Production of the Biodegradable Plastic PHB (Poly-3-hydroxybutyrate) using Recombinant Escherichia coli Bacteria.

by

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July 2000
Abstract

Disposal of plastic, in particular packaging, is a serious problem confronting many countries. Part of a solution lies in using biodegradable plastics such as poly-hydroxyalkanoates (PHAs), which can be produced by bacterial fermentation. Substantial developmental work is required to achieve economically-attractive PHA production.

The aim of this thesis is to improve the understanding of PHA production by recombinant Escherichia coli, with a view to guiding future developmental work. Such understanding is an important aid to achieving economic improvements.

This aim is achieved via the following methodology:
- Several experimental techniques are developed and tested. Quantitative, repeatable fermentation profiles are obtained for acetyl-CoA and 3-hydroxybutyryl-CoA concentration, and the production and consumption of major extracellular metabolites.
- An economic description of PHA production by E. coli is formulated, highlighting areas where significant cost reductions are possible and setting the direction for the remainder of the thesis.
- A quantitative analytical framework is developed for the cellular metabolism of XL1-Blue(pSY1.107) during PHB production. Metabolic flux analysis techniques are used to quantify the major fluxes.
- The in-vitro kinetics of the PHB pathway are adapted for use with in-vivo fermentation data. Likely ranges of concentration are established for enzymes, cofactors, and intermediates of the PHA pathway. The sensitivity of PHB production rate to a variety of metabolic changes is established using metabolic control analysis.
- The effects of different oxygen-supply regimes and different nutrient feeding strategies are also examined and explained using the metabolic and kinetic models.

The models are used to explain the underlying factors influencing the progress of a typical PHB fermentation. This thesis also suggests numerous areas where further improvements to PHB production are possible, and proposes ways in which they could be implemented.
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