SELECTION CRITERIA OF OXYGEN CARRIERS UTILIZABLE FOR AUTOTROPHIC GROWTH IMPROVEMENT OF *ALCALIGENES EUTROPHUS* UNDER LOWERED OXYGEN PARTIAL PRESSURE

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Carbon dioxide fixation by using *Alcaligenes eutrophus* under lowered oxygen partial pressure for safe reactor operation has been investigated^{11,12}. Addition of perfluorocarbon (PFC) as an oxygen carrier to the culture has successfully improved the oxygen supplying rate¹². Enhanced oxygen transfer rate by PFC addition in aerobic culture have also been reported^{1,4,5,7}. However, since PFC is too expensive (4×10^3 US\$ / L) to be applied to a large scale reactor, an inexpensive substitute for PFC should be found. Although some hydrocarbons have been considered possible oxygen carriers^{2,10}, systematic assays for their suitability as oxygen carriers has hardly been discussed. In this paper, the factors, together with biodegradability and microbial toxicity, which affect the culture of *A.eutrophus* aiming at CO₂ fixation, are discussed.

1. Requirements for Oxygen Carrier

An oxygen carrier must have high oxygen solubility and immiscibility with water. It must have a high boiling point to minimize its loss by vaporization during steam sterilization and cultivation. Its viscosity should be sufficiently low for easy mixing with an aqueous medium. In addition, the carrier must be non-toxic to the microbe and non-biodegradable.

For the preliminary screening of oxygen carrier candidates (OC), two biological indices - microbial toxicity and biodegradability - are proposed. The microbial toxicity index I_T defined by Eq. (1) indicates the ratio of cell growth rate ($\Delta X / \Delta t$) in the presence of OC to that in an OC-free state.

$$I_T = (\Delta X / \Delta t) / (\Delta X / \Delta t)_{oc-free} \quad (1)$$

If the cell growth environment is toxic, the ratio will be less than 1.

On the other hand, the biodegradability index I_B , defined by Eq.(2) as the ratio of cell yield against hydro-

gen consumption ($\Delta X / \Delta H_2$) in the presence of OC to that in an OC-free state, will become greater than 1 if the OC is metabolized heterotrophically. The reason why H₂ consumption is taken into consideration in the yield is that if the microbe doesn't consume H₂, it is considered to grow heterotrophically utilizing OC as energy and carbon sources, and vice versa⁶.

$$I_B = (\Delta X / \Delta H_2) / (\Delta X / \Delta H_2)_{oc-free} \quad (2)$$

Ideally both I_T and I_B will become unity, when evaluated under an appropriate cultivation condition.

2. Materials and Methods

2.1 Candidates for oxygen carrier

Candidates examined for oxygen carrier are shown in Table 1 together with their physical properties.

2.2 Microorganism and cultivation procedure

1) Microorganism *Alcaligenes eutrophus* H16 was cultured using an inorganic medium³.

2) Preliminary screening Refrigerated stock culture were inoculated into a 35 mL L-shaped test tube containing 5 mL inorganic medium and 0.02 mL of OC. The amount of OC was sufficient for its saturation in the aqueous medium. In order to quantify well the cell growth rate, the optimum gaseous substrate mixture (H₂:O₂:CO₂ = 75:10:15; in molar ratio) for the microbe³ was used. The tube was filled aseptically with the gaseous substrate was sealed with a rubber stopper. Cultivation was performed on a reciprocating shaker at 303 K for 40 h.

3) Examination of OC in a jar fermentor The OCs selected by preliminary screening were examined in a 3L jar fermentor (3-MJB, Mitsuwa, Japan), where a non-explosive gas mixture of H₂:O₂:CO₂ = 81:3:16 was continuously fed into the fermentor through a 0.2-μm filter. Agitational speed was 400 rpm and pH was controlled at 7.0.

2.3 Measurements

Cell concentration, gas composition and gas consumption amount were measured as described elsewhere¹².

