

# Alternative Polyhydroxybutyrate Solvent Extraction: a Process Simulation Study

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Polyhydroxybutyrate (PHB) is a possible sustainable alternative to fossil-based plastic, today the cause of one of the primary forms of pollution in the world, since it is biodegradable, recyclable and compostable. Furthermore, PHB can be accumulated in several bacterial strains as a product of their metabolic activity. Solvent extraction (SE) is the most used technique, thanks to the high recovery yields and purity, to extract PHB from bacteria. In particular, a two-step SE is required: the accumulated PHB in the bacterial biomass is firstly dissolved in a solvent to obtain a mixture rich in PHB; then, an anti-solvent is added to reduce the total solubility allowing the biopolymer to precipitate and to be recovered from the residual biomass. However, despite its wide application, the SE technique is characterised by several limitations, such as the high cost and the use of halogenated solvents (i.e. chloroform).

In this work, a non-halogenated based extraction process for PHB recovery from wet bacterial biomass is proposed. Simulations were carried out using butyl acetate (BA) as solvent and hexane (HEX) as anti-solvent. The process scheme also includes a distillation column and a cooling system (when necessary) to recover both the solvent and the anti-solvent. The effects of the PHB solubility during the precipitation step and the solvent recovery on process performance were investigated. The recovery yield was higher than 90% and increased as the solubility decreased. The simulation results highlighted that the proposed strategy is an effective and sustainable alternative to conventional processes for PHB recovery.

## 1. Introduction

Plastics, thanks to their properties, are widely used in many industrial, domestic, and environmental applications. However, they are not biodegradable, accumulate in the environment and contribute to pollution. Bioplastics are a sustainable alternative. They are biocompatible and biodegradable polymers with physical, chemical and mechanical properties similar to chemically synthesised plastics, such as poly- $\beta$ -hydroxybutyric (PHB) belonging to the polyhydroxyalkanoate (PHA) family (Byrom, 1987). PHAs are biopolymers accumulated in intracellular granules, under unfavourable growth conditions due to nutrient limitation (Kim et al., 1994), by a great number of prokaryotes (Lee and Choi, 2001; Ganjian A. et al., 2013). In addition, depending on their composition, PHA can be used for the fabrication of a wide range of products, including packaging material (Chen, 2009; Pietrosanto A. et al., 2021), household products (Philip et al., 2007), up to medical scaffolding (Shum-Tim et al., 1999), sutures (Shishatskaya and Volova, 2004) and other applications. Despite the advantages, high costs limit large-scale production: the only extraction and purification phases of biopolymer recovery from biomass represent up to 50% of the polymer production costs (Lee and Choi, 1998). In particular, the extraction phase consists of the disruption of the cells and the subsequent separation of the biopolymer from the non-biopolymeric material (non-PHB) (Braunegg et al., 1998). Sometimes, a pretreatment step may facilitate the subsequent extraction. This can be chemical (e.g. treatments with alkali) and/or acids and/or physical (e.g. heat treatment) (Jacquel et al., 2008). There are different extraction methods, the most diffused of which uses organic solvents (Kunasundari and Sudesh, 2011), that first modify the cell membrane permeability and then

dissolve the biopolymer. Then, the separation of PHB from the solvent is performed by solvent evaporation or precipitation in a non-solvent liquid. Currently, solvent extraction is the most widely used method for PHB recovery (Riedel et al., 2012). It stands out because it ensures a high recovery yield and maintains almost unchanged the characteristics of the extracted biopolymer. However, large-scale production is limited by still too high costs. However, this drawback can be reduced by recycling and recirculating the solvent/anti-solvent pair, reducing the costs of purchasing raw materials (Jacquel et al., 2008).

The most commonly used solvents are chlorinated hydrocarbons such as chloroform that, however, is toxic, non-recyclable and expensive. One way to minimise these disadvantages is to replace the chloroform with green solvents (Koller et al., 2009). In this regard, the solubility of PHB in solvents (Terada and Marchessault, 1999) determines the solvent/anti-solvent pair to use in the extraction and precipitation process.

