Isolation of Polyhydroxyalkanoates from Fermentation Broth

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1. ABSTRACT

Polyhydroxyalkanoates are excellent polymeric materials for production of environmentally degradable packaging devices and have potential applicability in medical uses. Aim of this project was the isolation of PHA from fermentation broth. Two general approaches for isolation of biopolymers from fermentation broth have been determined and compared. The first possibility is a multistage extraction process, the second possibility for isolating polymers is a cell disruption process realized by a combined mechanical chemical treatment. Economic efficiency and ecological feasibility of PHA production very much depend on the recycling rate of process streams. Possibilities to recycle all process streams have been found.
It was shown that process development contributes to an economically reasonable and environmentally safe overall process of biopolymers by optimization of downstream-processing.

2. INTRODUCTION

Polyhydroxyalkanoates are produced by specific strains of bacteria. Polyhydroxyalkanoates are excellent polymeric materials for production of environmentally degradable packaging devices with versatile shape, good oxygen barrier properties and broad application opportunities. PHA has potential in medical uses also; it can be degraded by the body. So PHA is applicable in the field of tissue engineering as well as in controlled drug release. [12]
The physical properties of PHA and the molecular structure can be altered by varying the fermentation conditions. This creates polymers with a desired molecular

structure. During downstream processing the morphology of PHA can be influenced too. [3]

In the ongoing project various downstream routes for isolation of PHA have been investigated and compared to contribute to an economically feasible and environmentally safe process.

3. Experimental Methods and Results

3.1. Multi step Extraction

The first route, a multi step extraction needs removal of water and lipids from the cell matrix prior to extraction of PHA.

3.1.1. Lipids Removal

Degreasing can be carried out with conventional solvents or supercritical carbon dioxide ($scCO_2$).[1] Both opportunities were investigated.
To gain data about the optimum extraction conditions with $scCO_2$ the operation pressure was varied between 150 bar and 300 bar and extraction temperature was varied between 40°C and 60°C. Figure 1 and Figure 2 show the influence of both parameters on the yield of fat extraction

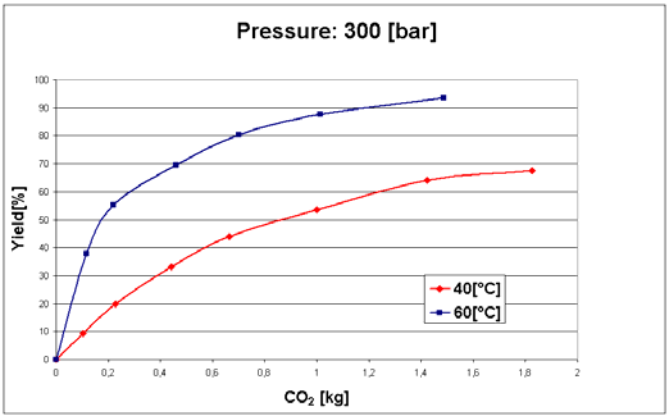

Fig. 1: Extraction yield of fat depending on the temperature of extraction

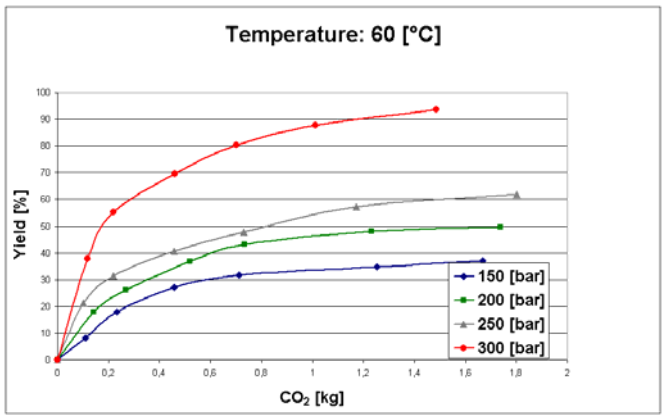

Fig. 2: Extraction yield of fat depending on the pressure of extraction[4]

Solvent recovery of $scCO_2$ from high pressure extraction processes is much less energy consuming compared to solvent extraction with liquid solvents. By

pressure swing complete separation of the solvent CO_2 is achieved, and as a consequence further separation steps for removing $scCO_2$ from the extract phase as well as from the solid residues are not necessary.
High pressure treatment may disrupt the structure of the cell matrix. The cells partially burst under rapid expansion of $scCO_2$ and enable access to the polymer material.

3.1.2. PHA EXTRACTION

After degreasing the extraction of polymers from the cell matrix is carried out in a second extraction step. PHA has very poor solubility properties in standard solvents such as esters, ketones and alcohols.[5] Solvent screening finally led to the selection of chlorinated solvents which have sufficient solvent capacity for PHA.
Dichloromethane was chosen as solvent because it is the only chlorinated solvent which is still used in the food industry. As shown in Figure 3 dichloromethane actually caused severe gelation in practical application.

Fig. 3: Gel formation during extraction of PHA from the cell matrix with dichloromethane Ratio 1:10:10

To suppress gelation, modification of the solvent by altering the ionic strength and the pH value of the crude feed was investigated. The results of these experiments indicated promising improvement by re-dispersing the biomass in aqueous carrier solvent.

3.2. ISOLATION BY CELL DISRUPTION

The second route of isolating biopolymers from fermentation broth, which was investigated, is a combination of chemical and mechanical treatment. Investigation focused on cell disruption through bursting the cells by careful destruction of the cell walls and diaphragms. The relative strength of the cell wall depends on the growth conditions. In nutrient-poor media and during growth limitation frequently the cell wall is strengthened: The mechanical firmness of micro organisms does not represent constant size, but depends on the growth conditions and the manipulation of the biomass.

Main purpose of the disruption procedure was the isolation of intracellular material without mechanical attack of the polymer structure. Disruption can either be carried out mechanically or chemically or by combination of both. [6]

During optimization of the cell disruption process chemical and mechanical treatment was considered. Mechanical disruption with chemical pre-treatment was tested under various conditions. The fermentation broth was re-dispersed in aqueous sodium hydroxide. The biomass to liquid ratio, the alkali to biomass ratio and the mechanical energy input was varied.
During cell disruption lipids saponify and dissolve at elevated pH and separate from the PHA granules. Therefore degreasing of the cells prior to PHA isolation is not necessary.
Because of the simple procedure mechanical disruption of cells at elevated pH is a very promising option for PHA isolation. PHA separation from the carrier solvent and product purification after disruption of the cells is possible by filtration and washing of the filter cake.

3.3. RECYCLE STREAMS

For economically reasons and environmentally safety a maximum recycle rate of the process liquors is eligible.
Based on PHA isolation by cell disruption, several electrolytes, cell debris and fermentation residues can be upgraded and recycled for reutilization. The results from bench scale tests are highly satisfactory and still have not shown a negative influence on overall process efficiency.

4. SUMMARY

Aim of this project was the isolation of PHA from fermentation broth. Two routes of isolation were investigated. The first route is a multi-step extraction process and the second route of isolating polymers is a cell disruption process based on combined mechanical chemical treatment. The type of isolation process determines the needs of pre-treatment and degreasing. Economic efficiency and ecological feasibility need a high recycle rate of all down stream process streams.

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