Recovery of Poly(3-hydroxybutyrate) from Coagulated Ralstonia eutropha Using a Chemical Digestion Method

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For economic recovery of poly(3-hydroxybutyrate) (PHB) from culture broths of Ralstonia eutropha containing PHB, Al-based and Fe-based coagulants were used in the pretreatment step. The coagulated cells were then separated by centrifugation, and PHB was extracted by chemical digestion with a sodium hypochlorite/chloroform dispersion solution. The practical upper limits of dosage were found to be 1,500 mg-Al/L and 1,000 mg-Fe/L, respectively, for Al- and Fe-based coagulants. When the harvested cells were treated with a 50% sodium hypochlorite/chloroform dispersion solution, PHB recovery and purity were 90–94% and 98–99%, respectively. The influence of the use of coagulants on the PHB recovery process was found to be insignificant. Despite the residual Al and Fe in the recovered PHB (less than 450 mg-Al/kg-PHB and 750 mg-Fe/kg-PHB, respectively), no detectable amounts of Al and Fe were leached from films made of the recovered PHB under acidic conditions. The use of Fe-based coagulants is less recommended because the Fe impurity can cause an unwanted colorization problem in the final product.

Introduction

Poly(3-hydroxyalkanoate) (PHA) is a group of biodegradable, biocompatible, microbial thermoplastics that are regarded as potentially useful polyesters replacing conventional petroleum-derived polymers. Poly(3-hydroxybutyrate) (PHB) is one of the best known members of PHA. Ralstonia eutropha, which accumulates PHB as intracellular carbon and energy storage material under unfavorable growth conditions, has been widely used for the production of PHB because it is easy to grow, it accumulates a large amount of PHB, and its physiology and genetic information are well understood (1). The use of biologically produced PHA and copolymers is currently limited as a result of their high production costs. In particular, the recovery process is the primary obstacle to commercial exploitation. Therefore, a number of solvent extraction processes have been developed to recover and purify PHB from biomass. PHB can be extracted from cells with solvents such as methylene chloride, propylene carbonate, dichloroethane, and chloroform (2–5). As an alternative to the solvent extraction, enzymatic digestion methods were developed by ZENECA (6). A differential digestion method employing sodium hypochlorite was proposed (7, 8). A dispersion method employing a sodium hypochlorite solution and chloroform that takes advantage of digestion and extraction was also developed (9, 10).

To commercialize PHA production processes, the development of efficient methods for cell separation from culture broth is also important. In our previous studies, pretreatment with Al-based or Fe-based coagulants was applied before the separation of PHB-containing R. eutropha cells from the culture broth by centrifugation (11, 12). The energy demand for cell recovery with coagulation was found to be only 3–11% of that without it (11). The cell recovery was higher than 95% (12). It was speculated, however, that PHB recovery efficiency and purity might be compromised by the use of coagulants. Therefore, in this study, the effects of coagulant type and concentration were investigated in recovering intracellular PHB by using hypochlorite/chloroform dispersion.

Materials and Methods

Cultivation of R. eutropha. R. eutropha (NCIMB 11599) was cultivated in a 60 L fermentor (Korea Fermentor Co., Korea) operated in a fed-batch mode (13). The medium composition for the seed culture was (per liter) 10 g of glucose, 1.5 g of KH2PO4, 9.0 g of Na2HPO4·12H2O, 1.0 g of (NH4)2SO4, 0.2 g of MgSO4·7H2O, and l mL of trace metal solution. The trace metal solution contained 10 g of FeSO4·7H2O, 2.0 g of CaCl2·2H2O, 2.25 g of ZnSO4·7H2O, 0.5 g of MnSO4·4H2O, 1.0 g of CuSO4·7H2O, 0.1 g of (NH4)6Mo7O24, and 0.2 g of Na2B4O7·7H2O in 1 L of a 5 N HCl solution. The initial medium composition for fed-batch culture was (per liter) 20 g of glucose, 4.44 g of KH2PO4, 4.0 g of (NH4)2SO4, 1.2 g of MgSO4·7H2O, 1.7 g of citric acid, and 10 mL of trace metal solution. The culture pH was adjusted to 6.8 with 28% NH4OH solution and 5 N HCl solution.

