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High Density Cell Cultivation of *Escherichia coli* in a Dual Hollow Fiber Bioreactor

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이중실관 반응기에서 *E. coli*의 고농도 배양

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The cell density and packing characteristics of *Escherichia coli* immobilized in a dual hollow fiber bioreactor consisting of outer silicone membrane for oxygen transport and three inner isotropic polypropylene hollow fibers for substrate transport were investigated. The cells have grown forming the layer like animal tissue in a nearly 100% packing density. The dry biomass density was 550 g/liter of void volume for cell growth, which was the highest among the biomass densities ever reported.

For the improvement of bioreactor productivity it is necessary to improve strain productivity through gene manipulation and mutation and the second approach is to keep more viable cells in a unit volume of bioreactor. In the case of bacterial cell culture 1-2 g/L can be attained in a shake-flask culture and about 10 g/L is obtained in a well-controlled fermenter[1,2]. Since this level is far lower than the maximum cell packing density of about 200-300 g/L, there is much room for improvement.

For the high density cell culture of *E. coli* the use of pure oxygen, temperature variation and carbon source control were attempted[3-7]. Shiloach and Bauer[4] cultured *E. coli* at 22°C and obtained the cell density of 55 g/L using pure oxygen and semi-continuous feeding of glucose. Wada et al.[8] immobilized *E. coli* in carrageenan beads and achieved a high density of 4.5×10^{10} viable cells/mL of gel. This is three times the maximum cell density obtained in the suspension culture. Recently Inloes et al.[9] grew *E. coli* in asymmetric hollow fiber wall and increased the cell density 10^{12} cells/mL and obtained a 100-fold productivity of β-lactamase compared to that in the shake flask culture.

In this study we cultured *E. coli* in a dual hollow fiber bioreactor developed by Kim and Robertson[10] and investigated several problems arising from the reacter.

MATERIALS AND METHODS

Materials and strains

Yeast extract and trypton were products of Difco Laboratory and glucose was from Hayashi Pure Chemical Industry. The strain used was *E. coli* K-12 from Sigma Chemical Co. (Sigma EC-1). This strain is an alkaline phosphatase-rich mutant.

Bioreactor system

The dual hollow fiber bioreactor used in this study was

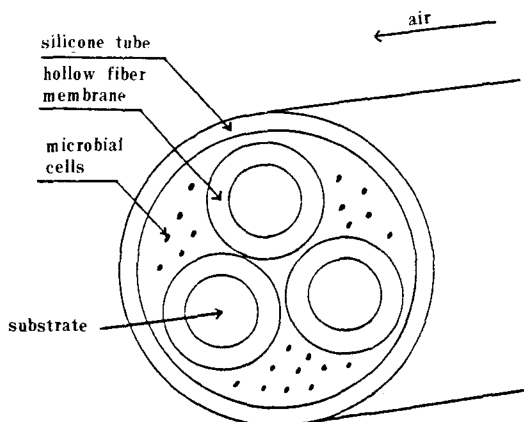


Fig. 1. Cross sectional diagram of a dual hollow fiber unit.

constructed differently from that of Kim and Robertson[10]. The outer layer was the silicone membrane (Dow Corning, U.S.A.; I.D. = 0.147 cm, O.D. = 0.196 cm) while the inner layer was silicone in the reactor devised by Kim and Robertson. Inside the silicone tubing three polypropylene hollow fibers (Enka, West Germany; I.D. = 0.03 cm, O.D. = 0.065 cm) were inserted as shown in Fig. 1. Two types of the reactor systems were used; DHFR1 with one dual hollow fiber unit in a glass tube and DHFR10 with 10 units in a glass tube. Fig. 2 shows a detailed specification of the reactor and a schematic diagram of the experimental setup was shown in Fig. 3.

Methods

Seed culture was grown in a 250-ml flask containing 50 ml of LB medium (yeast extract 10 g/L, trypton 10 g/L, NaCl 10 g/L, pH 7 adjusted with 1N NaOH). The seed culture was inoculated with lyophilized *E. coli* and cultivated on a rotary shaker with 250 rpm at 37°C. When the culture reached its exponential growth phase, it was diluted twenty-fold with distilled water and transferred into the reactor. In the reactor operation medium was supplied through the polypropylene tubes at 2 ml/hr and air was supplied at 100 cc/min. The temperature was maintained at 37°C. In order to see the degree of nutrient consumption LB medium containing 1 g/L of glucose was supplied to DHFR1 and 5 g/L of glucose in LB medium was used in DHFR10. Glucose was analyzed with glucose analyzer (YSI model 23A, U.S.A.)

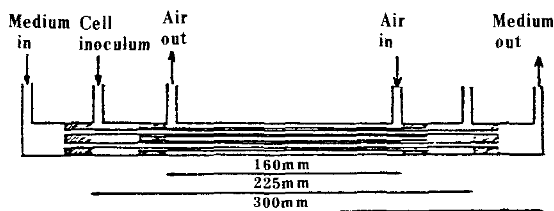


Fig. 2. Detailed specification of dual hollow fiber bioreactor.

