|  |  |
| --- | --- |
| cetlogo ***CHEMICAL ENGINEERING TRANSACTIONS*** ***VOL. , 2023*** | A publication ofaidiclogo_grande |
| The Italian Associationof Chemical EngineeringOnline at www.cetjournal.it |
| Guest Editor: Sauro PierucciCopyright © 2023, AIDIC Servizi S.r.l.**ISBN** 978-88-95608-98-3; **ISSN** 2283-9216 |

A SIMULATION STUDY FOR COMPARING HALOGENATED AND GREEN SOLVENTS DURING THE EXTRACTION OF POLY(3-HYDROXYBUTYRATE)

Teresa Abate1, Claudia Amabile1, Carmen De Crescenzo1\*, Antimo Migliaccio1, Giuseppe Capece2, Renato Ciampa3, Raul Muñoz4, Simeone Chianese1, Dino Musmarra1

1Department of Engineering, University of Campania “Luigi Vanvitelli”, Via Roma 29, 81031, Aversa, Italy

2Progest S.r.l., Zona ASI Aversa-Nord, Via della Stazione s.n.c., CE, Gricignano d'Aversa, 81030, Italy

3Centro Diagnostico Baronia S.r.l., Località Taverna Annibale SNC, Area PIP, Lotto n°7, AV, Frigento, 83040, Italy

4Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina, s/n, 47011, Valladolid, Spain

\*Corresponding author: carmen.decrescenzo@unicampania.it

Thanks to their properties, fossil fuel-based plastics are widely used daily (for industrial, domestic and environmental applications). However, they are not biodegradable, and their end of life is the cause of one of the most dangerous pollution in the world. Plastic, if recovered, can be recycled or used to recover energy, but it is not possible to recover all the tons of waste produced, so some end up in landfills. Therefore all non-recycled and non-collected plastic can take up to a thousand years to degrade. So the scientific idea is to produce biodegradable plastic capable of replacing conventional plastics. One possible solution is the poly(3-Hydroxybutyrate) (PHB) of the polyhydroxyalkanoates (PHA) family. PHB is a biopolymer accumulated in intracellular granules, under unfavourable growth conditions, by many prokaryotes and can be extracted with eco-friendly solvents. 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). Currently, solvent extraction is the most used method for PHB recovery because it guarantees a high recovery yield and keeps the characteristics of the extracted biopolymer almost unaltered.

In this study, the extraction of PHB with eco-friendly solvents was investigated. Simulations were carried out using 1,2-propylene carbonate as the solvent and acetone as the anti-solvent. Two process schemes were analyzed, without and with the recirculation of solvents (by distillation) and with two different types of biomass (wet and dry). The present work aims to study the final recovery yield in different operating conditions and the influence of PHB solubility on the process. The simulation results confirm the importance of solubility for optimizing the extraction process and underline the problem related to the lack of data in the literature.

1. Introduction

Plastic waste pollution is a serious threat to the environment. Non-biodegradable plastic accumulated in the environment can take hundreds of years to degrade and can cause harm to wildlife, habitat, and human health. Plastic pollution contaminates oceans and coastlines, poisoning marine wildlife food sources and limiting biodiversity. To combat plastic pollution, it is necessary to reduce the production and use of single-use plastic items, increase recycling and good waste management, and develop sustainable alternatives. Biodegradable plastics are plastic materials that microorganisms can break down in the environment within a reasonable time frame. These materials are designed to have a reduced environmental impact compared to traditional plastics, which can persist in the environment for many years. However, these plastics are expensive, low-quality, and often unsustainably produced. Researchers are investigating sustainable alternatives: examples are poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB-co-HV) of the polyhydroxyalkanoates (PHA) family. It is used in many industries, such as food packaging, household products, and biomedical. This material is appreciated for its biodegradability, environmental safety, and mechanical and thermal properties. PHB is produced through fermentation under adverse conditions of specific bacterial strains (Jung et al., 2000). Subsequently, the accumulated polymer is recovered from cellular biomass. Much of the focus has been on the production phase, while few works have focused on polymer recovery. In particular, several simulations have already been conducted for the production of PHA from renewable resources (Amabile et al., 2022c) in different operating conditions (Amabile et al., 2022b) to optimize the process (Amabile et al., 2022a). As reported in the literature, the solvent extraction method is the favourite because it allows the recovery of a large part of the polymer from biomass with high purity, good quality and low content of endotoxins. However, the extraction process is not as *green* due to the use of halogenated solvents; in addition, the very high costs of the extraction phase (over 50% of the total cost) also limit the large-scale deployment (Kunasundari and Sudesh, 2011). Usually, the polymer accumulated during the fermentation phase is very viscous (>5% (p/v) of PHA (Yang et al., 2015)), and large volumes of the solvent are required to remove cellular debris. Therefore, solvent recovery and recirculation are essential to reduce process costs (Jacquel et al., 2008). Finally, the extracted polymer is typically recovered by solvent evaporation or precipitation in an anti-solvent.