This work proposes an extraction scheme for PHB recovery from wet biomass based on solvent extraction and anti-solvent precipitation. Butyl acetate (BA) and hexane (HEX) were used as solvent and anti-solvent respectively. Two process patterns, with and without solvent/anti-solvent recovery and recirculation, and three different solubilities, 2 mg/ml; 1 mg/ml; 0.1 mg/ml, related to the precipitation step, were considered.

## 2. PHB solvent extraction process simulation

The following paragraph details the different steps of the solvent extraction and anti-solvent precipitation process simulation.

### 2.1 Biomass entering the plant

Extraction simulations were carried out using wet biomass, i.e. biomass without drying. In particular, the mixed bacterial strain *Methylocystis*-dominated methanotrophic enrichment as a PHB source was considered. The biomass composition used for simulating the extraction process is reported in Table 1.

Table 1: composition of the input biomass (Myung et al. 2015)

Components	Flow rate [kg/h]	Composition [% w/w]
Water	0.65	65
PHB	0.133	38 (on a dry basis)
non-PHB	0.217	

### 2.2 Solvent/anti-solvent pair choice

The choice of solvent/anti-solvent pair primarily depends on their physical properties and safety. For example, low-viscous solvents require less energy to pump (Boyce et al., 2008), while a low boiling temperature requires less energy to heat and cool streams. The most hydrophobic compounds are easily separable from water that enters the system in the biomass slurry. In addition, solvent and precipitant combinations with significant differences in boiling points will be easier to separate via distillation than pairs with similar boiling points. Compounds with high permissible exposure limits (PEL) are preferred for worker safety. Secondly, it is better to choose solvents that are easy to find and cheap. Finally, the choice is determined by the best solvent/anti-solvent combination for PHB recovery since the solubility of PHB in solvents is crucial to ensure the success of the extraction and precipitation process. In this regard, Terada and Marchessault (1999) analysed the solubility of PHB in the 38 most used solvents in the literature, showing that butyl acetate can be used as a solvent and hexane as an anti-solvent (Budde, 2010).

### 2.3 Process layout

PHB recovery was modelled as a continuous process in which enters 1 kg/h of biomass containing 65% of water and 38% of PHB on a dry basis (Myung et al., 2015). Butyl acetate and hexane were added for extraction and precipitation steps, respectively.

Two process patterns were considered: without solvents recovery and recycling (Figure 1a); with solvents recovery and recycling (Figure 1b). In both the patterns, the effects of three different solubilities (2 mg/l; 1 mg/l; 0.1 mg/l) on the final recovery of the PHB were studied. The main steps of the process are biopolymer extraction, biopolymer precipitation and solvent/anti-solvent recovery.

#### 2.3.1 Biopolymer extraction

The success of the extraction phase depends on the choice of both the solvent and the operating temperature. Usually, the samples are incubated at temperatures below the boiling point of their solvent used ( $T_{eb}$ : 126 °C). In the process simulations, the butyl acetate extraction was conducted in a mixer at 103 °C and 1 bar in order to operate below the solvent boiling point (Aramvash et al., 2015).

### 2.3.2 Biopolymer precipitation

The anti-solvent reduces the solubility of the PHB in the mixture to facilitate the removal of the biopolymer. From the literature, it is possible to know the main chemical compounds in which the PHB is mainly insoluble (Terada and Marchessault, 1999). However, the effectiveness of the precipitation phase depends not only on the attitude of the anti-solvent but on its combination with the solvent. So, the goal is to find the best solvent/anti-solvent pair. In the process simulation, the precipitation step was carried out feeding hexane in a crystalliser, operated at room temperature ( $T_{amb}$ : 20 °C) and a pressure of 1 bar. In the absence of direct experimental data, the solubility was hypothesised on the basis of the literature (Terada and Marchessault, 1999; Fei et al., 2016). In particular, three different solubilities were considered: 2 mg/ml; 1 mg/ml; 0.1 mg/ml.

