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Continuous Cultivation in Air-lift Fermentor for Production of Single Cell Protein

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단세포 단백질 생산을 위한 기거식 발효조 내에서의 연속배양

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Abstract

Air lift fermentor (ALF) is widely used for production of single-cell protein (SCP) from hydrocarbon and other carbon sources, because oxygen transfer efficiency is believed to be superior in the ALF to that in other conventional fermentors. However, the performance of ALF in terms of mixing is somewhat questionable. In this research, we studied about the performance of the ALF in SCP production using methanol fermentation process as a model system. The results show that ALF could be employed for SCP production or other fermentation processes when substrate is miscible and used at low concentration. With a high substrate concentration, it must be operated under high pressure or low dilution rate to meet the adequate oxygen transfer requirement.

Introduction

In conventional fermentation processes, oxygen transfer and mixing are accomplished by aeration and mechanical agitation. However, the mechanical agitation has many disadvantages in terms of maintenance and power consumption. For circumventing these problems, various fermentor designs have been proposed. The most promising one was found to be the air-lift fermentor (ALF). ALF where the mixing is done only by aeration enables us to supply a cheaper oxygen for the production of single cell protein (SCP) from hydrocarbon.

Hatch et al.[1] showed that the oxygen transfer efficiency (performance ratio) in ALF was higher than that of any other equipment by carring out the experiments in air-watrer system. But the fermentation broth is much different from water in rheological properties, so the results are not correct for all the fermentation processes. In this paper, we would like to present the results of our recent studies on the continuous cultivation of methanol bacteria in ALF with some emphasis on mass transfer problems. At present, there are varieties of air-lift designs applied in industry for the production SCP or for the waste-water treat-

ment using the activated sludge system.[2,3]

Recently, there is an increasing interest in methanol as a carbon source for single cell protein production (Table 1).[4~18] The interest is based not only on its relatively low cost but also on its advantages in terms of engineering aspects as compared with hydrocarbon and methane.

Table 1. Microbial Cultures Capable of Growing on Methanol

Cultures	Reference
Anaerobic Cultures:	
Unknown isolate	Stephenson and Stickkand (4)
Methanobacterium suboxidans	Toerien and Hatting (5)
Methanobacterium propionicum	〃 〃
Hyphomicrobium sp.	Sperl and Hoare (6)
Aeroqic Bacteria:	
Strain HR (mixed culture)	Vary and Johnson (7)
Pseudomonas methanica	Dworkin (8)
Methanomoas methanoxidans	Stocks, *et al.* (9)
Methylococcus capsulatus	Foster (10)
Pseudomonas C	Chalfan and Mateles (11)
Pseudomonas methylotropha	McClennan, *et al.* (12)
Methanomonas methylovora	Kouno, *et al.* (13)
Methylomonas methanolica nov. sp.	Amano (14)
Actinomyces sp.	Terui, *et al.* (15)
Aerobic Yeast:	
Kloeckera sp. No 2201	Ogata, *et al.* (16)
Torulopsis glabrata	Asthana, *et al.* (17)
Hansenrular polymorpha DL-1	Cooney, *et al.* (18)

Materials and Methods

Culture strain and media composition

Methylomonas sp. was employed throughout the experiment. This strain was isolated from soil and was characterized by Kim et al.[4] Table 2 shows the characteristics of this strain. They are gram negative rods of about $1\times1.5\mu$. The yield coefficient for methanol is nearly constant, 0.4g cell/g methanol. The composition of the culture media is shown in Table 3. Kim reported that magnesium ion in the concentration range of 0.2 to 0.35g/l significantly stimulated growth of this strain[19].

Inoculum

A lyophilized vial of the culture was used to develop the inoculum. 2~3% (volume/volume) of the fermentation broth was used as inoculum when the shaking culture reached biomass concentration of 1 g/l.

Analysis

The cell concentration was obtained from a previously prepared calibration curve, relating the concentration of cells (dry weight basis) and the optical density of the broth. The optical density was measured at 600 nm using a spectrophotometer. The concentration of methanol in the broth was determined by the same method as described by Kim[19].

Batch culture

Batch cultivations were carried out in a chemostat (New Brunswick Sci.) to study the maximum specific growth rate and Monod constant. Using this fermentor, continuous cultures were also carried out under the same conditions as those of ALF to assess the performance of ALF.

