18, 1973)

Dissolved oxygen consumption by ***Aerobacter aerogenes was*** determined by measuring dissolved oxygen change controlling carbon source consumption instead of oxygen consumption by the cells. Results were presented as the oxygen consumed per unit carbon source. The advantage of this method is that measurement was done quickly and precise experimental values were obtained. Oxygen consumption was determined for various carbon sources with the same strain. In these experiments, the values obtained by this method were compared with two other methods. Maltose, pullulan and soluble starch were used as carbon source for isoamylase formation by this strain. Glucose was also used for comparison with the above carbon sources, but it was not a suitable carbon source for isoamylase formation. For the results, coefficient ***A*** for oxygen consumption per unit carbon source were calculated for each carbon source and about the same value was obtained for maltose, pullulan and soluble starch respectively, while this value for glucose was half those of the other carbon sources.

INTRODUCTION

It can be considered that the consumption of disssolved oxygen by microbes during aerated cultivation arises from microbial respiration due to metabolism of a carbon source. Therefore, a quantiative relation may exist between carbon source consumption or dissolved oxygen consumption and the yield of microbial cells under uniform conditions of culture. The quantitative relation between carbon source consumption and cell yield can be determined experimentally under uniform conditions of medium composition and culture method. Studies on decrease of dissolved oxygen in broth during cultivation will play an important role in the design of fermentation vessels and in deciding industrial culture conditions, especially in cases of high cell concentration.

There are many papers (Cooper et ***al.,*** 1944 ; Yoshida ***et al.,*** 1960 ; Bandyopadhyay ***et al.,*** 1967) on the transfer of oxygen from gas to liquid phase, but, very little on actual consumption of dissolved oxygen by the cells. Generally, microbial cell concentration under aerobic conditions is the principle factor controlling dissolved oxygen concentration in broth. From a comparison of cell yield with consumption of carbon source and dissolved oxygen, carbon source concentration in broth can act as the controlling factor for dissolved oxygen

concentration with diluted carbon source and microbial cell at some concentration. Since the carbon source is instantaneously consumed by microbial cell under these conditions. The quantity of oxygen consumed can be calculated by measuring the change in dissolved oxygen concentration. These methods can be used to measure the difference in dissolved oxygen consumption for various carbon sources by the same microbial cells.

The purpose of this investigation is to measure quantitative relation between consumption of carbon sources and dissolved oxygen using a strain of ***Aerobacter aerogenes*** (Fujio ***et al.,*** 1970) whose powerful isoamyase formation has been quantitatively investigated for the purpose of industrial production of isoamylase. Oxygen consumption per unit carbon source has been measured for each of three carbon sources suitable for the formation of isoamylase and for glucose, which was unsuitable. These results have been compared with those obtained by other methods.

EXPERIMENTAL METHODS

(1) Apparatus

As shown in Fig. 1, the fermentor was made of pyrex. The total volume was 1000 ml and agitation was by magnetic stirrer and thermal regulation by water bath.

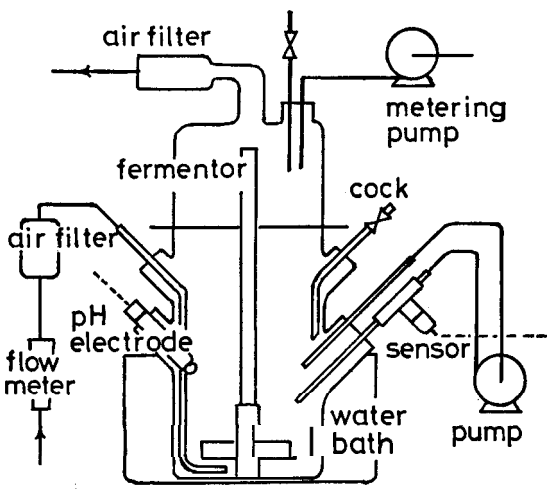


Fig. 1. Schematic diagram of experimental apparatus.

(2) Strain and medium

A strain of ***Aerobacter aerogenes*** was used. This strain was isolated for the purpose of industrial isoamylase production.

