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Pilot Scale Production of Poly(3-Hydroxybutyrate-*co*-3-hydroxyvalerate) by Fed-batch Culture of Recombinant *Escherichia coli*

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Abstract Production of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB/V)], by fed-batch culture of recombinant *Escherichia coli* harboring a plasmid containing the *Alcaligenes latus* polyhydroxyalkanoate (PHA) biosynthesis genes, was examined in two pilot-scale fermentors with air supply only. In a 30 L fermentor having a K_La value of 0.11 s^{-1}, the final P(3HB/V) concentration and the P(3HB/V) content obtained were 29.6 g/L and 70.1 wt%, respectively, giving a productivity of 1.37 g P(3HB/V)/L-h. In a 300 L fermentor having a K_La of 0.03 s^{-1}, the P(3HB/V) concentration and the P(3HB/V) content were 20.4 g/L and 69 wt%, respectively, giving a productivity of 1.06 g P(3HB/V)/L-h. These results suggest that economical production of P(3HB/V) is possible by fed-batch culture of recombinant *E. coli* in a large-scale fermentor having low K_La value.

Keywords: P(3HB/V), recombinant *E. coli*, pilot-scale fermentor, oxygen transfer rate

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are a carbon and/or energy storage material accumulated by various microorganisms under unbalanced growth conditions (Lee 996a). Recently, these microbial polyesters have been rawing much attention because of their biodegradabil-y and material properties similar to conventional non-egradable plastic materials [1,2]. However, the use of HAs in a wide range of applications has been ham-ered due to their high production cost compared with etrochemical-based polymers [3,4].

There have been many reports on the production of HA by bacterial fermentation to a high concentration vith high productivity [3]. Among many different bac-eria producing PHAs, recombinant *Escherichia coli* har-oring the heterologous PHA biosynthesis genes has een considered as a potential PHA producer for the ommercialization of PHA [3]. Recombinant *E. coli* has een shown to be able to produce a large amount of oly(3-hydroxybutyrate) [P(3HB)] with high productiv-y [5]. Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) P(3HB/V)], a more flexible member of the PHA group, an also be produced to a high concentration by recom-inant *E. coli* from glucose and propionic acid [6]. How-ver, these high performance fermentation results have een obtained only in a lab-scale fermentor with pure xygen supply. The use of pure oxygen increases the production cost substantially, particular in large-scale industrial fermentation.

In this paper, we report pilot-scale, fed-batch fermentation of recombinant *E. coli* for the production of P(3HB/V) with air supply only.

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MATERIALS AND METHODS

Bacterial Strain and Plasmid

The *Escherichia coli* strain used in this study was XL1-Blue (*supE44 hsdR17 recA1 endA1 gyrA96 thi relA1 lacF'* [*proAB*$^+$ *lacIq lacZ*Δ*M15 Tn10(tet^r)*]). The plasmid pJC4 harboring the *Alcaligenes latus* PHA biosynthesis genes and the *parB* locus of the plasmid R1 has been previously described [5].

Culture Conditions

Cells were maintained as a 20% (v/v) glycerol stock at -80°C after growing in Luria-Bertani (LB) medium (containing per liter: 10 g tryptone, 5 g yeast extract and 5 g NaCl). For the production of P(3HB/V), propionic acid was used as a co-substrate providing the precursor of 3HV monomer. Acetic acid induction strategy was used to stimulate the uptake and utilization of propionic acid [6]. The feeding solution contained per liter: 800 g of glucose, 15 g of $MgSO_4 \cdot 7H_2O$, 250 mg of thiamine, 1 g of oleic acid, and either 200 mM of propionic acid for fermentation A or 800 mM of propionic acid for fermentation B and C.

For the fermentation in a 30-L fermentor (KoBio Tech Co., Incheon, Korea; fermentation A and B), seed culture (1 L) was prepared in a 6.6-L fermentor (Bioflo 3000; New Brunswick Scientific Co., Edison, NJ, USA) by batch culture. Seed culture was prepared in a chemically defined MR (pH 6.9) medium as described previously [6]. In fed-batch culture, cells were first grown until an OD_{600} of 0.8 was obtained at 30°C in the 30 L fermentor containing 10 L of MR medium supplemented with 2 g/L of tryptone (Difco Laboratories, Detroit, MI, USA) and 10 mM acetic acid without glucose. Then the feeding solution was added by the pH-stat method. Upon the pH rise indicating the depletion of glucose, 0.25 L of feeding solution was added. Culture pH was controlled at 6.9, except for the periods of nutrient feeding, by the addition of 28% (v/v) ammonia water.

