Conditions of Oxygen Supply in L-Glutamic Acid Fermentation from Hydrocarbon

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A small jar fermentor was developed in order to investigate the effect of oxygen supply on hydrocarbon fermentation. Several indices to oxygen transfer were examined with this small jar fermentor. Conditions for suitable oxygen supply were examined in L-glutamic acid fermentation from hydrocarbon by use of shaking flasks and these small jar fermentors. The data indicated that the rate of oxygen transfer ought to be more than 14.3×10^{-7} mole/ml·min in order to obtain satisfactory results. The coefficient of oxygen transfer rate (K_{La}/H) decreased as the fermentation went on, so the supply of oxygen enriched gas mixture was effective to increase the production of L-glutamic acid.

It is well known that a great deal of oxygen is required in hydrocarbon fermentation. Darlington¹⁾ and Guenther²⁾ have calculated that about three times more oxygen was needed in hydrocarbon fermentation than in carbohydrate fermentation. Mimura³⁾ has demonstrated this fact with *Candida petrophilum* in cell mass production. Although there are investigations^{4~6)} concerning the oxygen demand of microorganisms in the field of production of cell mass, there are very few in other fields of hydrocarbon fermentation.

The present paper is concerned with the development of a small jar fermentor which is suitable to study the hydrocarbon fermentation, and conditions of oxygen supply in L-glutamic acid fermentation from hydrocarbon.

MATERIALS AND METHODS

Microorganism employed in this study was *Corynebacterium hydrocarboclastus* R-7 UV Pc.r.-10. This strain was a penicillin-resistant mutant and the isolation of this strain from the parent strain was described in the previous paper.⁷⁾ Cultivation of this bacterium was performed according to the method described in the

previous paper.⁷⁾ The composition of media is given in Table I. Partial pressure of oxygen in gas- and liquid-phase (P_f and P_L , respectively) was determined

TABLE I. COMPOSITION OF THE BASAL MEDIUM

For the shaken flask culture:	
<i>n</i> -Hexadecane	80 ml
K ₂ HPO ₄	5 g
(NH ₄) ₂ HPO ₄	20 g
MgSO ₄ ·7H ₂ O	0.5 g
FeSO ₄ ·7H ₂ O	0.01 g
MnSO ₄ · <i>n</i> H ₂ O	0.01 g
ZnSO ₄ ·7H ₂ O	0.088 g
Yeast extract	2 g
Thiamine hydrochloride	1 mg
Tap water	1000 ml
pH	6.75
For the S-type jar culture:	
<i>n</i> -Hexadecane	80 ml
K ₂ HPO ₄	5 g
MgSO ₄ ·7H ₂ O	0.5 g
FeSO ₄ ·7H ₂ O	0.01 g
MnSO ₄ · <i>n</i> H ₂ O	0.01 g
ZnSO ₄ ·7H ₂ O	0.088 g
Yeast extract	2 g
Thiamine hydrochloride	1 mg
Tap water	1000 ml
pH	6.75

using a Beckman 777 Oxygen Analyzer. Oxygen transfer rate r_{ab} (mole/ml·min) and oxygen demand KrM (mole/ml·min) were evaluated by the unsteady-state gas analysis method.⁸⁾ Sulfite oxidation experiments were performed with the reaction mixture of K. Yamada *et al.*⁹⁾ The other analytical methods were the same as already reported.^{7,10)}

RESULTS AND DISCUSSION

Development of a small jar fermentor

A small jar fermentor was developed in order to investigate the effect of oxygen supply on L-glutamic acid fermentation from hydrocarbon

FIG. 2. pH Control and Oxygen Analysis System of the S-Type Jar Fermentor.

as well as to obtain the data for scale-up.

This jar fermentor (hereafter called the S-type jar) was made of glass and the total volume was approximately 1000 ml as shown in Fig. 1. The S-type jar fermentor consisted of many equipments such as, the aeration and agitation, the sampling and feeding, the temperature and pH controlling as well as the oxygen analyzing modules. The diagram of a part of these systems was illustrated in Fig. 2.

Usually, a great effort and a long period were required for the scale-up of the fermentation from the shaking culture to the commercial tank culture, because there were many differences between them. So, a number of pilot-plant jar fermentors were employed in order to obtain the data for the scale-up. The effort and period would be saved by the use of S-type jar fermentors, because these small jar fermentors have almost the same functions as the pilot-plant jar fermentors. In addition, S-type jar fermentors occupy less space and less man-power.

The agitation was made by an impeller driven magnetic force. This agitation system had many advantages in hydrocarbon fermentation over the ordinary mechanical agitation system; it was free from the troubles such as the leakage of air and hydrocarbon vapor, the contamination of other microbes and the dissolution of grease for the mechanical seal of

FIG. 1. The S-Type Jar Fermentor.

the driving system.