Continuous culture in ALF

The ALF consists of two concentric acrylic

Table 2. Characteristics of *Methylomonas*, sp. Used

Shapes	Single, straight, rods
Dimensions	0.5~1.0 by 1.5~2.0
Type	Gram negative strict aerobes
Yield coefficient	0.4g cell/g methanol
Protein content	71.5%
Specific oxygen uptake rate	18m mole O_2/g cell/hr

Table 3. Media Composition

CH_3OH	0.2~1.0 % (v/v)
$(NH_4)_2SO_4$	2.0 g/*l*
KH_2PO_4	2.0 g/*l*
K_2HPO_4	7.0 g/*l*
$MgSO_4 \cdot 7H_2O$	200 mg/*l*
$FeSO_4 \cdot 7H_2O$	1 mg/*l*
Yeast extract	60 mg/*l*
Distilled water	to 1 *l*

cylinders equipped with an air sparger at the base of inner cylinder. The air sparger was made of 60~80 mesh syntered glass. In the inner cylinder, two perforated plates, whose open area was 40%, were placed to prevent the coalescence of gas bubbles. The working volume of this fermentor was 9-*l*. A simplified diagram of ALF type continuous culture system is shown in Fig. 1. Temperature was kept constant at 30°C by circulating the heating or cooling water through the jacket. Air sparged into the base of the inner column lowered immediately the density of the liquid in draft tube region which resulted in a pressure difference between the draft tube and annualar region. Oxygen probe, galvanic type consisting of a silver cathode and a lead anode, was employed for the measuring of dissolved oxygen concentration. This probe was submerged inside the draft tube. pH of the fermentation broth was automatically controlled and was fixed at 7.3 by adding 1 N-NaOH using the automatic controller (New Brunswick Sci model pH 22). Before starting the continuous culture, the cell concentration was built up by a batch cultivation in ALF during the first 10 hrs. Media was then fed into the bottom of

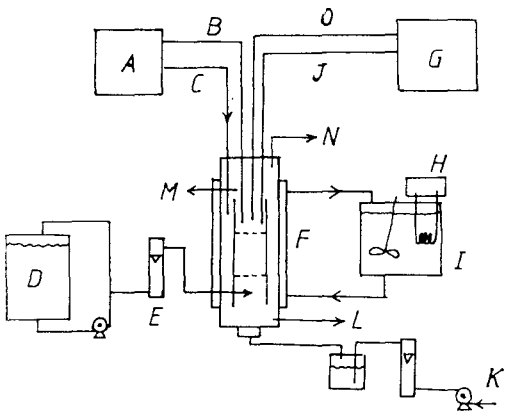

Fig. 1 Schematic Diagram of Continous Culture in ALF.
A : pH controller B : pH probe
C : Acid/Base feed D : Media tank
E : Rotameter F : ALF
G : Recorder H : Thermostat
I : Water bath J : Thermocouple
K : Compressed air L : Product
M : Level controller N : Condenser
O : D. O. Probe

the draft tube region and product was pumped out from the bottom of the annular part, and continuous culture commenced. Liquid level was held constant by continuously pumping out broth at a certain height. To avoid the foaming, antifoam agent (Silicone Antifoam, Corning) was used periodically. After one equivalent mean residence time, we sampled the product and measured the cell and residual methanol concentration to check the steady-state value. The total fermentation period was 2 to 3 times the mean residence time. The operating variables controlled were dilution rate, aeration rate, and feeding methanol concentrations.

Results and Discussion

Growth

A typical growth curve for *Methylomonas* sp. obtained with the apparatus and medium described is shown in Fig. 2. This organism was seldom contaminated and rapidly grew under the normal operating conditions. The pH of the culture broth

began to change after 5 to 6 hours from the beginning and the final pH was dropped to 6.5 from 7.3.

