Extraction and recovery process of Poly-β-hydroxybutyrate from recombinant *Escherichia coli*

Yao LING

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Department of Chemical Engineering

Faculty of Engineering

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STATEMENT

The work described in this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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SUMMARY

In response to the increasing concern about the disposal of petroleum-derived plastic, the development of biodegradable plastic has drawn much research attention. Poly-β-hydroxybutyrate (PHB) and its copolymer are considered to be the potential candidate. However, their high production cost has restricted their widespread use. PHB extraction and recovery has received less attention on a scientific basis comparing with PHB formation and processing of the purified product, despite its importance in determining overall production cost and altering properties of the final product. The industrial extraction method in use is complex in procedure and also involves the treatment with expensive chemical materials, which raised the production cost and also resulted in the low product quality. The aim of this study is to develop the process for PHB recovery from recombinant *E. coli*, using homogenization and centrifugation as the basic unit operations, with effects directed to reduce the PHB overall production cost.

The use of homogenization and centrifugation for PHB recovery was investigated and characterized. Homogenization was found efficient in PHB release from recombinant *E. coli* and high disruption efficiency was obtained after two passes of homogenization. Repeated centrifugation effected cell debris removal, while ensure a reasonable PHB collection efficiency. The fractionation was improved by incorporating NaOCl digestion into the process, which facilitated other cellular components removal such as protein and DNA as well. DNA denaturation and adherence to PHB granules during treatment were eliminated by restructuring unit operations sequence. A process which combined three homogenizer passes, three centrifuge passes, coupled with mild NaOCl treatment was established giving PHB purity of 96.5% and recovery rate of 79.5%, with negligible DNA and protein contamination levels.

For the simulation of the fractionation of cell debris and PHB, some fundamental work was conducted subsequently. Debris comminution of *E. coli* cell containing PHB by homogenization was characterized using Cumulative Sedimentation

Analysis (CSA), and PHB granules released were sized by an Analytical disc centrifuge (CDS). Both size distribution can be described by the Boltzmann function. The mathematical model for the prediction of cell debris size obtained after homogenization was validated. The effect of fermentation on cell debris comminution was discussed. The evidence about the effectiveness of freeze and thaw treatment of *E. coli* cells on following debris comminution by homogenization was presented. Chemical treatment (NaOH and NaOCl) on cell debris size reduction was assessed. NaOCl was recommended for the use of PHB recovery because of the impact of NaOH on PHB aggregation. NaOCl at mild condition showed less effect on cell debris micronisation compared with NaOH, but more efficient than that by having additional homogenization pass.

Particle fractionation in a disc-stack centrifuge was described by grade-efficiency curves. The simulation of the fractionation of cell debris and PHB granules confirmed that repeated centrifugation effected the fractionation of cell debris with PHB granules and indicated that cell debris removal and PHB collection in a given operation condition vary with the centrifugation feedrate and the homogenization passage prior to centrifugation. The importance of the first centrifugation on the overall cell debris removal was identified. The significance of the micronisation of cell debris prior to the centrifugation on the overall purification was highlighted. The significance of NaOCl treatment on cell debris fractionation was identified.

Cell debris digestion by NaOCl was modelled using response surface methodology, and expressed as a function of NaOCl concentration, cell concentration and the number of homogenization passes prior to centrifugation. PHB stability during the treatment was also investigated and modelled. Cell concentration (B) and NaOCl concentration (C) were identified as major effects on cell debris micronization, and their effect can be well summarized by the ratio B/C. It was recommended that the sole use of NaOCl treatment was unlikely to provide sufficient cell debris size reduction, while maintaining a high PHB collection, and other unit operations such as multiple homogenization should be included in association with NaOCl treatment. The Boltzmann function was also

reassessed statistically for the expression of cell debris size distribution and it was found that the Boltzmann function might not be the best model for the expression of cell debris size digested with NaOCl.

Based on the information obtained in the preceding studies, the PHB recovery process was finally optimized through the simulation of cell debris and PHB fractionation. The optimal process which met the cell debris removal target (〈95% cell debris removal) at the minimum overall production cost was then identified, involving 6 passes homogenization and 2 passes centrifugation, incorporating NaOCl treatment at a concentration of 0.85 g/L active chlorine. The process was demonstrated at pilot scale, a cell debris removal of 96.11% and PHB recovery rate of 93.65%, were achieved, and the estimated PHB production cost was US$6.57 /kg PHB. The efficiency on DNA and protein removal was also revealed. The results obtained can be simply extrapolated to full-scale PHB manufacture. It is believed that the optimal process was competitive to the existing industrial method in use in terms of PHB purity and recovery, but with significant reduction on production cost.

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