A sustainable extraction process with green solvents was simulated, and different operating conditions were compared: with wet and dry biomass, with and without solvent recirculation.

2. PHB solvent extraction process simulation

All simulations were performed using AspenPlus, which consists of four main blocks that must be completed before running the simulation. In particular, it is necessary: to define the property method; set the component list that interacts in the simulation; define the main flowsheet (operative units and streams); set operative conditions for all the blocks; reading the results.

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

2.1 Choice of input biomass

The simulations were conducted assuming only one type of biomass but under two different conditions, i.e. wet and dry (in the last case, biomass was thermally pre-treated). The composition of the biomass used was supposed to be equal to that obtained during fermentation of the mixed bacterial strain Methylocystis-dominated methanotrophic enrichment was used as a reference (Myung et al., 2015). In particular, 1 kg of input biomass containing 38% w/w of PHB (on a dry basis) was considered in this research work: the biomass composition used for simulating the extraction process is reported in Table 1.

2.2 Choice of pre-treatment

Cell breakdown is required to facilitate the release of PHB because of its intracellular nature. In this regard, in the literature, a pre-treatment phase is sometimes proposed before the extraction phase. Some studies report on chemical methods, physical methods(Jacquel et al., 2008) or their combination (Fiorese et al., 2009).

The same biomass was used for two different simulations, with and without thermal pre-treatment at 60 ºC. The different composition of biomass entering the extraction phase is shown in Table 1.

Table 1: Composition of the input biomass without and with thermal pre-treatment (Myung et al. 2015)

|  |  |  |
| --- | --- | --- |
| Components | Flow rate[kg/h] | Composition[% w/w] |
| Water | 0.65 (wet biomass) | 65 |
| 0,0045 (dry biomass) |
| PHB | 0.133 | 38 (on a dry basis) |
| non-PHB | 0.217 |  |

2.3 Choice of the extraction method

PHA is an intracellular product, therefore, the methods adopted for its recovery are focused either on its solubilization or the solubilization of non-polymeric cellular materials (non-PHB). Until now, the most used methods have been solvent extraction; enzymatic digestion; digestion of sodium hypochlorite and mechanical extraction (Jacquel et al., 2008). Several recovery methods have already been tested in the literature, but each of them has disadvantages: recovery with sodium hydroxide or sodium hypochlorite (Hahn et al., 1994); chemical recovery with detergents (Choi and Lee, 1999); enzymatic digestion (Kunasundari and Sudesh, 2011). To date, solvent extraction is the most widely used method thanks to the high recovery yields and purity and the highest quality of the final product. Several methods have also been tested for the precipitation phase: anti-solvent, which involves adding an anti-solvent to the solvent to induce PHB precipitation; temperature change, which consists in lowering the temperature of the solvent to induce PHB precipitation; solvent evaporation, which involves evaporating the solvent at a controlled temperature to induce PHB precipitation; centrifugation, which consists in using centrifugal force to separate PHB from the solvent; filtration, which consists in using a filter to separate PHB from the solvent, and pressure increase precipitation, which involves increasing pressure to induce PHB precipitation. Among the methods mentioned above, anti-solvent addition was chosen for the precipitation phase for the simulative activity performed. After the growth and accumulation phases of the polymer within the biomass, the PHB is recovered by solid-liquid separation (i.e centrifugation) and dissolved in a solvent in which it is solubilized. In this way, cellular breakdown and the dissolution of the polymer within the solvent are favored, while the non-polymer cellular material is removed (filtration or centrifugation). After the solubilization, the addition of the anti-solvent reduces the solubility of the system and promotes the precipitation of the polymer inside the mixture. The anti-solvent can be immiscible (that is, less than about 10% of solubility) with the solvent used during the extraction phase or miscible (that is, greater than about 90% of solubility). The PHB can be separated from the mixture by solid-liquid separation or solvent evaporation. Whenever possible, the solvents are recovered by distillation and recirculated into the system; this step is essential to reduce the costs of the recovery process. However, this method also presents some limits to overcome, such as the high cost due to the price of the solvent and the recovery process and the negative environmental impact due to the emission of pollutants into the environment: so, two sustainable and safe solvents were chosen in this simulation study to solve them, while solvent recirculation is also proposed to reduce the final process costs.