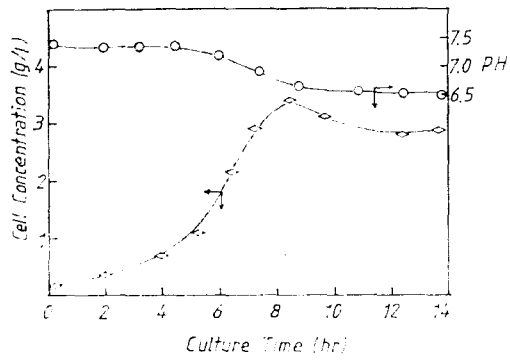

Fig. 2. Growth Curve in Batch Culture.
30°C, 600rpm aeration rate 2 vvm, methanol concentration (S_0) = (8 g/*l*), working volume 350m*l*.

The maximum specific growth rate was obtained by graphical method. Assuming the constant yield coefficient, 0.4, the residual methanol concentration at the maximum specific growth rate was calculated. The data from the set of runs in batch culture is plotted in Fig. 3. The values of μ_m and K_s determined are 0.526 hr^{-1} and 0.085 g/*l* respectively.

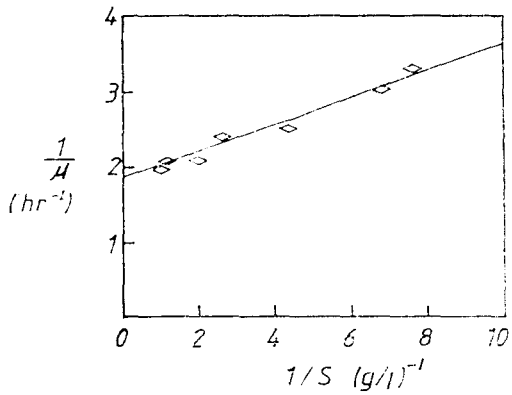

Fig. 3. The Relation between the Methanol Concentration and the Specific Growth Rate.
Maximum specific growth rate, $\mu m = 0.526\ hr^{-1}$, growth constant, $K_s = 0.085$ g/*l*.

Continuous culture

When the feed concentration of methanol was 0.2% (1.6g/*l*), the cell concentration did not change although the aeration rate was widely

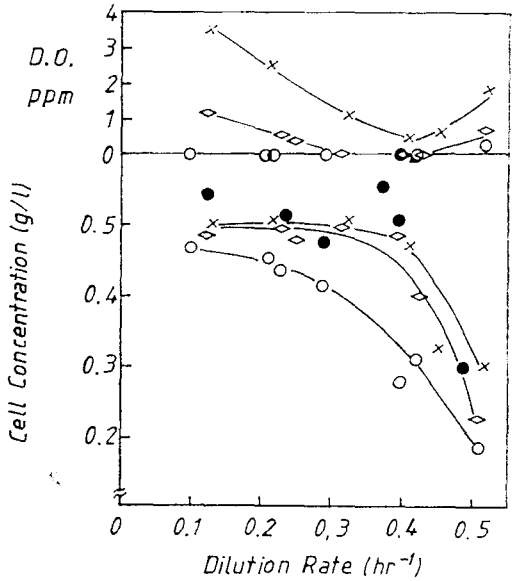

Fig. 4. Variation of Cell Concentration at Various Dilution Rate.
S_0=0.2%,
—●— : chemostat,
—○— : 0.5 vvm,
—◇— : 1.0 vvm,
—×— : 2.0 vvm.

varied (Fig. 4). If the aeration rate was 0.5 vvm, oxygen concentration was not high enough for cell cultivation except when the dilution rate was as low as 0.1 hr^{-1}. In the case of higher methanol concentration, the cell concentration was dropped with a slightly increased dilution rate. These phenomena seemed to be caused by the lack of oxygen for microbial growth (Fig. 5).

Our results agree well with the Pirt's result[20] where he showed the continuous culture data under oxygen limiting condition. Caution should be taken to prevent severe foaming since it lowers the oxygen transfer rate while operating the ALF. The amount of methanol evaporated should not be ignored when the aeration rate was high. The rate of evaporation of methanol was proporional to the gas velociety and to the residual methanol concentration. When the aeration rate was 3 vvm,