Figure 3 shows the values of sulfite r_{ab} under the various conditions of aeration and agitation. The sulfite r_{ab} of the S-type jar fermentor was affected strongly by the number of revolution in comparison with that of the pilot-plant jar fermentor. Another characteristic was that a greater part of oxygen transfer was made from liquid surface into the liquid. This was due to the existence of the large free surface per unit liquid volume. Values of $Kd(=K_La/H)$ where K_La was overall oxygen transfer coefficient across gas-liquid interface (liter/min)

FIG. 3. Sulfite r_{ab} under the Various Conditions.

- Air flow rate 1/4 v/v.min, turbine type impeller
- Air flow rate 1/4 v/v.min, paddle type impeller
- Air flow rate 1/2 v/v.min, turbine type impeller
- Air flow rate 1/2 v/v.min, paddle type impeller
- ▲—▲ Air flow rate 1/1 v/v.min, turbine type impeller
- ▲—▲ Air flow rate 1/1 v/v.min, paddle type impeller
- △—△ Air flow rate 2/1 v/v.min, turbine type impeller
- △—△ Air flow rate 2/1 v/v.min, paddle type impeller

and H was Henry's constant (atm·ml/mole) were estimated under two conditions; the usual aeration through the sparger and the ventilation of gaseous phase over the liquid phase. Supposing that Kd value of the latter indicates the oxygen transfer from liquid surface, the percentage of oxygen transfer from liquid surface in overall oxygen transfer could be calculated from these two Kd values. More than 90% of oxygen was transferred from liquid surface in the S-type jar fermentor, whereas its percentage in the pilot-plant jar fermentor was less than 10%, as shown in Table II. Accordingly, three types of oxygen supply were possible; through the sparger, ventilation of gaseous phase over the liquid of the vessel and through the cotton

TABLE II. COMPARISON OF THE S-TYPE JAR FERMENTOR AND THE PILOT-PLANT JAR FERMENTOR IN THE MODE OF OXYGEN TRANSFER

	The S-type jar fermentor	The pilot-plant jar fermentor
Liquid volume (ml)	300	30×10^3
Free surface per unit volume (cm^2/liter)	185	31.8
Depth of liquid (cm)	5.4	31.5
Sulfite $Kd(t)$ (mole/ml.min.atm)	10.6×10^{-6}	13.2×10^{-6}
Sulfite $Kd(g)$ (mole/ml.min.atm)	9.9×10^{-6}	1.2×10^{-6}
$Kd(g)/Kd(t) \times 100$	93	9

S-type jar fermentor: 1/2 v/v.min, 1500 rpm, 1 kg/cm².

70 liter jar fermentor: 1/2 v/v.min, 370 rpm, 1.5 kg/cm².

$Kd(t)$: Total Kd value measured under the aeration through the sparger as shown in Fig. A.

$Kd(g)$: Kd value measured under the ventilation of gaseous phase as shown in Fig. B.

FIG. A.

FIG. B.

FIG. 4. Comparison of Three Types of Aeration.

- Aeration through the sparger
 ●—● Ventilation of gaseous phase
 ▲—▲ Aeration through the cotton plug (5.1 cm in diameter, 8.5 cm in length and 0.16 g/cm³ in density was equipped in a modified top of S-type jar)

at 31.5°C, 1/1 v/v·min of air flow rate

plug. K_d values under these conditions are shown in Fig. 4.

Effects of oxygen supply on L-glutamic acid production and cell growth

L-Glutamic acid fermentation was performed under various conditions of oxygen supply which were attained by selecting suitable volumes of culture fluid and employing different types of cotton plugs of shaking flasks. The condition of oxygen supply was expressed in sulfite r_{ab} .¹¹⁾ As shown in Table III, the condition of oxygen supply exerted a great influence on L-glutamic acid production and cell growth. When sulfite r_{ab} was less than 14×10^{-7} mole/ml·min, the sufficient production of L-glutamic acid was not accomplished as shown in Fig. 5. Effects of oxygen supply at the earlier phase (growth phase) and at the later phase (production phase) of fermentation were examined. Fermentations were carried out under the conditions of oxygen supply at 5.3 , 10.5 and 14.3×10^{-7} mole/ml·min as sulfite r_{ab} in the growth phase (before the addition of penicillin) and then oxygen supply was changed to the various levels in order to know effects at the production phase. As shown in Fig. 6,

TABLE III. EFFECTS OF OXYGEN SUPPLY ON L-GLUTAMIC ACID PRODUCTION AND CELL GROWTH

Gas phase characteristics	V_L (ml)	Sulfite r_{ab} (mole/ml·min)	L-Glutamic acid (g/liter)	Dried cell weight (g/liter)
Cotton plugs of type A were equipped	40	1.2×10^{-7}	1.0	4.1
"	20	2.3×10^{-7}	0.9	5.6
Cotton plugs of type B were equipped	40	3.0×10^{-7}	1.0	5.3
"	20	5.3×10^{-7}	6.3	7.0
Cotton plugs of type C were equipped	40	6.5×10^{-7}	10.6	6.0
"	20	10.5×10^{-7}	21.6	9.7
Air was employed for purging flasks	80	7.1×10^{-7}	17.6	7.3
"	40	10.0×10^{-7}	21.3	7.7
"	20	14.3×10^{-7}	23.7	10.3
50% of oxygen gas was employed for purging flasks	80	16.9×10^{-7}	21.8	9.1
"	40	23.9×10^{-7}	20.9	8.7
"	20	34.1×10^{-7}	23.0	8.9

Cotton plugs, type A: 0.4 cm in diameter and 3 cm in length, 0.52 g/cm³ in density.

