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EVALUATION OF SOLVENT PERFORMANCE IN THE EXTRACTION OF P(3HB) FROM GRAM-POSITIVE BACTERIA *Bacillus megaterium*

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ABSTRACT

Polyhydroxybutyrate (P(3HB)) is a biodegradable polymer, which has similar characteristics to some petrochemical polymers. P(3HB) is synthesized by many bacteria and stored inside the cell. The most commonly used technique for the recovery is the solvent extraction. P(3HB) extraction from Gram-negative bacteria have been the subject of several studies, but little is known about its extraction from Gram-positive ones. This study evaluated the recovery of P(3HB) from Bacillus megaterium *using chloroform (Chl), as well as to two non-halogenated solvents; dimethylsulfoxide (DMSO) and propylene carbonate (CP). A mechanical rupture pretreatment method for the CP extraction was also evaluated. The recovery found for Chl was lower than the previously reported in the literature for Gram-negative bacteria. The DMSO was not a selective solvent, extracting P(3HB) with low purity. CP recovered 17.6 ± 0.2 % of total P(3HB) at 140 °C and 15 min of contact with untreated biomass, recovering up to 27 % for the best evaluated pretreatment condition.*

1. INTRODUCTION

The polyhydroxyalkanoates (PHA) are a class of polymers synthesized by many bacteria. Among the PHA family, polyhydroxybutyrate (P(3HB)) stands out because its characteristics resemble those of polypropylene. (Kulpreecha et al., 2009). Since it is an intracellular product, the recovery step of this polymer causes a direct impact on the final cost of the product. Among the possible recovery methods, the most usual is solvent extraction, with chloroform being the most used solvent. However, chloroform is highly toxic and can cause health effects, such as hepatic disease (Flanagan and Pounder, 2010). Therefore, finding a solvent with low environmental impact and toxicity is very interesting for the P(3HB) processing. *Bacillus megaterium* is a high versatile microorganism, since it grows using several carbon and nitrogen sources, including low cost industrial by-products (Sathiyanarayanan et al., 2013). In addition, as a Gram-positive bacterium, it does not present

lipopolysaccharide (LPS), an endotoxin that can cause immunological response in medical applications. However, Gram-positive bacteria have thicker cell walls (Naranjo et al., 2013). No previous reports were found comparing quantitatively the recovery of P(3HB) under the same biomass conditions between Gram-negative and Gram-positive bacteria. Therefore, the present study determined the extraction kinetics of P(3HB) from *B. megaterium* using chloroform (Chl), as well as verifies the recoverability of the polymer with three non-halogenated solvents, isoamyl propionate (PIso), dimethylsulfoxide (DMSO), and propylene carbonate (CP).

2. Materials and Methods

Microorganism and conditions of cultivation: The mass of the cells used was homogenized from previously lyophilized biomasses of *B. megaterium* cultures with whey made by Hassemer (2016). The percentage of polymer in the homogenized biomass was quantified using the methodology proposed by Riis and Mai (1988).

Preliminary tests with non-halogenated solvents: The solubility range of P(3HB) for isoamyl propionate (PIso), DMSO and CP, was determined by progressive addition of P(3HB) (Sigma-Aldrich, The United States of America) in the solvents, under magnetic stirring at 140 ^{o}C, until turbidity appeared.

Biomass extraction tests: Biomass and solvent were added at a ratio of 1 % (m/v) in glass tubes. Each tube was kept under agitation and temperature for a set time, Chl (30, 60, 120, or 180 min, 60 ^{o}C) CP and DMSO (15 min, 140 ^{o}C). Thereafter, each mixture was vacuum filtered and the filtrate was dried to constant mass. The purity of the P(3HB) was evaluated according to the methodology of Riis and Mai (1988).

Pretreatment tests: The methodology of cellular rupture by abrasion with sand grains, developed by Sahin *et al.* (2016), was applied as a pretreatment of the biomass before extraction with CP. River sand, previously sanitized, was used in two particle size ranges, 0.250 - 0.295 mm and 0.295 - 1.00 mm. The mixture (biomass, sand and solvent) was vortexed for 15 min.

3. RESULTS AND DISCUSSION

Biomass characterization: P(3HB) content in the *B. megaterium* biomass was of 46,7 ± 0,8 %.