Table 1 shows the medium composition and carbon sources used. In this medium, this strain formed cell-bound isoamylase. Carbon sources were glucose,

Table 1. Medium and carbon source used.

	%	Carbon sources
$(NH_4)_2\ SO_4$	0.80	Glucose
K_2HPO_4	0.10	Maltose
$MgSO_4\ 7H_2O$	0.05	Pullulan
KCl	0.05	Soluble starch
$FeSO_4\ 7H_2O$	0.01	
Pepton	0.05	

maltose, soluble starch and pullulan. Of these carbon sources, glucose was not suitable for isoamylase formation (Fujio et al., 1970).

(3) **Measurement of dissolved oxygen**

The sensor of oxygen analyzer was set in a small pipe (I.D 3 mm), and the fermentation broth was circulated through this pipe by use of a peristaltic pump. Thus, measurements were obtained at constant flow rate. This circulating method (Fujio *et al.*, 1970) provided more reliable and reproducible values for dissolved oxygen concentration than the usual method.

(4) **Analytical methods for cell and carbon source concentration** (Fujio et *al.*, 1970)

Cell concentration was measured spectrophotometrically as optical density at 660 mμ and the dry weight was calculated from these values with a standard curve.

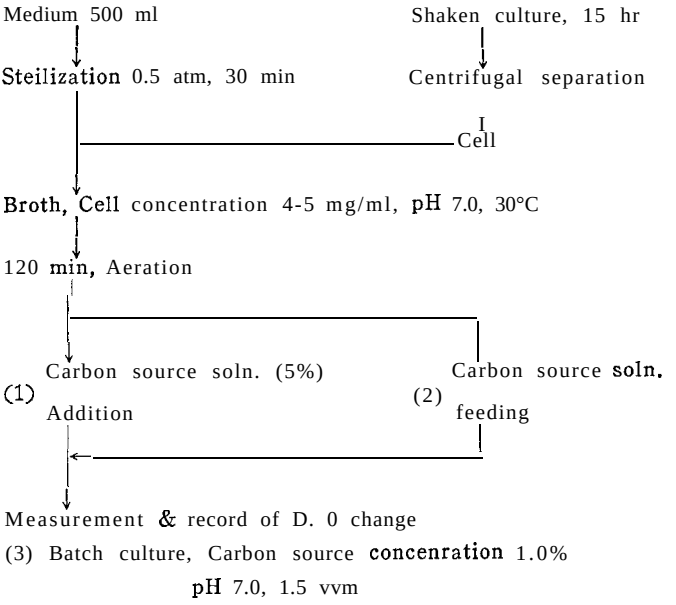


Fig. 2. Experimental methods.

The concentration of carbon source in the broth was analyzed by micro Bertrand's method, after acid hydrolysis if necessary.

(5) Experimental procedures

Fig. *2* shows experimental methods. Three different methods were used for the determination of oxygen consumption. In Fig. 2-method(1), the cells, after 15-hour cultivation in shaken Flask (working vol. 100 ml), were harvested by centrifugation and added to 500 ml medium without carbon source to a concentration of 4-5 mg/ml. This cell concentration was also obtained in batch culture for isoamylase formation. After a 2-hour aeration at 30°C, a small amount of carbon source was added into the broth from the burette set at the top of the fermentor. The added carbon source was immediately consumed and the oxygen concentration in the broth decreased as carbon source consumed by cells. The change in oxygen concentration was measured by oxygen analyzer.

In Fig. 2-method(2), carbon source solution was added into the broth at constant rate with peristaltic pump. The broth for this experiment was prepared by the same procedure described in experiment (1). Method (2) should be done under the same conditions as method (1), since the dissolved oxygen concentration was controlled by the rate of addition of carbon source.

In experiments (1) and (2), cell growth due to consumption of carbon source could be neglected because of relatively small amount of carbon source. These experiments were done at the aeration rate of 1.0 and 1.5 vvm.