### 2.3.3 Solvent recycling

In the proposed process, butyl acetate and hexane are easily separable by distillation. In fact, according to the Doherty and Perkins (1978) report, it is possible to separate the two components by simple distillation since azeotropic points are not formed by this mixture. Thus, the mixture can be considered ideal. The recovery of the light key (the most volatile: hexane) and the recovery of the heavy key (the least volatile: butyl acetate) were set at 99% and 1%, respectively. After fixing the recoveries, distillation was conducted at 1 bar and under the assumption of total condensation.

### 2.4 Extraction simulation

Without the recovery of solvent/anti-solvent pair (Figure 1a), butyl acetate was added to dissolve the PHB (A1). In the process, 95% of the non-PHB, 10% of PHB and 5% of solvents were separated from the mixture (A2 – SOLID), and 80% of the solvents were recovered (A5). Hexane was added (A3) to precipitate the PHB, making the solution less soluble and allowing the removal of the biopolymer (A4). At this point, the solid PHB was separated and washed with distilled water (A6).

With the recovery of solvent/anti-solvent pair (Figure 1b), the solvent and the anti-solvent were separated by distillation (A7), cooled, if necessary, and recycled, resulting in the butyl acetate recovered stream (BAREC), and hexane recovered stream (HEXREC).

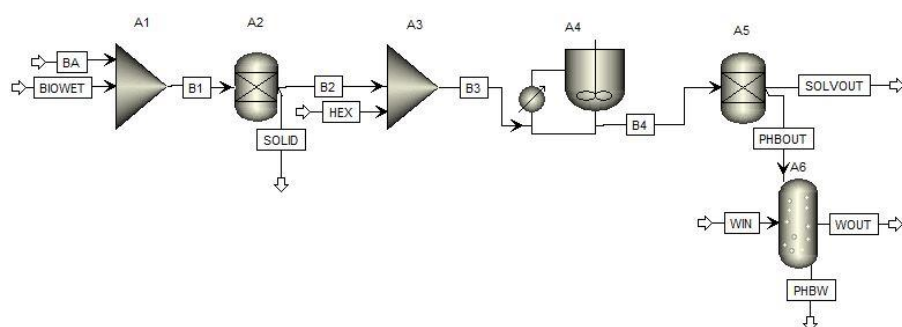
According to the process scheme, 99% of the flows from the distillation tower (A8 - A9) were recycled, while the remaining 1% was discarded (BAOUT; HEXOUT), avoiding the accumulation of these compounds over time. Fresh streams of BA and HEX were added to replace the discharged material. BAREC contained 99% of BA. Since this stream was at 125 °C, it was cooled at 80 °C with a heat exchanger (A10) before the reuse to prevent the vaporisation of water contained in the fed wet biomass. HEXREC contained 97% of HEX. In this case, cooling was not necessary ( $T_{HEXREC}$ : 43 °C).

Operative conditions of the units involved in the extraction process, defined according to Levett et al. (2016) and Terada and Marchessault (1999), are reported in Table 2.

Table 2: Details of the operative conditions of the units used in the PHB recovery process (Terada and Marchessault, 1999; Levett et al., 2016)

Unite ID	A1	A2	A3	A4	A5	A6
Unite Type	Extractor	Solid/liquid separator 1	Mixer	Precipitator	Solid/liquid separator 2	PHB washing
Input	T [°C] 103 p [bar] 1	R [%] 95 non-PHB 10 PHB 5 W 5 B	p [bar] 1	T [°C] 20 p [bar] 1	R [%] 80 BA 80 HEX	p [bar] 1
Unite ID	A7	A8 / A9	A10			
Unite Type	Distiller	Splitter	Heat exchanger			
Input	Reboiler p [bar] 1 Condenser p [bar] 1 Light key recovery [%] 99 Heavy key recovery [%] 1	99% of input goes to recycle streams p [bar] 1	T [°C] 80 p [bar] 1			

