J. Microbiol. Biotechnol. (2000), **10**(5), 628–631

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Increased Poly(3-Hydroxybutyrate) Accumulation in Recombinant *Escherichia coli* from Whey by Agitation Speed Control

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Received: April 26, 2000
Accepted: September 1, 2000

Abstract The timing of poly(3-hydroxybutyrate) (PHB) biosynthesis was controlled by varying the agitation speed of a stirred tank fermentor during the pH-stat fed-batch culture of recombinant *Escherichia coli* strain GCSC 6576 harboring pSYL107. Using a concentrated whey solution containing ca. 200 g/l lactose as the nutrient feed, the PHB content was only 57% after 35 h due to volumetric limitation of the fermentor. However, by limiting the oxygen by maintaining the agitation speed at 300 rpm, the final PHB content increased to 70% after 70 h with a cell concentration of 15 g/l. When the agitation speed was increased up to 500 rpm, a cell concentration of 31 g/l with 80% PHB was obtained after 52 h. A further increase in the maximum agitation speed increased the cell concentration, PHB concentration, and PHB productivity, however, the PHB content decreased to 56–58%.

Key words: Poly(3-hydroxybutyrate), PHB, whey

Polyhydroxyalkanoates (PHAs) are biodegradable polyesters accumulated by many microorganisms. They have attracted considerable attention from academia and industry due to their various technical applications in industry, agriculture, medicine, pharmacology, and other areas [7, 8, 13, 14]. The use of waste products such as dairy whey from cheese industry decreases the high production cost of PHA since about 40% of the total production cost is for raw materials [2, 4]. Recently, one large Australian dairy company invested AU$18M in drying technology for disposing of dairy whey (personal communication from A. P. J. Middelberg, Cambridge University, U.K.). It was believed that a full PHA process could be constructed with approximately half of this cost.

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Recombinant *Escherichia coli* strains harboring the heterologous PHA biosynthesis genes are good candidates for the production of PHAs because they have several advantages over other wild-type PHA producers [3, 9]. One advantage is that *E. coli* strains can utilize cheap carbon sources. Lee *et al.* [11] examined various recombinant *E. coli* strains for their ability to accumulate a large amount of poly(3-hydroxybutyrate) (PHB), a homopolymer of 3-hydroxybutyrate, in a whey-based medium. They obtained a high cell concentration of 87 g/l with 80% PHB after 49 h using a fed-batch culture of the selected recombinant *E. coli* strain [16]. During the fermentation, however, portions of the culture broth had to be removed due to volumetric limitation of the fermentor caused by the relatively low concentration of lactose in whey.

In the present study, we examined the effects of the maximum agitation speed on PHB accumulation and a new operational method to control the timing of PHB biosynthesis in recombinant *E. coli*. By limiting the maximum agitation speed during the cultivation, cells containing 70–80% PHB were produced without removing any culture broth.

MATERIALS AND METHODS

Microorganism and Growth Condition

E. coli strain GCSC 6576 harboring pSYL107 [6] was grown on an LB medium supplemented with 100 mg/l of ampicillin. For the fed-batch culture, the initial medium was (per liter): whey powder containing 11.5% (w/v) proteins and 74% (w/v) lactose (Binggrae, Korea), 30 g; $(NH_4)_2SO_4$, 4 g; KH_2PO_4, 13.3 g; $MgSO_4 \cdot 7H_2O$, 1.2 g; citric acid, 1.7 g; and a trace element solution [5], 10 ml. The initial pH of the medium was adjusted to 7.0 with NaOH. The pH was controlled with 28% (v/v) NH_4OH solution, and the temperature was controlled at 30°C. The fermentation was

carried out in a 2.5-l jar fermentor (Korea Fermentor Company, Inchon, Korea). The initial volume of the culture was 0.8 l. The feeding nutrient solution was prepared by dissolving 310 g of whey powder and 0.36 g of $MgSO_4 \cdot 7H_2O$ in 1 l of distilled water. The solution was autoclaved, cooled down to room temperature, and then centrifuged in a sterilized bottle to remove the precipitates. The feeding strategy in the fed-batch culture was a pH-stat with a high limit. When the pH rose above 7.1, 80 ml of the feeding solution was automatically added.