In experiment (3) in Fig. 2, the usual batch culture method was used. That is, this experiment was carried out at aeration rate of 1.5 vvm at 30°C with 1.0 % carbon source. The concentration of pullulan was reduced to 0.3% because of its high viscosity.

RESULTS AND DISCUSSION

(1) Theoretical analysis of carbon source consumption and dissolved oxygen consumption

In the fermentation broth, oxygen transfer into liquid phase was determined by difference in saturated dissolved oxygen concentration C_o^* and observed concentration C_o, that is $(C_o^* - C_o)$. Factor controlling $(C_o^* - C_o)$ is the cell concentration generally. However, if there is a significant cell concentration and no carbon source, carbon source added bit by bit will become a controlling factor of dissolved oxygen concentration in the broth. The relation between carbon source consumption and dissolved oxygen concentration can be calculated as follows ;

Initially, equation (1) holds experimentally for cell yield and consumed carbon source concentration.

$$C_{x\,\text{yield}} = mC_{s\,\text{used}} \tag{1}$$

For dissolved oxygen consumption, the same relation might hold as follows,

$$C_{x\,\text{yield}} = m' C_{o\,\text{used}} \tag{2}$$

From eqs. (1) and (2),

$$C_{o\,\text{used}} = A C_{s\,\text{used}} \quad A = \frac{m}{m'} \tag{3}$$

For dissolved oxygen, the material balance in a unit volume of broth is as follows.

$$\frac{dC_o}{dt} = k_L a (C_o^* - C_o) - A \frac{dC_s}{dt} \tag{4}$$

For dC_s/dt in eq. (4), the following relation holds experimentally in batch culture.

For pullulan, maltose and glucose,

$$\frac{dC_s}{dt} = kC_{so} \exp(-kt) \tag{5}$$

The rate constant (Fujio *et al., 1970) k* were experimentally *0.2, 0.4* and *0.6* for pullululan, maltose and glucose respectively.

Since soluble starch can not be considered as the single component of carbon source, in this case the rate equation (Fujio et *al.* 1970) *is* as follows.

$$\frac{dC_s}{dt} = kC_{so} \exp\left(-\frac{k'Rt}{m}\right) \tag{6}$$

$$R = C_{xo} + mC_{so}$$

But in the case of soluble starch, as consumption of the small amount of soluble starch supplied takes place in sufficiently short time periods, eq. (6) may be treated the same as eq. (5). From eqs. (4) and (5),

$$\frac{dC_o}{dt} = k_L a (C_o^* - C_o) - AkC_{so} \exp(-kt) \tag{7}$$

Integration and rearrangement of eq. (7) gives

$$(C_o^* - C_o) = \frac{Ak C_{so}}{(k_L a - k)} (\exp(-kt) - \exp(-k_L a t)) \tag{8}$$

The extremal value of eq. (8) becomes as follows.

$$(C_o^* - C_o)_m = AC_{so} \left(\frac{k}{k_L a}\right)^{\frac{k_L a}{(k_L a - k)}} \tag{9}$$

The total amount of oxygen transfer from gas to liquid phase holds as follows.

$$k_L a \cdot I = k_L a \int_0^\infty (C_o^* - C_o)\, dt = AC_{,,} \tag{10}$$

Experiments in this paper were analyzed by equations (4) and (10).

(2) **$k_L a$ and its experimental values**

For the calculations of *oxygen transfer* in the broth, $k_L a$ of the fermentor

must be decided experimentally. There were many methods for determinin, of $k_La.k_La$ used in this paper was determined as follows. A small amount o Na_2SO_3 solution (10 %) was added into 500 ml of distilled water in a constantly agitated and aerated fermentor, at 30°C. The dissolved oxygen in the water of fermentor was consumed by reaction with Na_2SO_3, and then, after the disappearance of Na_2SO_3, dissolved oxygen in water increased gradually, and the increasing value was measured by oxygen analyzer. k_La values were calculated by using these measurement at each aeration rate. Results are shown in Fig. 3.