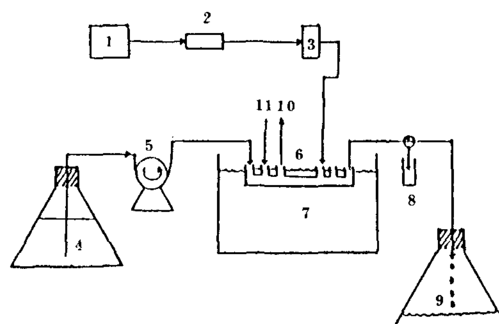


Fig. 3. Schematic diagram of experimental setup.

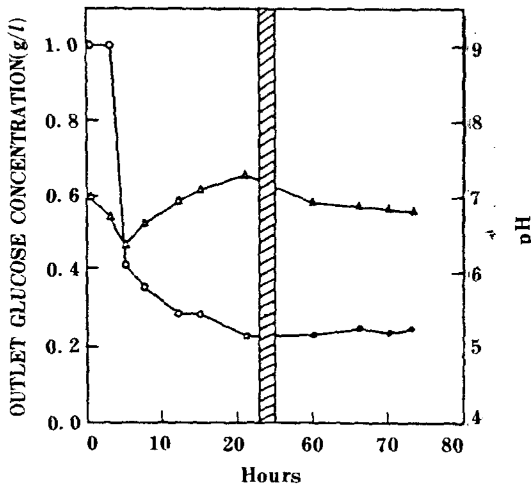
1. air generator
2. air filter
3. rotameter
4. medium reservoir
5. peristaltic pump
6. reactor
7. water bath
8. sampling bottle
9. effluent reservoir
10. air out
11. inoculum port

The cell mass was measured by drying at 90°C for 72 hours the reactor segement cutted in 10 cm and subtracting the fiber weight in the absence of the cell. The space between the inner polypropylene tubes and the outer silicone tube is considered as the volume for the cell growth. A segment of the fiber was used for the electron micrograph in order to observe the cell growth. The procedure for the electron micrograph was the same as that by Inloes et al.[9]

RESULTS AND DISCUSSION

Reactor operation

Fig. 4 shows the glucose concentration and pH change in the DHFR1 and DHFR10 during the operation. When it reached stationary phase, 0.8 g/L of glucose was consumed in DHFR1 and 4.5 g/L of glucose was consumed in DHFR10. Since DHFR10 was the 10-fold scale-up of DHFR1, 8 g/L of glucose should have been consumed. The consumption was lower than that expected. DHFR10 reacter was cut into pieces and examined. Cells were grown densely only in 4 out of 10 fibers and in the rest of the fibers the cell growth was very poor. This is similar to result by Inloes et al.[11]



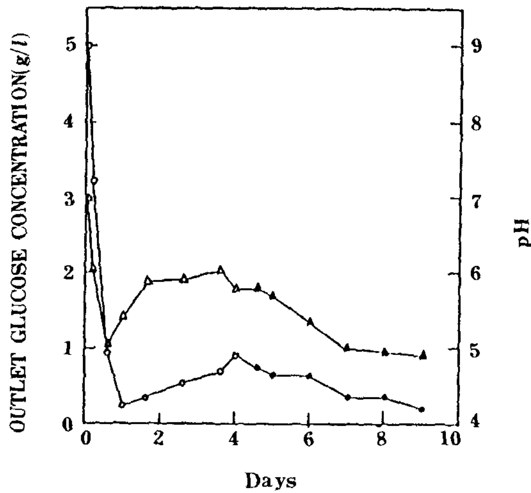


Fig. 4. Glucose and pH profiles in the effluent during the dual hollow fiber reactor operation.

−○−, −●−; glucose concentration.
−△−, −▲−; pH. (a) DHFR1,
(b) DHFR10.

when yeast was grown in hollow fibers in their study. This may be due to the *entrapment of* CO_2 produced and *nonuniform distribution of flow among the fibers*[12]. The maximum pore size of the polypropylene hollow fiber used in this experiment was 0.41 μm, thus no leakage of *E. coli* cells should have occurred, but the densely grown *E. coli* cells leaked through the isotropic polypropylene fiber and was detected in the effluent. In Fig. 4 turbid effluent was denoted by black circles and triangles.