For the fermentation in a 300-L stirred tank fermentor (KoBio Tech Co., Incheon, Korea; fermentation C), seed culture (10 L) was prepared in a 30-L fermentor by batch culture as described above. Cells were first grown until an OD_{600} of 0.8 was obtained at 30°C in 100 L of MR medium containing 2 g/L of tryptone and 10 mM acetic acid. Again, the pH-stat feeding strategy was employed. Upon the pH rise, 2.5 L of feeding solution was added.

In all fermentations, the dissolved oxygen concentration (DOC) was initially maintained above 30% of air saturation by varying the agitation speed up to 520 rpm and 200 rpm, in the 30-L and 300-L fermentors, respectively. When DOC could not be maintained above 30% of air saturation even at maximum agitation speed, cultivation was simply continued at that agitation speed. Aeration rate was maintained at 1 vvm (600 L/h for the 30-L fermentor and 6,000 L/h for the 300-L fermentor).

Analytical Procedures

Cell growth was monitored by measuring the absorbance at 600 nm (OD_{600}) (DU® Series 600 Spectrophotometer, Beckman, Fullerton, CA, USA). Cell concentration, defined as dry cell weight (DCW) per liter of culture broth, was determined by weighing the dry cells in the following manner: 4 mL of culture broth was collected in two pre-weighted tubes and cells were harvested by centrifugation. Harvested cells were washed twice with distilled water and dried in an oven at 95°C to a constant weight. P(3HB/V) concentration and monomer composition were determined by gas chromatography (HP5890, Hewlett-Packard, Wilmington, DE) with *n*-benzoic acid as an internal standard [7]. The P(3HB/V) content (wt%) was defined as the percentage of the ratio of P(3HB/V) concentration to cell concentration.

K_La Measurement

The K_La values of the fermentors were measured by the gassing-in method with oxygen-enriched air [8]. The total gas flow rate was measured with a rotameter and was kept constant so that mixing conditions remain undisturbed. An oxygen electrode (Mettler-Toledo GmbH, Switzerland) was used to monitor the oxygen concentration in the liquid. In the 30-L fermentor, the maximum K_La value was 0.11 sec^{-1} under the conditions of P(3HB/V) production (working volume of 10 L, aeration rate of 1 vvm, agitation speed of 520 rpm, and temperature of 30°C). In the 300-L fermentor, the maximum K_La value was only 0.03 sec^{-1} under the conditions of P(3HB/V) production (working volume of 100 L, aeration rate of 1 vvm, agitation speed of 200 rpm and temperature of 30°C).

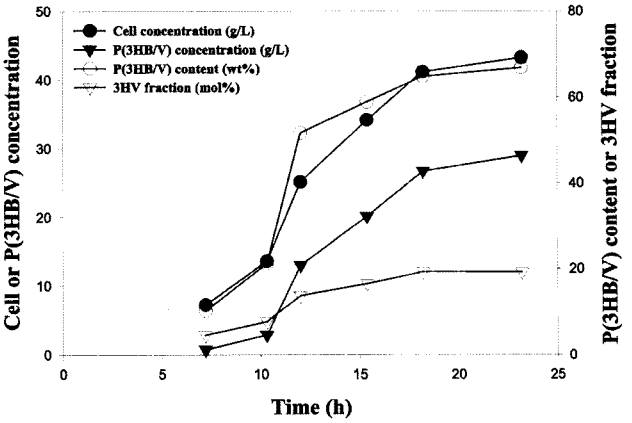
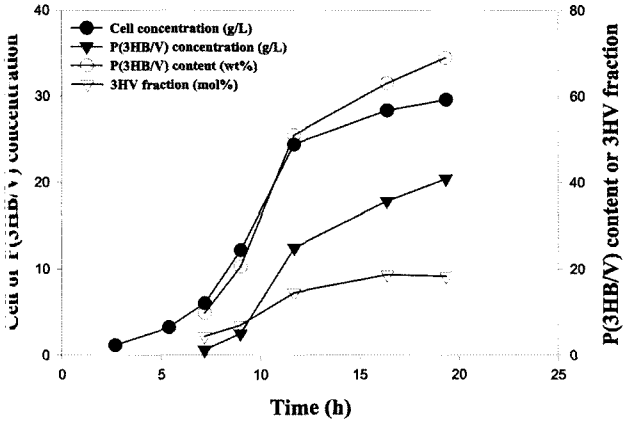


Fig. 1. Time profiles of (A), cell and P(3HB/V) concentration, and (B), P(3HB/V) content (wt%) and 3HV fraction in P(3HB/V) (mol%), during the fed-batch culture of E. coli XL1-Blue (pJC4) in the 30-L fermentor (fermentation B).

