Effects of Cell Fermentation Time and Biomass Drying Strategies on the Recovery of Poly(β-hydroxybutrate) from *Ralstonia eutropha* Using Supecritical Disruptron

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Abstract

This investigation concentrates on the rupture of the Gram-negative bacterium *R. eutropha* by supercritical CO_2 for recovery of poly(β-hydroxybutrate) (PHB). The parameters affecting cell disruption such as operating pressure, temperature, volume of modifier, as well as the effect of culture age and drying strategies were studied. Bacterial cultures which were grown in the logarithmic phase exhibited less resistance to rupture than nutrient limited cultures in the stationary phase. Experiments also indicated that the wet biomass could be utilized to recover PHB, but purity of the product was lower than using lyphilized biomass.

Keywords: Poly (β-hydroxy butyrate) (PHB); Recovery; Supercritical Disruption;

Ralstonia eutropha.

1.Introduction

Products of recombinant technology, the overproduction of proteins as inclusion bodies and the potential use of the intracellular storage product e.g. poly(β-hydroxy butyrate) (PHB) have led to the increase interests in efficient and cost effective cell rupture to enable the recovery of intracellular microbial products.

PHB accumulates as distinct inclusions in the cell and comprises up to 80% of cell dry weight for strains of *Ralstonia eutropha*, under conditions of nitrogen and phosphate limitation and excess of carbon source [1]. This biodegradable and biocompatible thermoplastic, has broadly similar physical properties to polypropylene. It has many applications in medicine, veterinary practice, and agriculture due to its biodegradability [2]. Currently the main problem, which limits the widespread use of PHB and its copolymers, is its relatively high costs compared to polypropylene. The fermentation process, substrates and product recovery are the major costs [3]. A variety of disruption techniques have been developed for PHB recovery [4-6] and some are available commerically. Mechanical methods appear to be favored at the present time for their economic advantages, although some nonmechanical methods, particularly enzymatic lysis, have attracted great attention [7]. Recently, some biological applications of supercritical fluids might also provide solutions to drastic problems related to cell lysis, sterilization and cell inactivation [8-12].

A technique to exploit supercritical (SC) carbon dioxide for disruption of *R. eutropha* has recently been developed in this laboratory [13]. To date, rigorous study of the use of supercirtical fluid in cell rupture over a wide range of operating conditions has been confined to the yeasts.

The process involves a sudden release of the applied fluid pressure that follows penetration of SC-CO2 into the cells. The expansion of gas within the cells, when it is released, forces the breakage of microorganisms. It is simple, inexpensive and more importantly, noninjurios to enzyme activities. The other advantages of