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Table 1. Physical properties of candidates for oxygen carrier

Material	O ₂ solubility under air saturation at 309 K [mg·L ⁻¹]	Melting point [K]	Boiling point [K]	Viscosity [mPa·s]
Perfluorotributylamine (PFC)	100	273	414	5.1
n-Decane	43	243	447	0.82
n-Undecane	44	247	469	1.0
n-Dodecane	51	261	488	1.2
n-Hexadecane	47	291	560	2.7
n-Heptadecane	49	295	576	3.3
n-Hexadecanol	8>	323	493	20>
i-Octadecanol	70	329	483	20>
n-Eicosanol	13	308	393>	20>
n-Tetraeicosanol	11	<273	393>	20>
2-Hexyl-1-octadecanol	9	<273	393>	20>
cis-9-Octadecene-1-ol	8>	<273	483	32
Hexane	8>	280	354	3.0
Toluene	8>	177	384	4.1

Fig. 1 Relationship between I_B and I_T of oxygen carrier candidates
 1 PFC 2 *n*-Decane 3 *n*-Undecane 4 *n*-Dodecane
 5 *n*-Heptadecane 6 *n*-Hexadecane 7 *n*-Hexadecanol
 8 *n*-Eicosanol 9 *n*-Tetraeicosanol 10 2-Hexyl-1-octadecanol
 11 *i*-octadecanol 12 Oleyl alcohol
 13 Hexane 14 Toluene

3. Results and Discussion

Figure 1 shows the relationship between the observed indices I_B and I_T in the preliminary screening cultures. All hydrocarbons, four higher alcohols and PFC were situated in the very vicinity of coordinate (1, 1), which implied their possible candidates for oxygen carrier. On the other hand, both indices of oleyl alcohol and *i*-octadecanol were much greater than 1, while those of toluene and hexane were less than 1. The former two alcohols are believed to have been degraded by the microbe. Toluene and hexane were toxic to the microbe, as expected.

For further examination, considering large scale economy and higher oxygen solubility, *n*-hexadecane (HD) (66US\$/L) and *n*-decane (87US\$/L) were selected from

Fig. 2 Effect of *n*-hexadecane addition on growth of *A. eutrophus*

Table 2. Characteristics of jar fermentor cultures of *A. eutrophus*

OC	Φ_p [-]	E* as a function of Φ [-]					
		0	0.3	0.6	0.7	0.8	0.9
free	-	1	-	-	-	-	-
PFC	0.60-0.65	-	0.748	1.14	4.39	10.8	17.5
<i>n</i> -hexadecane	0.64-0.78	-	0.683	0.494	-	3.54	8.93
<i>n</i> -decane	0.75-0.88	-	1.98	3.23	-	-	11.2

* enhancement factor defined as the ratio of linear cell growth rate under the presence of OC to that under OC-free. $E = (dX/dt)/(dX/dt)_{OC-free}$

among the possible OCs picked up by the preliminary screening.

Figure 2 shows the growth curves of *A. eutrophus* cultured in the jar fermentor containing the inorganic medium mixed with various amounts of HD (Φ). Cell mass grew linearly with time after the observed dissolved oxygen concentration (DO) decreased to zero. The observed non-zero DO at Φ above 0.8 implied that the probe detected the DO in the continuous HD phase. The effects of HD addition amount on the growth behavior were similar to those of PFC addition¹². For example, remarkable increase in the cell growth rate was observed in the region where the aqueous medium was dispersed in the continuous HD phase. Similar results were observed in the case of *n*-decane addition.

Growth characteristics under the presence of OC in the jar fermentor are summarized in **Table 2**. Higher growth rates were observed in the range where the medium was dispersed dropwise in the OC phase. Enhancement in the growth rate differed depending on the OCs examined. For example, the enhancement factors E at $\Phi = 0.9$ were 8.93 and 11.2 for *n*-hexadecane and *n*-decane,

respectively. These values are a little less than, but are of the same order to, that of PFC addition case. This difference in enhancement factor is not considered to have been due to microbial toxicity, but rather to mass transfer resistance around the medium droplet and air bubble. Although maximum enhancement was a little less than that of the PFC addition case, *n*-hexadecane and *n*-decane are promising substitute candidates for PFC considering their cost.

The Φ at which the continuous liquid phase changed from the medium to OC, or vice versa, in the fermentor (denoted as Φ_p) varied a little, depending on the OCs. A smaller Φ_p is preferable because the fermentor efficiency depends largely on the medium volume.

The preliminary OCs' selection procedure proposed in this paper was applicable. To seek an oxygen carrier appropriate for a specific microbial culture, preliminary selection should be followed by a comprehensive examination in a large fermentor system because of the complicated effects of many factors.

Nomenclatures

E	= enhancement factor of cell growth rate	[-]
I_B	= index of biodegradability	[-]

I_T	= index of microbial toxicity	[-]
t	= time	[h]
X	= cell concentration	[g·L ⁻¹]
Φ	= volume fraction of oxygen carrier candidate	[-]
Φ_p	= Φ at occurred phase inversion	[-]

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