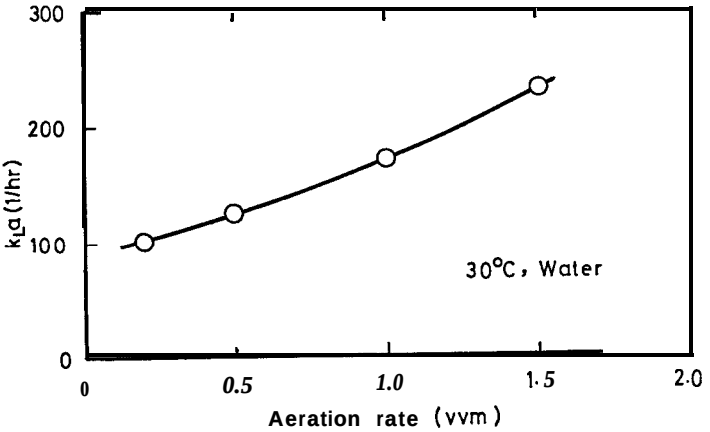


Fig. **3.** Volumetric coefficients of oxygen transfer from experiments using water.

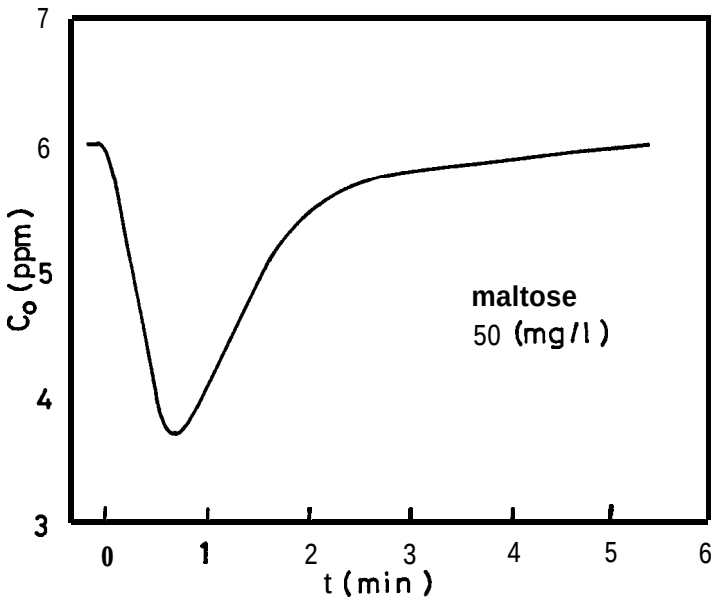
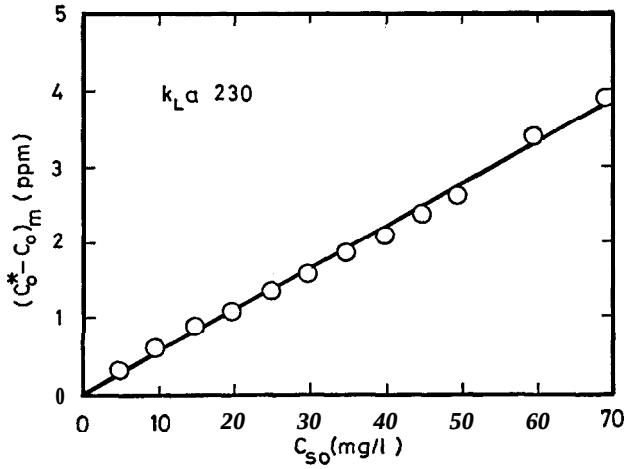


Fig. 4. Dissolved oxygen trace for addition of maltose.

(3) Experiment with maltose

For an example of experimental analysis, experimental and analytical results were shown in the case of maltose.

Fig. 4 shows the results measured by method (1) in Fig. **2.** Total consumed oxygen for eq. (10) was calculated by the graphical integration method for the curve in Fig. 4 and with k_La under the corresponding condition. From the peak of the curve in Fig. 4, extremal value of $(C_o^* - C_o)$ was obtained. To check equations used in this analysis, $(C_o^* - C_o)_m$ was plotted against the amount of each added carbon source C_{so} as shown in Fig. 5. The results in Fig. 5 shows the validity of equations. Fig. 6 shows the analytical results obtained by applying the experimental data C_{so} and C_o, to eq. (10). For results, coefficient



Fig, 5. Quantitative relationship of equation 5 for consumption of maltose.