Analytical Procedures

The microbial growth was monitored by measuring the absorbance of the culture broth at 600 nm. The dry cell weight was determined gravimetrically. The PHB was determined by gas chromatography [1]. The cell concentration was defined as the dry cell weight per liter of culture broth. The residual biomass was defined as the cell concentration minus the PHB concentration. The PHB content was defined as the ratio of the PHB concentration to the cell concentration given as a percentage.

Results and Discussion

Figure 1 shows the result of the fed-batch fermentation without any oxygen limitation. The dissolved oxygen was maintained above 10% by increasing the agitation speed up to 1,300 rpm and supplying pure oxygen thereafter. The operation was stopped after 35 h because the culture volume (2.19 l) approached the total fermentor volume (2.5 l). At that time, the cell and PHB concentrations were 55 and 32 g/l, respectively, resulting in a PHB content of 57%. Such a low PHB content is economically unfavorable since a high purification cost results in a high overall production cost [2]. Wong and Lee [16] obtained a cell concentration of 87 g/l containing 80% PHB in a pH-stat fed-batch culture by removing some of the culture broth intermittently during the cultivation. If the fermentation had been carried out without removing any culture broth, they estimated that the final culture volume would have increased to 28 l after 49 h from an initial volume of 1.6 l. This was because a highly concentrated whey solution, like glucose syrup (ca. 700 g/l), could not be prepared as the feeding nutrient solution due to the low solubility of lactose (ca. 200 g/l), the major component in whey.

Accordingly, to overcome this problem, we developed in this study a new operation method to control the timing of PHB biosynthesis in recombinant *E. coli*, so that the cells can produce PHB earlier and accumulate it to more than 70% of the dry cell weight without the removal of any of the culture broth. It is known that recombinant *E. coli* does not require a nutrient limitation for PHB biosynthesis and can accumulate PHB during growth [10]. In this study, it was noticed that PHB biosynthesis increased as the cell growth was gradually decreased. This condition was achieved by limiting the oxygen transfer rate to the culture broth. Also, a sudden switching to an insufficient oxygen supply condition while maintaining the dissolved oxygen concentration at 1-5% did not significantly increase the PHB biosynthesis [15]. Thus, it would appear that under an oxygen limiting condition, the gradual decrease of cell growth was achieved by increasing the agitation speed up to a certain value while continuously supplying air.

Figure 2 shows the result of a fed-batch fermentation with limited oxygen when the agitation speed was maintained at 300 rpm. After 70 h, the cell concentration was as low as 15 g/l since the oxygen supply was insufficient. However, the final PHB content reached 70% without the removal of any of the culture broth during the cultivation. To obtain a higher cell concentration, the agitation speed was increased up to 500 rpm, and the result is shown in Fig. 3. After 52 h,

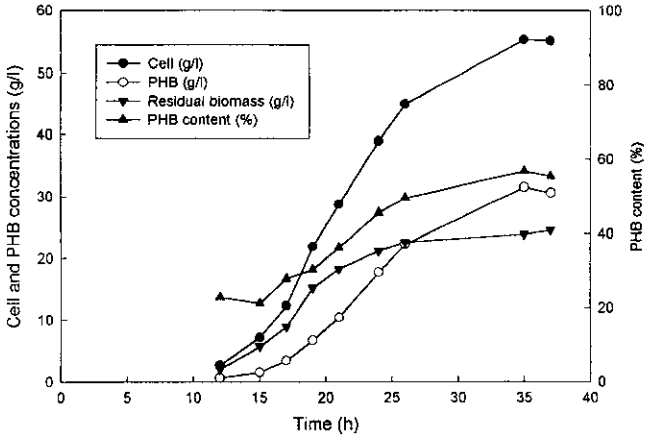

Fig. 1. Fed-batch culture of recombinant *E. coli* without oxygen limitation.
The dissolved oxygen was maintained above 10% by increasing the agitation speed up to 1,300 rpm and supplying pure oxygen thereafter.