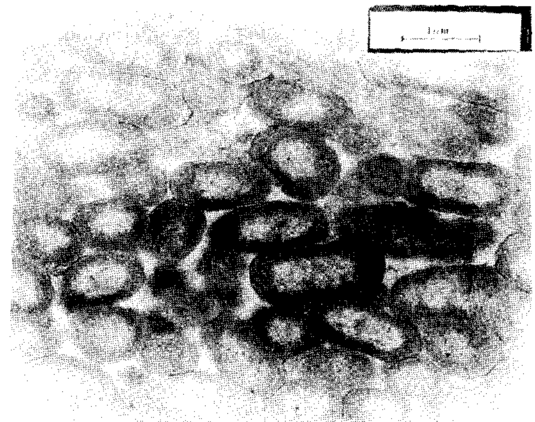
Cell Packing Characteristics

The dry biomass of 550 g/L was obtained in the operation

Table 1. Comparison of cell mass of *E. coli* in various fermentations.

System	Cell density	Reference
○ Shake-flask culture	1−2g/l	
○ Submerged-culture under controlled conditions	10g/l	(1−2)
○ Submerged-culture with pure oxygen supply and semicontinuous feeding of glucose at 22℃.	55g/l	(4)
○ Immobilization with carrageenan beads	4.5×10^{10} viable cells/ml	(8)
○ Immobilization on hollow fiber wall	10^{12} cells/ml	(9)
○ Immobilization in a dual hollow fiber biereactor	550g/l	This work

of DHFR10. This is the highest biomass density ever reported in the literature as shown in Table 1. The electron micrographic examination shows that the cell packing characteristics is similar to that by Inloes et al.[9] Fig. 5(a) is the electron-micrograph of *E. coli* cells grown in the space between silicone tube and polypropylene hollow fiber. *E. coli* cells formed layers like animal tissue and the packing density was nearly 100%. Fig. 5(b) shows the cells packed near the polypropylene fiber. The cells were packed very densely near the fiber and some cells penetrated into the fiber wall. Normal *E. coli* cells retain a rod-shape as shown in Fig. 5(a), but the cells penetrated into the fiber have a spherical shape. Deformation of the cell morphology has occurred to grow in the small space in the pore. Fig. 5(c) shows the amplification of the cells in Fig. 5(b). Some dividing cells are observed.



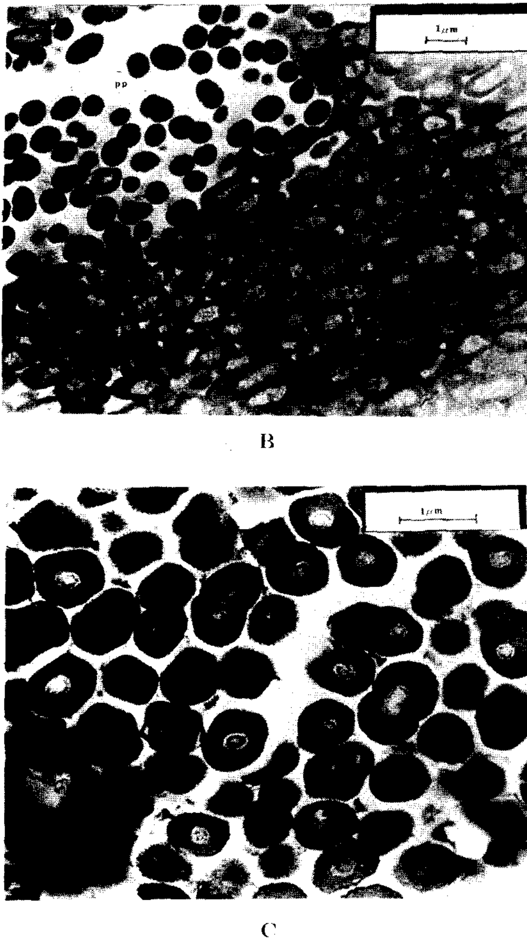


Fig. 5. Electron micrograph of densely packed. *E. coli* cells.

(a) The cells at the interface between silicone tube and polypropylene hollow fiber.
(b) The cells near the polypropylene hollow fiber (pp; polypropylene hollow fiber wall).
(c) The cells leaked into the polypropylene hollow fiber wall.

Conclusion

High density of 550 g/L of *E. coli* was obtained in the culture of dual hollow fiber bioreactor. Examination of the electron micrograph showed that the penetration of *E. coli* into the membrane pore was also possible in the case of isotropic polypropylene membrane. The cells packed nearly 100% of the space forming the layer like animal tissue. In the case of multitubular reactor uniform flow distribution among the fibers was necessary for the successful operation of the reactor.

요 약

산소 투과율이 좋은 silicone tube안쪽에 nutrient 공급을 위한 3 개의 isotropic polypropylene hollow fiber 3 개를 넣어 제작된 이중 실관 반응기에서 *E. coli* cell을 immobilization하여 cell density와 packing characteristics를 조사해 보았다. *E. coli* cell들은 거의 100% packing되어 동물조직에서 처럼 층을 이루면서 자랐고 cell density를 측정해 본 결과 약 550 g/l의 고농도 세포배양이 가능하였다.

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