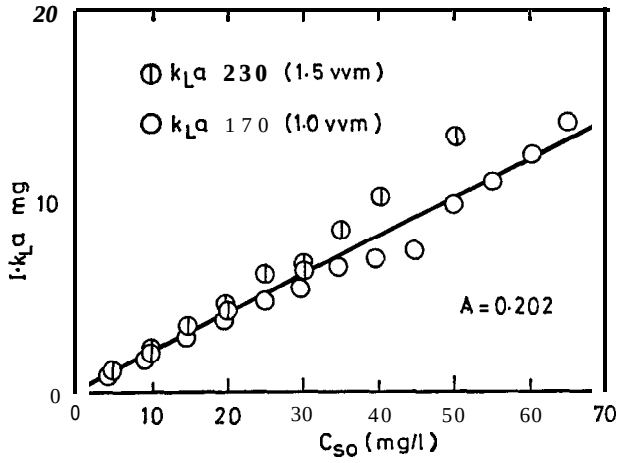


Fig. **6.** Quantitative relationship between consumed oxygen and maltose.

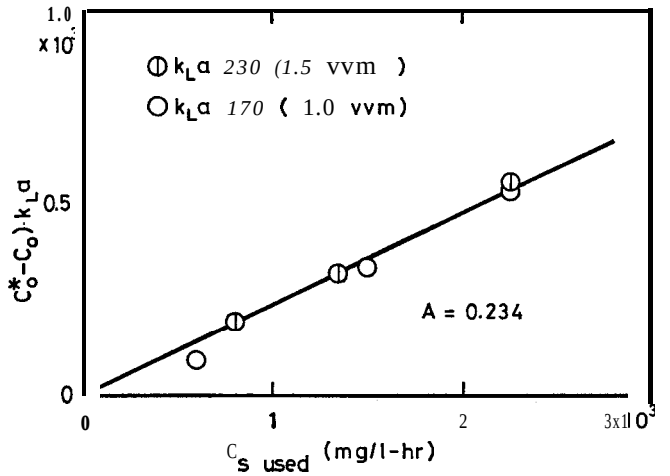


Fig. 7. Consumed oxygen from feeding experiments of maltose.

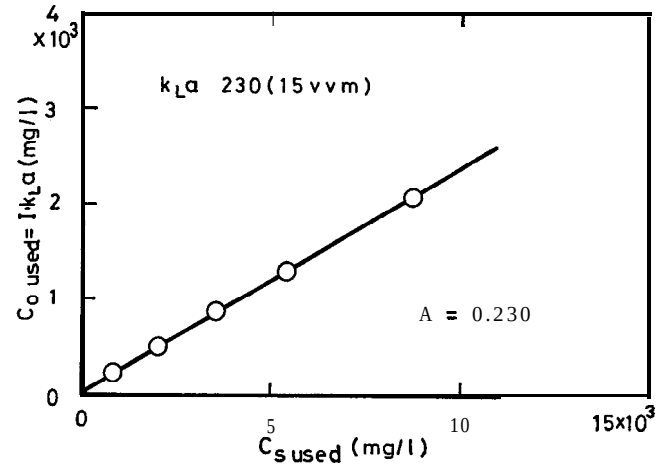


Fig. 8. Consumed oxygen for consumed maltose from batch cultivation.

A, oxygen required per unit of maltose in eq. (10), was calculated to be 0.202 (mg/mg).