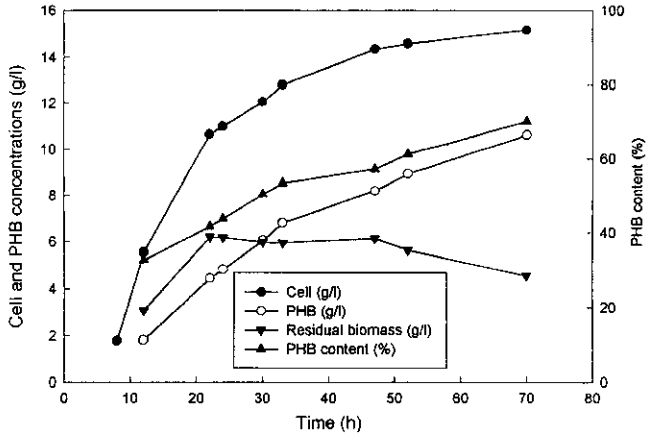

Fig. 2. Fed-batch culture of recombinant *E. coli* with oxygen limitation (maximum agitation speed=300 rpm).

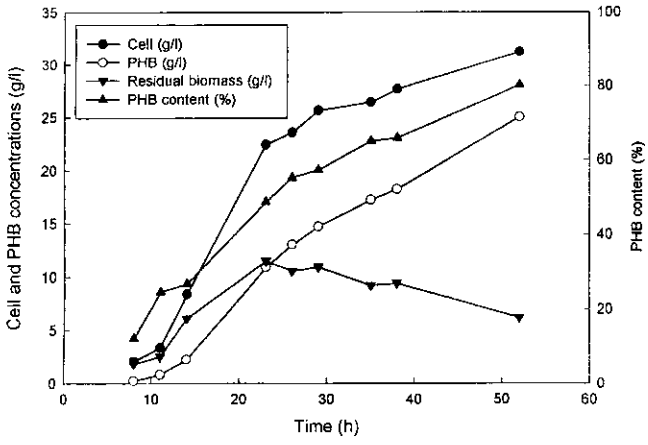

Fig. 3. Fed-batch culture of recombinant *E. coli* with oxygen limitation (maximum agitation speed=500 rpm).

the cell concentration and PHB content were 31 g/l and 80%, respectively.

In other fed-batch fermentations, the maximum agitation speeds were increased up to 700 and 900 rpm. Table 1 summarizes the final cell concentrations, PHB concentrations, PHB contents, and PHB productivities obtained under various fed-batch conditions. The cell concentration, PHB concentration, and PHB productivity increased as maximum agitation speed increased, and the highest PHB content was 80% when the agitation speed was 500 rpm. When the maximum agitation speed was above 700 rpm, the PHB content was as low as 56–58%, because the operations were stopped by the increased culture volume. Choi and Lee [2] examined several factors affecting the production cost of PHB. They concluded that the PHB content was the most important, because it had multiple effects on the PHB yield and recovery efficiency, while the PHB productivity affected only the equipment-related costs. From the above results using the proposed fermentation system and in terms of economic PHB production, the optimum culture condition would seem to be at a maximum agitation speed of 500 rpm. It is also apparent that oxygen limitation can enhance PHB biosynthesis in recombinant *E. coli* by decreasing the cell growth rate. This is the first report that the timing of PHB biosynthesis in recombinant *E. coli* can be artificially controlled, similar to the timing of nitrogen or phosphate limitation controlling PHB biosynthesis in *Ralstonia eutropha* [5, 12]. Since it was necessary to select either a high PHB content or high PHB productivity, the former was chosen for its greater economic benefit for the whole process. The advantage of this culture technique is that cells containing a high PHB content can be produced without the use of expensive pure oxygen.

Table 1. Effect of maximum agitation speed on cell concentration, PHB concentration, PHB content, and PHB productivity.

Maximum agitation speed (rpm)	Cell conc. (g/l)	PHB conc. (g/l)	PHB content (%)	PHB productivity (g/l/h)
300	15	11	70	0.15
500	31	25	80	0.48
700	34	20	58	0.68
900	37	21	56	0.87
1,300 and supplying pure oxygen	55	32	57	0.90

Acknowledgments

This work was supported by the Korea-Australia International Cooperative Research Program run by the Korean Ministry of Science and Technology. We are grateful to Mi Ae Lim and Taek Wan Kim for their help.

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