Preliminary tests with non-halogenated solvents: It was possible to identify that the solubility limit for the solvents PIso, DMSO and CP would be among the concentration ranges presented in Table 1.

Table 1. Concentration range in which the solubility limit of P(3HB) in the evaluated non-halogenated solvents is found.

Solvent	Concentration range containing solubility limit	
Isoamyl propionate (PIso)	1.2 (g/L)	2.5 (g/L)
Dimethylsulfoxide (DMSO)	65 (g/L)	70 (g/L)
Propylene Carbonate (CP)	100 (g/L)	Unidentified

Biomass extraction tests: The recovery of P(3HB) (Figure 1) found for Chl was lower than that reported in the literature for *C. necator* (Fiorese et al., 2009, Dalcaton, 2006). It is assumed that this higher resistance to permeability observed in Gram-positive bacteria is due to the wall structure formed by several layers of cross-linked peptide-glycols. This same structure exists in Gram-negative bacteria, but in a much lower number of layers (Hogg, 2005). DMSO was not selective when applied for extraction of P(3HB), presenting a recovery equal to 138.1 ± 2.7 %. Recovery of more than 100 % may have occurred by digestion of a portion of the biomass. Vizcaino-Caston *et al.* (2016) have demonstrated that P(3HB) bacterial cells undergo cell lysis on contact with DMSO at 70 ºC. The recovery of P(3HB) achieved using CP was 17.6 ± 0.2 %, a lower value than that previously reported for the Gram-negative bacteria extraction (Fiorese et al., 2009).

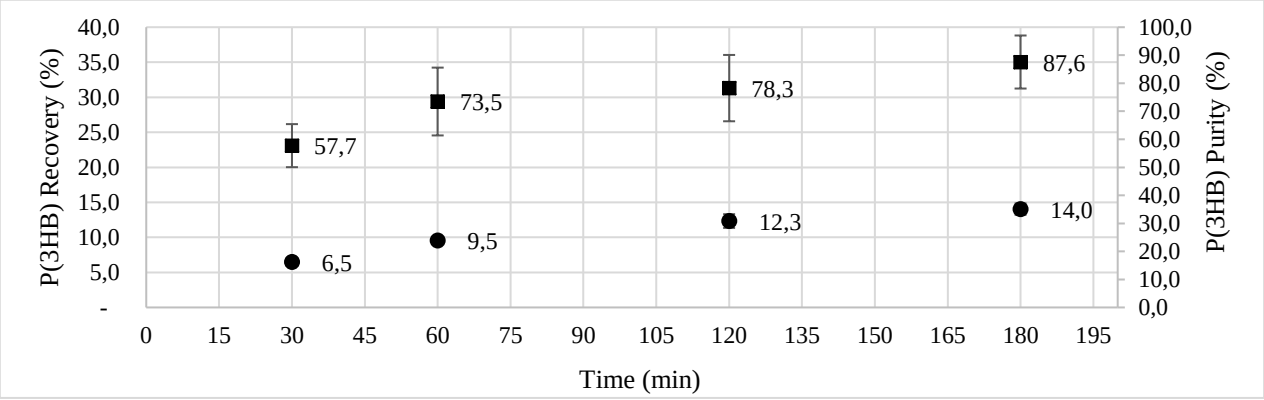

Figure 1. Kinetic recovery curve and purity of P(3HB) extracted from B. megaterium with chloroform (Chl) up to 180 min. Recovery percentage (●). Purity percentage (■).

Prior processing of biomass tests: The pretreatment with sand of the granulometry between 0.295 and 0.250 mm resulted in a recovery of 19.6 ± 0.5 %. While the pretreatment with granulometry between 1.00 and 0.295 mm resulted in higher recovery (27.0 ± 1.7 %).

4. CONCLUSIONS

The recovery of P(3HB) from *B. megaterium* using chloroform (Chl), dimethylsulfoxide (DMSO) and propylene carbonate (CP) was possible. More studies are needed to improve purity and

increase recovery. With respect to recovery it was possible to observe that the cell wall of Gram-positive bacteria offer great resistance to mass transfer in the solvent extraction process. The implementation of pre-treatment with abrasive grains of sand, using CP as solvent, increased the recovery of P (3HB) from *B. megaterium*.

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