Fig. 7 shows analytical results of method (2) in Fig. 2. In this experiment, the value of A was **0.234**, which showed nearly agreement with the value obtained from method (1). In method (3) in Fig. **2**, the value of A was calculated by measurement of dissolved oxygen concentration during batch culture. Calculation method was as follows. Oxygen consumption was calculated over a given interval from the area including between the curve for the concentration of saturated dissolved oxygen and that for the observed concentration of oxygen during cultivation. At the same time, consumption of maltose was determined by sampling analysis. Fig. 8 shows the results obtained according to the above procedure. The A value was **0.230.** But this analysis was accompanied

with some difficulties, because the saturated concentration of dissolved oxygen changed during cultivation. In this report, the saturated concentration of oxygen was determined by graphical extrapolation of the curves for cell and dissolved oxygen concentrations in batch culture. Value of k_La in Fig. 3 was used for this calculation.

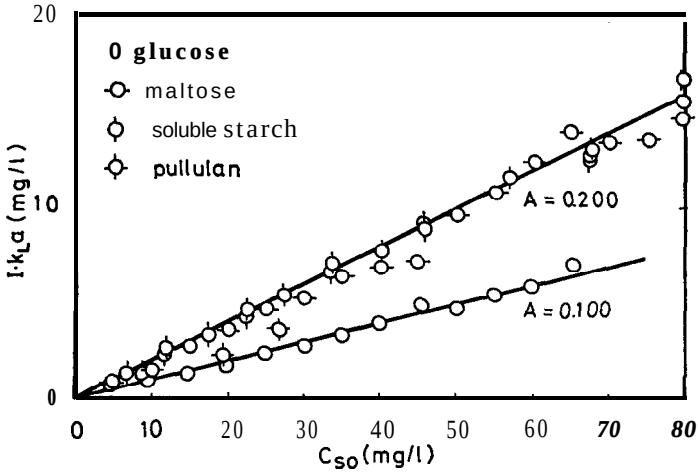


Fig. 9. Quantitative relationship between consumed oxygen and consumption of added various carbon sources.

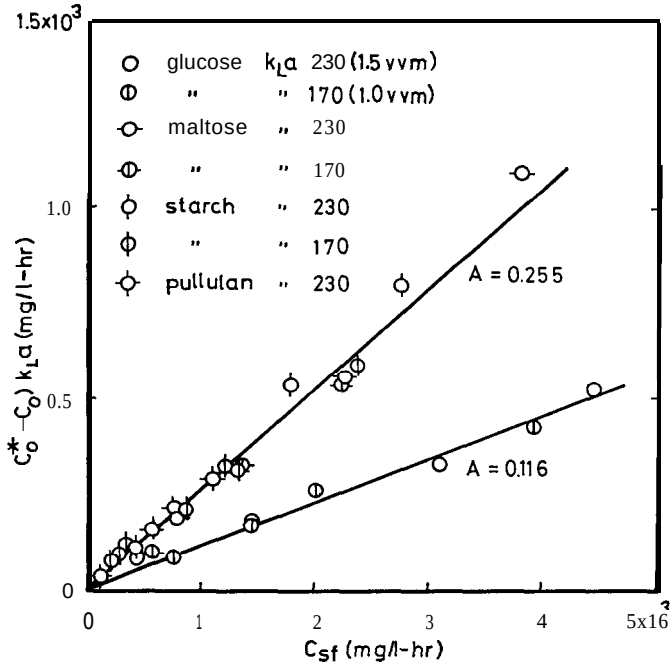


Fig. 10. Consumed oxygen from feeding experiments of various carbon sources.

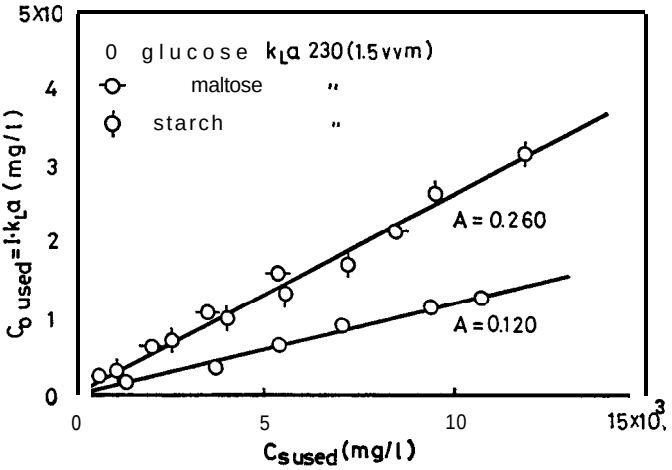


Fig. 11. Consumed oxygen for consumption of various carbon sources from batch cultivation.