Coagulation. Three Al-based and two Fe-based coagulants were used in this study: aluminum sulfate (Al2(SO4)3; concentration, 8% Al2O3 equivalent), polyalu-
minum hydroxide chloride silicate (AlaSib(OH)cCld; concentration, 17% Al2O3 equivalent; PACS), polyaluminum hydroxide chloride (Ala(OH)bClcH2O; concentration, 17% Al2O3 equivalent; Hi-PAX), ferric sulfate (Fe2(SO4)3), and Ferix-3 (FeClxOHy, x + y = 3). The basicity and density of PACS and Hi-PAX were 45–50% and 1.39, respectively. The coagulation experiments were carried out in 100 mL beakers, and the volume of culture broth treated was 25 mL. Cell concentration in the used culture broth is about 83 g-dry cell weight/L. After the pH of culture broth was adjusted to 11–12 by using 10 N NaOH, a coagulant was added to the culture broth. The solution was mixed vigorously at first for 1 min and then gently for 3 min.

**PHB Recovery from Coagulated Cells.** A coagulated cell sample of 10 mL was placed in a 15 mL centrifugal tube and the cells were harvested by centrifugation at 45 × g for 10 min. The harvested cells (1.25 g-dry cell weight) were treated with a dispersion of 50 mL chloroform and 50 mL sodium hypochlorite solution. The concentrations of sodium hypochlorite solution used were 30%, 35%, 40%, 45%, and 50% (v/v). After the mixture was agitated at 30 °C for 90 min, it was centrifuged at 8,000 rpm for 10 min. The resulting PHB-containing bottom phase (chloroform phase) was withdrawn and added into a nonsolvent solution (70% methanol in water). The PHB precipitated was recovered by filtration (Whatman no. 1 filter paper; pore size 11 μm; VWR Scientific, USA), and then dried at 70 °C for 5 h. All experiments were duplicated.

**Leaching Experiments of Aluminum and Iron from Purified PHB.** The leaching experiment of coagulants from purified PHB was carried out in 500 mL shaking flasks. PHB powder (2.5 g) and a PHB film (2 mm thickness, 70 mm × 70 mm, 2.5 g) made of the purified PHB by a hot press (Samho Co., Korea) were treated with a 0.1 N HCl solution (200 mL) at 30 °C and 200 rpm for 8 h, respectively.

**Analysis.** Purity and recovery (%) of PHB were determined by using a gas chromatograph (HP5890, Hewlett Packard Co., USA) (14). The standard PHB sample with a purity of 99.9% was from Marborough Biopolymer (MW 643,000). Residual concentrations of aluminum and iron in the recovered PHB were measured using an inductively coupled plasma atomic emission spectrophotometer (Plasma 40, Perkin-Elmer Co., USA) after digestion with 5 N HCl and 5 N HNO3 (15). Aluminum and iron concentrations in acid leachates were measured by a colorimetric method with a spectrophotometer (DR/4000, Hach Co., USA).

**Results and Discussion**

**Effects of Coagulant Dosage on PHB Recovery and Purity.** The effects of coagulant dosage on PHB recovery and purity are shown in Figures 1 and 2. The sodium hypochlorite concentration was 30%. In the case of AI-based coagulants, the PHB recovery decreased with coagulant dosage (Figure 1). When the polymeric coagulants (PACS and Hi-PAX) were used, the PHB recovery decreased more significantly with coagulant dosage. It seems likely that coagulant in cell coagulates hampered contacts between cells and hypochlorite, slowing down the digestion of cell envelope. However, it was also observed that the PHB recovery was not compromised when the dosage was lower than 1,500 mg-Al/L for all of the AI-based coagulants tested. These results imply that the practical upper limit of coagulant dosage is about 1,500 mg-Al/L, although a higher dosage would save more energy cost in the subsequent step of cell separation by centrifugation.

The data for PHB recovery are shown in Figure 2 when the Fe-based coagulants were used. As with AI-based coagulants, the PHB recovery decreased with coagulant dosage, but much less significantly. It appears that the PHB recovery was not affected significantly when the coagulant dosage was lower than 1,000 mg-Fe/L for both Fe-based coagulants tested. The purity of recovered PHB was over 98% regardless of coagulant type and dosage without being compromised by the addition of coagulant.