RESULTS

Fed-batch cultures of recombinant *E. coli* XL1-Blue (pJC4) were carried out in the 30-L fermentor for the production of P(3HB/V) with air supply only. In fermentation A, a fixed volume of nutrient solution (0.25 L for each dose) was added to increase the concentrations of glucose and propionic acid in the medium to approximately 20 g/L and 5 mM, respectively. The cell concentration, P(3HB/V) concentration, P(3HB/V) content and 3HV fraction obtained in 21.7 h were 42.2 g/L, 29.6 g/L, 70.1 wt% and 5.7 mol%, respectively, giving a productivity of 1.37 g P(3HB/V)/L-h.

To increase the 3HV fraction in P(3HB/V), feeding solution containing higher propionic acid concentration (800 mM) was used (fermentation B). A fixed volume of nutrient solution (0.25 L for each dose) was added by pH-stat strategy to increase glucose and propionic acid concentrations to approximately 20 g/L and 20 mM, respectively. Cell concentration, P(3HB/V) concentration, P(3HB/V) content and 3HV fraction obtained in 23.2 h were 43.3 g/L, 29 g/L, 66.9 wt% and 19.2 mol%, respectively, giving a productivity of 1.25 g P(3HB/V) L/h (fermentation B; Fig. 1). Increased feeding of propionic acid resulted in an increased 3HV fraction in



ig. 2. Time profiles of (A), cell and P(3HB/V) concentration, ıd (B), P(3HB/V) content (wt%) and 3HV fraction in (3HB/V) (mol%), during the fed-batch culture of E. coli XL1- lue (pJC4) in the 300-L fermentor (fermentation C).

(3HB/V). However, the PHA productivity was reduced ue to the toxic effect of the high concentration of ropionic acid in the medium as previously reported [6].

P(3HB/V) production by fed-batch culture of recombinant *E. coli* in a 300-L fermentor was carried out next. When cells were cultivated under the conditions that increased glucose and propionic acid to approximately 0 g/L and 20 mM after each feeding, cell concentration, (3HB/V) concentration, P(3HB/V) content and 3HV action in 19.4 h were 29.6 g/L, 20.4 g/L, 69 wt%, 18.4 mol%, respectively, giving a P(3HB/V) productivity of .06 g P(3HB/V) L^{-1} h^{-1} (fermentation C; Fig. 2).

DISCUSSION

Many fermentation strategies for the efficient production of PHA have been developed in order to commercialize PHAs as a biodegradable plastic material [9,10]. In the case of P(3HB/V), fed-batch culture of recombinant *E. coli* allowed production of 158.8 g/L of P(3HB/V) with a productivity of 2.88 g P(3HB/V) L^{-1} h^{-1} [6]. However, such high performance was achieved in a lab-scale fermentor with pure oxygen or oxygen-enriched air supply. Because it is economically undesirable to supply pure oxygen or oxygen-enriched air for the production of PHA in industrial scale fermentation, we examined (3HB/V) production in a pilot-scale fermentor with air supply only.