2.4 Choice of solvent/anti-solvent pair

The choice of solvent for PHB extraction depends on the solubility of the material and its compatibility with the subsequent purification process. Factors considered in selecting the solvent include solubility, boiling point, toxicity, recoverability, and compatibility with the following process. Thus, in this simulation study, 1,2-propylene carbonate (PC) was used as the extraction solvent (PHB is soluble in this solvent; it has a high boiling temperature and is non-toxic), while acetone (AC) was used as an anti-solvent for the precipitation phase. In addition, they form an ideal mixture, so it is possible to recover solvents by simple distillation and recirculate them within the system.

2.5 Process layout

All simulations proposed in this research study were conducted considering a 65% wet biomass input and a PHB content of 38% on a dry basis (Myung et al., 2015). Here, the polymer accumulated within the bacterial biomass was extracted with 1,2-propylene carbonate and recovered by precipitation in acetone.

Four different conditions were modelled and compared:

* 1. wet biomass without solvent recirculation (Figure 2a);

1.2) dry biomass without solvent recirculation (Figure 2a);

2.1) wet biomass with solvent recirculation (Figure 2b);

2.2) dry biomass with solvent recirculation (Figure 2b).

2.5.1 Biopolymer extraction and precipitation

For safety reasons, extraction is usually conducted at a temperature below the boiling point (Teb) of the solvent used (1,2-propylene carbonate - Teb = 242°C). For this reason, recovery with 1,2-propylene carbonate was simulated at a maximum temperature of 130 ºC and atmospheric pressure. In the absence of direct experimental activity, the data useful to the realization of the process was referred to in the report of Fiorese *et al.* (2009).

The precipitation phase, however, was conducted using acetone as an anti-solvent at room temperature and atmospheric pressure. Other studies have used acetone (Choi and Lee, 1999), but there is no data on the solubility of PHB. However, based on the studies proposed by Terada and Marchessault (1999), a solubility of about 0.2 mg/ml has been assumed.

2.5.2 Solvent recycling

The mixture PC and AC is ideal because it doesn’t have azeotropic points. For this reason, according to the Doherty and Perkins (1978) report, they can be separated by simple distillation and recirculated within the system to reduce the final process costs. The recoveries of the light key (the most volatile: acetone) and the heavy key (the least volatile: 1,2-propylene carbonate) were set at 99% and 1%, respectively. After fixing the recoveries, distillation was conducted at 1 bar and under the assumption of total condensation.

2.6 Recovery simulation

In the absence of solvent recirculation, 1kg of biomass was directly mixed (M1) with PC (Figure 2a without H) or previously thermally pre-treated (H) at 60 ºC (Figure 2a). Based on data reported by Abate et al. (2022), it has been suggested, in both cases, that 95% of the non-polymer cellular material, 10% of biopolymer and 5% of PC and AC were separated from the mixture (S1 – SOLID), and 80% of the solvents have been recovered and recirculated within the system (D). Subsequently, acetone was added to the mixture (M2) to promote the next precipitation phase of PHB within the PC/AC mixture (P). Finally, the crystallized polymer was recovered by solid-liquid separation (S2) and rinsed with distilled water (W). In the scheme of Figure 2b, PC and AC were separated by distillation (D), cooled to 80 ºC (C) (if necessary to avoid the evaporation of water contained in the fed wet biomass) and recirculated within the system (PCRAFF and ACREC). To prevent the accumulation of solvents over time, it is always recommended to avoid recovery of 100%. For this reason, 99% of the flows from the distillation column (SP1 – SP2) were recycled, while the other 1% was removed (PCOUT - ACOUT): lost solvents are constantly replaced with fresh streams at room temperature (PC – AC).

Table 2 details the operating conditions of the entire process of recovery of the PHB, defined according to Levett *et al.* (2016) and Terada and Marchessault (1999).