In our previous work, when high cell density broth of 40–210 g-DCW/L was pretreated with tested coagulants, the optimum dosages of AI- and Fe-based coagulants for 95–98% cell recovery by centrifugation at 45 × g have
been found in the range of 1,000–1,500 mg-Al/L and 1,000 mg-Fe/L, respectively (12). Thus, the effect of coagulant concentration on PHB recovery over the optimum coagulant dosage used for cell recovery is insignificant.

We studied the effects of sodium hypochlorite concentration under a fixed coagulant concentration of 2,160 mg-Al/L. The PHB recovery data for various sodium hypochlorite concentrations (30%, 35%, 40%, 45%, and 50%) are presented in Figure 3. The PHB recovery monotonically increased with sodium hypochlorite concentration without showing a maximum up to 50% of sodium hypochlorite concentration. The purity slightly increased with sodium hypochlorite concentration. The treatment by sodium hypochlorite of above 50% concentration resulted in a significantly reduced molecular weight and also caused difficulties due to increased viscosity (10).

When the concentration of sodium hypochlorite in dispersion solution was fixed at 50%, the effect of coagulant concentration on recovery and purity of PHB was investigated. The PHB recovery changes with the concentration of PACS, Hi-PAX, and Ferix-3 are given in Figures 4 and 5. In the tested range of 0–1,500 mg-Al/L or 0–2,000 mg-Fe/L, the PHB recovery and purity were over 90% and 98%, respectively, for three coagulants. It should be noticed that the PHB recovery is quite insensitive to the dosage of coagulant, being different from the case of 30% hypochlorite concentration (Figure 1). The purity slightly decreased with the coagulant concentration.

Residual Coagulants in Purified PHB. As it was considered that coagulants used in cell separation might remain as impurities in the purified PHB, the content of residual coagulants was measured. Figure 6 shows the residual aluminum and iron contents in the purified PHB when the sodium hypochlorite concentration was 50%. Even if no coagulants were added, the purified PHB included 86 mg-Al/kg-PHB and 100 mg-Fe/kg-PHB. It is considered that the source of these impurities was the culture medium and the solvent for PHB extraction from cells. As mentioned earlier, a coagulant dosage up to 1,500 mg-Al/L would be practical if we want to avoid a significant loss of PHB. As shown in Figure 6, the residual aluminum was less than 450 mg-Al/kg-PHB when up to 1,500 mg-Al/L of Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, PACS, or Hi-PAX was used. In the case of Fe-based coagulants, residual Fe increased in proportion to coagulant dosage. It was less than 750 mg-Fe/kg-PHB when up to 1,000 mg-Fe/L of coagulant was added. In addition, the PHB recovery with Fe-based coagulants was found to be problematic as a result of the coloration (light red) of recovered PHB caused by the residual Fe. Therefore, we recommend Al-based polymeric coagulant such as Hi-PAX or PACS as coagulants for cell recovery.

The potential applications of biodegradable polymers are bags and sacks for waste disposal, shopping, food packaging, cosmetic products, infant products, products for fishing and leisure, and mulching films. When containers made of PHB are used for solutions, residual Al in PHB may be leached into the solution depending upon the conditions. This can be harmful to human health, especially when the container is used for food storage. Therefore, the amount of Al and Fe eluted from a piece of film and powder of the recovered PHB were measured under acidic conditions (Figure 7). No detectable amount

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of Al and Fe were eluted from the film sample. On the contrary, when powder PHB was treated, a significant amount of aluminum and iron was leached into the solution. PHB tinted by residual Fe could be decolorized with acid washing.

Conclusions

When up to 1500 mg-Al/L of Al-based coagulants or up to 1000 mg-Fe/L of Fe-based coagulants was used before cells were separated by centrifugation and PHB was extracted by chemical digestion with a 50% sodium hypochlorite/chloroform dispersion solution, PHB recovery and purity were 90–94% and 98–99%, respectively. Residual Al and Fe contents in the recovered PHB were less than 450 mg-Al/kg-PHB and 750 mg-Fe/kg-PHB, respectively. It was observed that no detectable amounts of Al and Fe were leached from the films made of the recovered PHB when they were treated with a 0.1 N HCl solution for 24 h at 30 °C. The use of Fe-based coagulants is less recommended because the Fe impurity can cause an unwanted colorization problem in the final product.

References and Notes


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