1a



1b

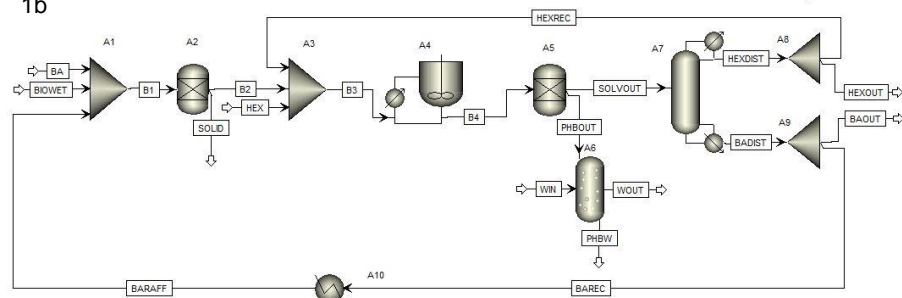


Figure 1: Process scheme without (1a) and with solvent recycling (1b)

### 3. Results

Table 3 shows temperatures, pressures and mass flow rates (total and partial) resulting from the process schemes proposed (Figures 1a and 1b). BA and HEX were fed as pure compounds at 20 °C and, when recirculating, their temperatures achieved 80°C and 41°C, respectively, after cooling (Figure 1b). Due to the recycling of streams, an increase of the temperature of the mixer (A3) from 64°C to 77°C happened, and the amount of fresh solvent/anti-solvent pair to be purchased was reduced by 25%w/w for butyl acetate (from 15 kg/h to 3.8 kg/h) and 21%w/w for hexane (from 33.75 kg/h to 7 kg/h). Despite these differences, the amount of biopolymer obtained and the final recovery yield were equal for both the process schemes. In any case, it was necessary to recirculate solvents to reduce costs and make the process applicable on a large scale.

Process efficiency is a function of solubility. It increases as the solubility of the PHB in the solvent increases and the solubility in the anti-solvent decreases. From wet biomass containing 0.133 kg/h of PHB (BIOWET), the PHB extracted (PHBS) increased from 0.018 to 0.115 kg/h (B4), with a recovery yield that grew from 15% to 96% by reducing the solubility in the anti-solvent from 2 mg/ml to 0.1 mg/ml (Table 4). The recovery yield obtained with a solubility of 0.1 mg/ml is comparable with those reported in the literature for similar works. Aramvash et al. (2015) used butyl acetate as the extraction solvent and obtained recovery yields of 96%. Budde (2010) achieved a recovery of about 95% using butyl acetate as the extraction solvent and three volumes of hexane for the precipitation step.

Table 3: Stream characteristics resulting from the PHB recovery process

Stream Name	BIOWET	BA	B1	SOLID	B2	HEX	B3
T [°C] (Figure 1a)	20	20	103	103	103	20	64
T [°C] (Figure 1b)	20	20	103	103	103	20	77
p [bar]	1	1	1	1	1	1	1
Mass Flow [kg/h] (Figure 1a)	1	15	16	1.0020	14.9981	33.75	48.748
Mass Flow [kg/h] (Figure 1b)	1	3.8	16.986	1.0024	15.983	7	49.876
Water [kg/h] (Figure 1a)	0.65	0	0.65	0.0325	0.6175	0	0.6175
Water [kg/h] (Figure 1b)	0.65	0	0.65	0.0325	0.6175	0	0.88
non-PHB [kg/h]	0.217	0	0.217	0.2062	0.0108	0	0.0108
PHB [kg/h]	0.133	0	0.133	0.0133	0.1197	0	0.1197
BA [kg/h] (Figure 1a)	0	15	15	0.75	14.25	0	14.25
BA [kg/h] (Figure 1b)	0	3.8	15.72	0.75	14.968	0	15.09
PHBS [kg/h]	0	0	0	0	0	0	0
HEX [kg/h] (Figure 1a)	0	0	0	0	0	33.75	33.75
HEX [kg/h] (Figure 1b)	0	0	0.268	0.0004	0.267	7	33.78