Table 2. Oxygen consumption coefficient ***"A"*** from experiments.

Experimental methods	Carbon sources	
	maltose soluble starch pullulan	glucose
(1) Addition	0.200 (mg/mg)	0.100 (mg/mg)
(2) Feeding	0.255	0.116
(3) Batch	0.260	0.120
Average	0.238	0.112

For results of experiments with maltose, oxygen consumption coefficient ***A*** could be obtained from experiments (1), (2) and (3). The same experiments were carried out for various carbon sources with the same strain. Glucose, maltose, pullulan and soluble starch were used as carbon sources. These results are shown in Figs. 9, 10 and 11 for experiments (1), (2) and (3) respectively and calculated numerical values are shown in Table 2. In each case, ***A*** was calculated about same value for maltose and soluble starch. While, this value for glucose was about half that for maltose and soluble starch. The reason for this can be considered the difference in metabolic path-ways for each carbon sources. For the strain used in this experiments, average oxygen consumption coefficient *A were* 0.238 for maltose, soluble starch and pullulan, and 0.112 for glucose.

It is seen from these results that it is possible to determine very easily the consumed oxygen per unit carbon source, as long as dissolved oxygen concentration in broth was controlled by carbon source concentration. Experimental

method (1) in Fig. 2 is the most useful for this purpose, since the measurement can be quickly and accurately.

However, there remain many problems for k_La determination in medium with microbial cells, and these may be the subjects for a future study.

NOMENCLATURES

A	oxygen consumption coefficient per unit carbon source	(mg/mg)
C_s	carbon source concentation in broth	(mg/l)
C_{sf}	rate of addition of carbon source	(mg/l-hr)
C_{so}	initial carbon source concentration in broth	(mg/l)
$C_{s\ \text{used}}$	consumed carbon source by cell	(mg)
C_o	dissolved oxygen concentration	(mg/l)
C_o^*	saturated dissolved oxygen concentration	(mg/l)
$C_{o\ \text{used}}$	consumed oxygen by cell	(mg)
C_x	cell concentration in broth	(mg/l)
C_{xo}	initial cell concentration in broth	(mg/l)
$C_{x\ \text{yield}}$	cell yield obtained from carbon source consumed	(mg)
Z	integral value of right hand of eq. 9	(mg-hr/l)
k	rate constant for cell growth	(1/hr)
k'	rate constant for cell growth	(l/mg-hr)
k_La	volumetric coefficient for oxygen transfer	(1/hr)
m	cell yield constant for carbon source	(−)
m'	cell yield constant for consumed oxygen	(−)
R	$C_{xo} + mC_{so}$	(mg/l)
t	time	(hr)

REFERENCES

Bandyopadhyay, B., A. E. Humphrey and II. Taguchi 1967. Dynamic measurement of the volumetric oxygen transfei coefficient in fermentation systems. *Biotechnol. and Bioeng., 9, 533-544*

Cooper, C. M., G. A. Fernstrom and S. A. Miller 1944. Performance of agitated gas-liquid contactors. *Ind. Eng. Chem., 36, 504-509*

Fujio, Y., M. Shiosaka and S. Ueda 1970. Isoamylase production by *Aerobacter aerogenes*, part (I) effect of nitrogen source. J. *Ferment. Technol., 48, 8-13*

Fujio, Y., M. Sambuichi and S. Ueda 1970 Isoamylase production by *Aerobacter aerogenes*, part (II) *some* quantitative results by batch culture. *J. Ferment. Technol., 48, 601-606*

Yoshida, F., A. Ikeda and S. Imakawa 1960 Oxygen absorption rates in stirred gas-liquid contactors. *Ind. Eng. Chem., 52, 435-438*