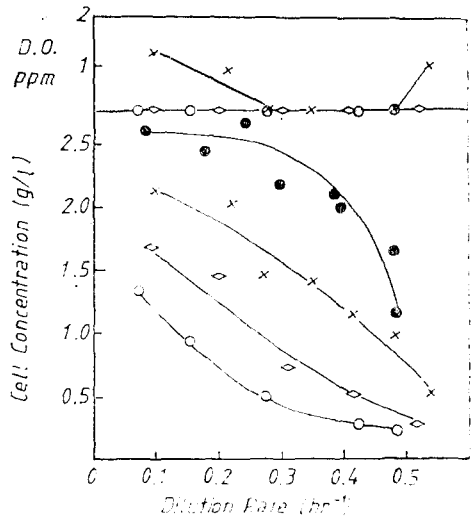

Fig. 5. Variation of Cell Concentration at Various Dilution Rate.
S_0=1.0%,
—●— : chemostat,
—○— : 0.5 vvm,
—◇— : 1.0 vvm,
—×— : 3 vvm.

the amount of methanol evaporated was about 10% of the feeding methanol (Table 4).

The maximum productivities were evaluated to compare the performance of ALF with that of

Table 4. Residual Methanol Concentration (g/l)

S_0 (g/l)	aeration rate (vvm)	dilution rate (hr^{-1}) 0.1	0.2	0.3	0.4	0.5
2.0	0.5	0.154	0.294	0.311	0.724	0.623
	1.0	—	—	—	0.111	0.574
	2.0	—	—	—	0.035	0.280
10.0	0.5	4.72	6.05	6.205	7.38	6.43
	1.0	2.17 (1.53)	5.55 (0.49)	5.27 (0.635)	5.45 (1.18)	6.58 (0.57)
	3.0	1.95 (0.82)	2.21 (1.11)	3.13 (1.37)	4.15 (0.84)	).18 (0.89)

The numbers in parentheses represent the amount of methanol evaporatod in each case.

chemostat equipped with mechanical mixer (Fig. 6). If S_0=0.2%, the maximum productivity of SCP in ALF was 0.2 g/l/hr and that of chemostat was 0.21 g/l/hr. When S_0=1.0%, maximum productivity in chemostat, but when the methanol concentration was high, oxygen transfer rate must be increased for higher biomass productivities (Fig. 6). The maximum biomass productivity

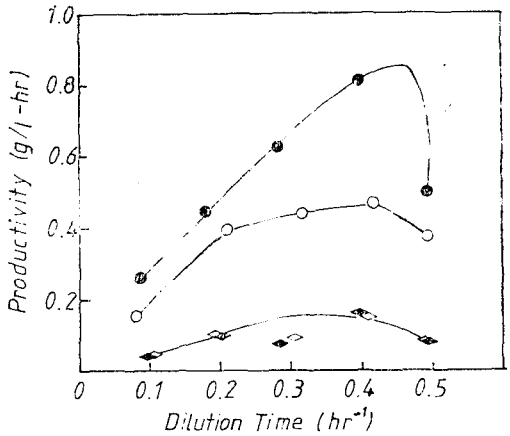

Fig. 6. Productivity of Biomass in ALF.
—●— : chemostat, S_0=1.0%,
—○— : ALF, S_0=1.0%,
—◆— : chemostat, S_0=0.2%,
—◇— : ALF, S_0=0.2%.

obtained by Kim using a standard continuous fermentor was reported as about 0.6 g cell/liter/hr.[19] This productivity is within the productivity range found in this study. Based on these results, we can conclude that ALF can be employed for production of SCP when miscible substrate is used at low concentration. But with higher substrate concentration, fermentation in ALF is somewhat disadvantageous, because of oxygen supply problem.

요 약

단세포 단백질의 합성이나 환성오니법에 의한 폐수처리 등에 응용되고 있는 Air-lift Fermentor (ALF)는 일반적인 기계식 교반 발효조에 비하여 산소전달 효과가 우수한 것으로 알려져 있다. 그러나 미생물의 성장반응에는 적절한 교반이 필요하므로 발효조 내에서는 높은 산소 농도와 함께 활발한 교반이 이루어져야 한다.

본 연구에서는 ALF 내에서의 산소전달에만 국한해오던 연구 방향에서 실제로 *Methylomonas*, sp

를 이용한 methanol fermentation을 행하여 이 발효조의 기능을 조사하였다. 그 결과 교반은 활발히 일어나고 있으나 높은 농도의 산소를 얻기 위해서는 높은 압력하에서나 낮은 dilution rate에서 운전하여야 하는 것으로 나타났다.

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