Stream Name	B4			PHBOUT			SOLVOUT
Solubility [mg/ml]	2	1	0.1	2	1	0.1	-
T [°C]	20	20	20	20	20	20	20
p [bar]	1	1	1	1	1	1	1
Mass Flow [kg/h] (Figure 1a)	48.748	48.748	48.748	9.858	9.858	9.858	38.89
Mass Flow [kg/h] (Figure 1b)	49.876	49.876	49.876	10.4	10.4	10.4	39.47
Water [kg/h] (Figure 1a)	0.6175	0.6175	0.6175	0.1275	0.1275	0.1275	0.49
Water [kg/h] (Figure 1b)	0.88	0.88	0.88	0.615	0.615	0.615	
non-PHB [kg/h]	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0
PHB [kg/h]	0.102	0.051	0.0051	0.102	0.051	0.0051	0
BA [kg/h] (Figure 1a)	14.25	14.25	14.25	2.85	2.85	2.85	11.4
BA [kg/h] (Figure 1b)	15.09	15.09	15.09	2.93	2.93	2.93	12.16
PHBS [kg/h]	0.018	0.069	0.115	0.0178	0.069	0.115	0
HEX [kg/h] (Figure 1a)	33.75	33.75	33.75	6.75	6.75	6.75	27
HEX [kg/h] (Figure 1b)	33.78	33.78	33.78	6.73	6.73	6.73	27.05

Stream Name	WIN	WOUT		PHBW			
Solubility [mg/ml]	-	2	1	0.1	2	1	0.1
T [°C]	20	20	20	20	20	20	20
p [bar]	1	1	1	1	1	1	1
Mass Flow [kg/h] (Figure 1a)	2	11.83	11.778	11.731	0.03	0.08	0.127
Mass Flow [kg/h] (Figure 1b)	2	12.37	12.32	12.27	0.03	0.08	0.127
Water [kg/h] (Figure 1a)	2	2.127	2.127	2.127	0	0.0001	0.00023
Water [kg/h] (Figure 1b)	2	2.61	2.61	2.61	0	0.0002	0.00027
non-PHB [kg/h]	0	0	0	0	0.0108	0.0108	0.0108
PHB [kg/h]	0	0.102	0.051	0.0051	0	0	0
BA [kg/h] (Figure 1a)	0	2.85	2.85	2.845	0	0.0002	0.0003
BA [kg/h] (Figure 1b)	0	2.93	2.93	2.93	0	0.0002	0.0003
PHBS [kg/h]	0	0	0	0	0.02	0.069	0.115
HEX [kg/h] (Figure 1a)	0	6.75	6.75	6.750	0.00016	0.0005	0.0007
HEX [kg/h] (Figure 1b)	0	6.73	6.73	6.73	0.00016	0.0004	0.0007

Table 4: Recovery yields at different solubilities

Solubility [mg/ml]	2	1	0.1
R [%]	15	58	96

#### 4. Conclusions

The simulation results confirm the possibility of extracting PHB using non-halogenated based solvents, which are sustainable and non-toxic, such as butyl acetate and hexane, from wet biomass. However, PHB recovery is highly affected by the solubility related to the precipitation step since the lower the solubility, the higher the recovery. In particular, with a solubility of 0.1 mg/ml, a recovery of 96% was achieved.

Solvent/anti-solvent pair recycling is feasible and reduces the amount of butyl acetate and hexane by 25%w/w and 21%w/w, respectively. Recycling and recirculation of the solvent/anti-solvent pair are crucial to reduce the cost of the process and make it more competitive.

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