The K_La value of the fermentor is an important parameter in the aerobic fermentation process. The K_La values of most lab-scale fermentors range from 0.2 sec^{-1} to 0.5 sec^{-1}, while those of industrial scale fermentors are often lower [11]. Recently, metabolic pathway analysis during P(3HB) biosynthesis in recombinant *E. coli* revealed that much carbon flux was directed to the (3HB) biosynthetic pathway under oxygen limitation [2,13], which is a condition often found in pilot-scale fermentors due to low K_La value. During fermentation for PHA production in a pilot-scale fermentor having low K_La value, it is important to suppress the accumulation of too much PHA in the early growth stage because it results in reduced cell growth and low final PHA concentration [14]. With the feeding strategy used in this study, the P(3HB/V) content could be maintained as low as 20 wt% until the cell concentration reached 14 g/L, which was found to be the optimal cell concentration to support high PHA concentration in a pilot-scale fermentor [14]. Consequently, the final P(3HB/V) concentration could be increased to 29.6 g/L with a high P(3HB/V) content of 70.1 wt% in the 30-L fermentor. In the 300-L fermentor, although the K_La value was only 0.03 sec^{-1}, a high P(3HB/V) content (69 wt%) could still be obtained with a productivity higher than 1 g P(3HB/V) L^{-1} h^{-1}. A higher productivity obtained in 30-L fermentor having higher K_La value than that in 300-L fermentor seems to be mainly due to that a higher residual cell mass could be obtained under the former condition. From these results, it was concluded that a high P(3HB/V) content with a high P(3HB/V) productivity can be achieved by fed-batch culture of recombinant *E. coli* in a pilot-scale fermentor. If a pilot-scale fermentor having a higher K_La value was used, a higher P(3HB/V) concentration could be obtained with higher P(3HB/V) productivity mainly because a higher residual cell mass can be obtained. In the process analysis and economic evaluation of the processes for the production of PHA, PHA content was the one of the most important factors for the economical production of PHA by bacterial fermentation [4]. Therefore, the results obtained in this study suggest that P(3HB/V) can be economically produced by recombinant *E. coli* in a large-scale process.

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REFERENCES

[1] Lee, S. Y. (1996) Bacterial polyhydroxyalkanoates. *Biotechnol. Bioeng.* 49: 1-14.

[2] Steinbüchel, A. and B. Füchtenbusch (1998) Bacterial and other biological systems for polyester production. *Trends Biotechnol.* 16: 419-427.

[3] Lee, S. Y. (1996) Plastic bacteria? Progress and prospects for polyhydroxyalkanoate production in bacteria. *Trends Biotechnol.* 14: 431-438.

[4] Choi, J. and S. Y. Lee (1997) Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. *Bioprocess Eng.* 17: 335-342.

[5] Choi, J., S. Y. Lee, and K. Han (1998) Cloning of the *Alcaligenes latus* polyhydroxyalkanoate biosynthesis genes and use of these genes for enhanced production of poly(3-hydroxybutyrate) by recombinant *Escherichia coli*. *Appl. Environ. Microbiol.* 64: 4897-4903.

[6] Choi, J. and S. Y. Lee (1999) High level production of

poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by fed-batch culture of recombinant *Escherichia coli*. *Appl. Environ. Microbiol.* 65: 4363-4368.

[7] Braunegg, G., B. Sonnleitner, and R. M. Lafferty (1978) A rapid gas chromatographic method for the determination of poly-β-hydroxybutyric acid in microbial biomass. *Eur. J. Appl. Microbiol. Biotechnol.* 6: 29-37.

[8] Chang, H. N., B. Halard, and M.-Y. Murray (1989) Measurement of K_La by a gassing-in method with oxygen-enriched air. *Biotechnol. Bioeng.* 34: 1147-1157.

[9] Lee, S. Y. and S. J. Park (2002) Fermentative production of SCL-PHAs. pp. 262-290. In: Y. Doi and A. Steinbüchel (eds.). *Biopolymers*. Vol. 3a. Wiley-VCH, Germany.

[10] Lee, S. Y. and S. J. Park (2002) Biosynthesis and fermentative production of SCL-MCL-PHAs. pp. 317-336. In: Y. Doi and A. Steinbüchel (eds.). *Biopolymers*. Vol. 3a. Wiley-VCH, Germany.

[11] Mehta, V. D. and M. M. Sharma (1971) Mass transfer in mechanically agitated gas-liquid contactors. *Chem. Eng. Sci.* 26: 461-479.

[12] van Wegen, R. J., S. Y. Lee, and A. P. J. Middelberg (2001) Metabolic and kinetic analysis of poly(3-hydroxybutyrate) production by recombinant *Escherichia coli*. *Biotechnol. Bioeng.* 74: 70-80.

[13] Wong, H. H., R. J. van Wegen, J. Choi, S. Y. Lee, and A. P. J. Middelberg (1999) Metabolic analysis of poly(3-hydroxybutyrate) production by recombinant *Escherichia coli*. *J. Microbiol. Biotechnol.* 9: 593-603.

[14] Park, S. J., J. P. Park, and S. Y. Lee (2002) Production of poly(3-hydroxybutyrate) from whey by fed-batch culture of recombinant *Escherichia coli* in a pilot-scale fermenter. *Biotechnol. Lett.* 24: 185-189.

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