Cotton plugs, type B: 0.8 cm in diameter and 3 cm in length, 0.16 g/cm³ in density.

Cotton plugs, type C: 2.4 cm in diameter and 4.5 cm in length, 0.16 g/cm³ in density.

FIG. 5. Relationship between r_{ab} Values and Fermentation Results.

FIG. 6. Effects of Oxygen Supply in Production Phase on L-Glutamic Acid Fermentation.

Sulfite r_{ab} in growth phase
 ●—● 5.3×10^{-7} mole/ml·min
 ○—○ 10.5×10^{-7} mole/ml·min
 ▲—▲ 14.3×10^{-7} mole/ml·min

the amount of L-glutamic acid was determined by the level of oxygen supply at the production phase irrespective of that at the growth phase. To attain the maximum productivity, the rate of oxygen transfer ought to be more than 14.3×10^{-7} mole/ml·min.

L-Glutamic acid fermentation under sufficient oxygen transfer

L-Glutamic acid fermentation was performed using S-type jar fermentors under the aeration of 1/1 v/v·min and the agitation of 1500 rpm which gave sulfite r_{ab} of 20×10^{-7} mole/ml·min. Although pH was maintained at the optimum level of 6.75 and oxygen supply was improved, L-glutamic acid production in the S-type jar fermentor did not increase so much as compared with the results obtained in the shaken flask culture. Shortage of oxygen supply was observed in the course of fermentation. Dissolved oxygen became zero even though biological r_{ab} was less than 20×10^{-7} mole/ml·min. Disagreement in values of sulfite r_{ab} and biological r_{ab} suggested a difference in the property of the fermentation liquid. Biological K_d was calculated according to the following equation.

$$\text{Biological } r_{ab} = \text{Biological } K_d \times (P_f - P_L)$$

As shown in Fig. 7, biological K_d value decreased as fermentation went on and it became about 1×10^{-6} mole/ml·min·atm after 30 hr

FIG. 7. Time Course of K_d Value.

FIG. 8. Effects of P_f on L-Glutamic Acid Production.

- ▲—▲ $P_f = 0.40$ atm
 ○—○ $P_f = 0.30$ atm
 ●—● $P_f = 0.21$ atm

FIG. 9. Effects of P_f on Biological r_{ab} .

- ▲—▲ $P_f = 0.40$ atm
 ○—○ $P_f = 0.30$ atm
 ●—● $P_f = 0.21$ atm

cultivation.

Improvement of oxygen supply was attempted by means of the aeration using oxygen enriched gas mixture. Oxygen and nitrogen gas mixtures which contained 21%, 30% and 40% of oxygen respectively were introduced into the fermentation liquid. L-Glutamic acid production and biological r_{ab} value increased as the oxygen content of gas mixture increased (Figs. 8, 9). Maximum L-glutamic acid production was 35 g/liter and maximum biological r_{ab} value was 26×10^{-7} mole/ml·min. The oxygen supply seemed to be a limiting factor in this fermentation and the supply of oxygen enriched gas mixture gave good results.

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REFERENCES

- 1) W.A. Darlington, *Biotechnol. Bioeng.*, **6**, 241 (1964).
- 2) K. R. Guenther, *ibid.*, **7**, 445 (1965).
- 3) A. Mimura, *J. Ferment. Technol.*, **49**, 245 (1971).
- 4) T. L. Miller and M. J. Johnson, *Biotechnol. Bioeng.*, **8**, 567 (1966).
- 5) M. J. Johnson, *Chem. Ind. (London)*, **1964**, 1532.
- 6) D. C. Wang, *Chem. Eng.*, **75**, 99 (1968).
- 7) K. Kobayashi, S. Ikeda, K. Takinami, Y. Hirose and T. Shiro, *Agr. Biol. Chem.*, **35**, 1241 (1971).
- 8) Y. Hirose, H. Sonoda, K. Kinoshita and H. Okada, *ibid.*, **30**, 49 (1966).
- 9) K. Yamada, J. Takahashi and H. Okada, *J. Agr. Chem. Soc. Japan*, **27**, 703 (1953).
- 10) S. Ikeda, Y. Hirose, K. Kobayashi and K. Kinoshita, *Agr. Biol. Chem.*, **33**, 1042 (1969).
- 11) Y. Hirose, H. Sonoda, K. Kinoshita and H. Okada, *ibid.*, **30**, 583 (1966).