Table 2: Operating conditions for PHB recovery from wet and dry biomass, with and without solvent recirculation (Levett et al., 2016; Terada and Marchessault, 1999)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Unite ID | H  | M1 | S1 | M2 | P | S2 |
| Unite Type | Heat exchanger | Extractor | Solid/liquidseparator 1 | Mixer | Precipitator | Solid/liquid separator 2 |
| Input | T [°C] 60 | T [°C] 130 | R [%] | 95 non-PHB | p [bar] 1 | T [°C] 20 | R [%] 80 PC |
|  |  | p [bar] 1 | p [bar] 1 |  | 10 PHB |  | p [bar] 1 | 80 AC |
|  |  |  |  |  |  | 5 W/PC/AC |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Unite ID | W | D | SP1 / SP2 | C |
| Unite Type | PHBwashing | Distiller | Splitter | Heat exchanger |
| Input | p [bar] 1 | Reboiler p [bar] 1 | 99% of input goes torecycle streams | T [°C] 80 |
|  | Condenser p [bar] 1 | p [bar] 1 |
|  |  | Light key recovery [%] 99 | p [bar] 1 |  |
|  |  | Heavy key recovery [%] 1 |  |  |

**

2a



2b

Figure 2: Process scheme with dry biomass: without (2a) and with solvent recycling (2b)

3. Results

The main results of this simulation work are the recovery yield and solvent flow rates under the different conditions analyzed. In the absence of recirculation (Figure 2a), 16 kg/h and 21 kg/h of fresh PC and AC are needed, respectively. Recirculation (Figure 2b) reduces fresh solvent flows by 70% for 1.2-propylene carbonate and 78.6% for acetone. In other words, of the total solvent required, 11.2 kg/h and 16.5 kg/h, respectively, shall be reused. However, the PC is fed back into the system at 80 ºC (after cooling), while the acetone at 56 ºC, so the system consumes more energy to cool currents. In any case, solvent recirculation is essential to reduce costs and make the process economically feasible. Thermal pre-treatment was ineffective for the final yield of the process. Therefore, with equal recovery yield and solvent flows, working directly with wet biomass is economically more convenient. The results obtained in this work are in line with those reported in the literature for similar works (Fiorese et al., 2009; Yabueng and Napathorn, 2018). These results suggest that PHB within wet cells is more soluble and amorphous than crystallized (de Koning and Lemstra, 1992; Fei et al., 2016). The recovery yield of the different configurations is reported in Table 3, according to which the final recovery yield is about 95% for all the cases analyzed. These results are consistent with previous studies (Fiorese et al., 2009).

Table 3: Recovery yield, fresh and recirculated solvent flow rates for the process patterns

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Configuration | R [%] | Fresh PC[kg/h] | Recirculated PC[kg/h] | Fresh AC[kg/h] | Recirculated AC[kg/h] |
| wet biomass - withoutsolvent recirculation | 95 | 16 | - | 21 | - |
| wet biomass – withsolvent recirculation | 95 | 4.8 | 11.2 | 4.5 | 16.5 |
| dry biomass - withoutsolvent recirculation | 95 | 16 | - | 21 | - |
| dry biomass - withsolvent recirculation | 95 | 4.8 | 11.2 | 4.5 | 16.5 |

4. Conclusions

The results obtained in these simulations confirm the possibility of using green solvents to extract the accumulated PHB within the biomass. Moreover, in line with other works in the scientific literature, they do not report benefits in the pre-treatment phase. However, the importance of solvent recirculation to reduce the final process costs, i.e. to increase the cost-effectiveness of the process, has been highlighted. In any case, the absence of solubility data limits the simulative activity: the solubility of PHB in solvents is necessary to optimize the extraction process.

Acknowledgements

This work was supported by “Fondo per la Crescita Sostenibile – Sportello “Agrifood” PON I&C 2014-2020”, di cui al D.M. 5 marzo 2018 Capo III. Prog. n. F/200125/01-03/X45. Authors would like to thank VALERE “VAnviteLli pEr la RicErca” PROGRAMME by the University of Campania Luigi Vanvitelli. The Regional Government of Castilla y Leon and the EU-FEDER (CLU 2017-09, CL-EI-2021-07, and UIC 315) are also gratefully acknowledged.

References

Abate T., Amabile C., De Crescenzo C., Sabbarese S., Capece G., Ciampa R., Chianese S., Musmarra D., 2022, Alternative Polyhydroxybutyrate Solvent Extraction: a Process Simulation Study. Chem. Eng. Trans. 92, 265–270;

Amabile C., Abate T., De Crescenzo C., Sabbarese S., Migliaccio A., Chianese S., Musmarra D., 2022, Poly(3-hydroxybutyrate) production from methane in bubble column bioreactors: Process simulation and design optimization. N. Biotechnol. 70, 39–48;

Amabile C., Abate T., De Crescenzo C., Sabbarese S., Capece G., Ciampa R., Chianese S., Musmarra D., 2022b, Elucidating the Effects of Superficial Gas Velocity on Poly(3hydroxybutyrate) Production. Chem. Eng. Trans. 92, 271–276;

Amabile C., Abate T., De Crescenzo C., Sabbarese S., Muñoz R., Chianese S., Musmarra D., 2022d, Sustainable Process for the Production of Poly(3-hydroxybutyrate- co-3-hydroxyvalerate) from Renewable Resources: A Simulation Study. ACS Sustain. Chem. Eng. 10, 14230–14239;

Choi J., Lee S.Y., 1999, Efficient and economical recovery of poly(3-hydroxybutyrate) from recombinant Escherichia coli by simple digestion with chemicals. Biotechnol. Bioeng. 62, 546–553;

de Koning G.J.M., Lemstra P.J., 1992, The amorphous state of bacterial poly[(R)-3-hydroxyalkanoate]in vivo. Polymer (Guildf). 33, 3292–3294;

Doherty M.F., Perkins J.D., 1978, On the dynamics of distillation processes-I. The simple distillation of multicomponent non-reacting, homogeneous liquid mixtures. Chem. Eng. Sci. 33, 281–301;

Fei T., Cazeneuve S., Wen, Z. Wu, L., Wang T., 2016, Effective recovery of poly-β-hydroxybutyrate (PHB) biopolymer from Cupriavidus necator using a novel and environmentally friendly solvent system. Biotechnol. Prog. 32, 678–685;

Fiorese M.L., Freitas F., Pais J., Ramos A.M., De Aragão G.M.F., Reis M.A.M., 2009, Recovery of polyhydroxybutyrate (PHB) from Cupriavidus necator biomass by solvent extraction with 1,2-propylene carbonate. Eng. Life Sci. 9, 454–461;

Hahn S.K., Chang Y.K., Kim B.S., Chang H.N., 1994, Optimization of microbial poly(3‐hydroxybutyrate) recover using dispersions of sodium hypochlorite solution and chloroform. Biotechnol. Bioeng. 44, 256–261;

Jacquel N., Lo C.W., Wei Y.H., Wu H.S., Wang S.S., 2008, Isolation and purification of bacterial poly(3-hydroxyalkanoates). Biochem. Eng. J. 39, 15–27;

Jung Y.M., Park J.S., Lee Y.H., 2000, Metabolic engineering of Alcaligenes eutrophus through the transformation of cloned phbCAB genes for the investigation of the regulatory mechanism of polyhydroxyalkanoate biosynthesis. Enzyme Microb. Technol. 26, 201–208;

Kunasundari B., Sudesh K., 2011, Isolation and recovery of microbial polyhydroxyalkanoates. Express Polym. Lett. 5, 620–634;

Lee S.Y., Choi J. Il, 1998, Effect of fermentation performance on the economics of poly(3-hydroxybutyrate) production by Alcaligenes latus. Polym. Degrad. Stab. 59, 387–393;

Levett I., Birkett G., Davies N., Bell A., Langford A., Laycock B., Lant P., Pratt S., 2016, Techno-economic assessment of poly-3-hydroxybutyrate (PHB) production from methane - The case for thermophilic bioprocessing. J. Environ. Chem. Eng. 4, 3724–3733;

Myung J., Galega W.M., Van Nostrand J.D., Yuan T., Zhou J., Criddle C.S., 2015, Long-term cultivation of a stable Methylocystis-dominated methanotrophic enrichment enabling tailored production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate). Bioresour. Technol. 198, 811–818;

Steinbüchel A., 2001, Perspectives for Biotechnological Production and Utilization of Biopolymers: Metabolic Engineering of Polyhydroxyalkanoate Biosynthesis Pathways as a Successful Example. Macromol. Biosci. 1, 1–24;

Terada M., Marchessault R.H., 1999, Determination of solubility parameters for poly(3-hydroxyalkanoates). Int. J. Biol. Macromol. 25, 207–215;

Yabueng N., Napathorn S.C., 2018, Toward non-toxic and simple recovery process of poly(3-hydroxybutyrate) using the green solvent 1,3-dioxolane. Process Biochem. 69, 197–207;

Yang Y.H., Jeon J.M., Yi D.H., Kim J.H., Seo H.M., Rha C.K., Sinskey A.J., Brigham C.J., 2015, Application of a non-halogenated solvent, methyl ethyl ketone (MEK) for recovery of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(HB-co-HV)] from bacterial cells. Biotechnol. Bioprocess Eng. 20, 291–297.