Design and Optimization of Poly(hydroxyalkanoate)s Production Plants using Alternative Substrates

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Abstract
In this work, we propose a Mixed Integer Nonlinear Programming (MINLP) model to determine the optimal design of a poly(hydroxyalkanoate)s (PHAs) production plant configuration. The superstructure based optimization model considers different carbon sources as raw material: glycerol (crude and purified), corn starch, cassava starch, sugarcane sucrose and sugarcane molasses. The PHA extraction section includes four alternatives: the use of enzyme, solvent, surfactant-NaOCl or surfactant-chelate. Model constraints include detailed capital cost for equipment, mass and energy balances, product specifications and operating bounds on process units. The resulting MINLP model maximizes the project net present value (NPV) as objective function and it is implemented in an equation oriented environment. Optimization results show the sugarcane-enzyme option as the most promising alternative (NPV = 75.01 million USD) for PHAs production with an energy consumption of 22.56 MJ/kg PHA and a production cost of 3.02 US$/kg PHA. Furthermore, an economic sensitivity analysis is performed.

Keywords: MINLP; Modeling; Optimization; PHA; Superstructure.

1. Introduction
Poly(hydroxyalkanoate)s (PHAs) arise as a sustainable alternative to fossil fuel based polymers (Mohapatra et al., 2017). PHAs are biomaterials that can be intracellularly produced from renewable sources by microorganisms. They are natural biopolymers, non-toxic and completely biodegradable polyesters whose physical and thermal properties resemble synthetic thermoplastics such as polyethylene, polypropylene and polystyrene (Mannina et al., 2019).

A broad range of substrates has been historically used as a carbon source for PHA production at the industrial and semi-industrial level, some examples of which are presented by Levett et al. (2016). It is well known that the cost of substrates can represent up to 50 % of the production cost for biomaterials (Koller et al., 2017). In fact, several factors like microorganism productivity, substrate yield, raw material cost or extraction methods influence the final product price (Dietrich et al., 2017). Therefore, even though individual advances in these factors will improve the final value of PHAs, cost reductions at industrial scale, will strongly depend on optimization of the integral process (Ramos et al., 2017).

The simultaneous determination of an industrial process optimal flowsheet and its operating conditions is a discipline known as process synthesis. Two main approaches can be found in the literature to solve this optimization problem. In the conceptual design strategy, presented by Douglas (1988), the problem is solved sequentially, by fixing a set of process variables and using heuristics to reach an improved solution. Alternatively, a simultaneous mathematical optimization based strategy can be followed (Grossmann, 1996; Biegler et al., 1997). In this strategy, several technological alternatives for the process stages are embedded within a superstructure. Then, a mathematical programming problem is formulated, involving continuous and discrete variables for the selection of the process optimal configuration. Finally, the model formulated as a Mixed Integer Nonlinear Programming (MINLP) problem is solved.
An alternative for representing problems that include continuous and discrete variables is the use of models with disjunctions and logical propositions (Grossmann and Harjunkoski, 2019). A particular case for process synthesis modeling including nonlinear constraints is the Generalized Disjunctive Programming (GDP) formulation. In this framework, three types of constraints can be found: (a) global constraints which are not related to the discrete decisions; (b) conditional constraints, represented by disjunctions where “OR” operators are involved; and (c) purely logical constraints involving only Boolean variables. In order to use existing MINLP solvers it is usual to reformulate GDP models as MINLP problems, by the use of Big-M or convex hull formulations (Grossmann and Ruiz, 2012).

In this sense, many authors propose the development of superstructures for process synthesis optimization within this framework. Martin and Grossmann (2012) formulate an MINLP problem which simultaneously optimizes process configuration and heat integration for biodiesel production using several oils as raw materials. Gong and You (2014) develop a superstructure for an algae-based biorefinery, minimizing the cost of the carbon capture unit, mitigating the process carbon footprint. Rizwan et al. (2015) present a superstructure and formulate the corresponding MINLP problem to find an optimal processing pathway for biodiesel production from microalgae. More recently, Dheskali et al. (2017) propose a mathematical model for the optimal design and scheduling operation of a biotechnological process section minimizing the annual cost.

Regarding PHAs production, many techno-economic assessments can be found in the literature (Posada et al., 2011; Lopez-Arenas et al., 2017; Shahzad et al., 2017), most of them using commercial simulators. To the best of our knowledge, the design of PHAs production processes using mathematical programming through the formulation of a superstructure has not been presented so far.
In this work, a superstructure is presented for the optimal design of a PHA production process, including mass and energy balances, design and sizing equations, as well as detailed correlations for equipment units capital cost. The objective function for the optimization problem is the maximization of the net present value (NPV). The model has into account several alternative carbon sources as substrates and includes different technological alternatives for the biosynthesis, extraction and purification stages. The resulting MINLP model has 8,249 continuous variables, 25 discrete variables, 7,456 constraints and it is implemented in GAMS (McCarl et al., 2017). Numerical results show that PHA production can become economically attractive if the proper combination of technologies is selected.

2. Materials and Methods

2.1. Process description

The PHA production process has three main stages: upstream processing (raw material pretreatment), biosynthesis and downstream processing (biopolymer extraction and purification). Figures 1-3 show the proposed superstructure, including different technological alternatives. A detailed description of the different stages is presented hereunder.

2.1.1. Upstream processing

As shown in Fig. 1, three main raw materials are considered, including some of their derivatives which could be potentially used as substrates for PHA production. The following sub-sections describe the technologies involved in the pretreatment of these different carbon sources: glycerol, corn and cassava starch, sugarcane sucrose and sugarcane molasses, as well as the associated operating conditions and specifications considered in the model.

2.1.1.1. Glycerol
Raw glycerol, main by-product from the biodiesel industry, can be employed as an economical carbon source for several microbiological processes such as PHAs production (López et al., 2012). Nevertheless, impurities in the glycerol stream resulting from the biodiesel production process (60.1 wt % glycerol, 22.6 wt % methanol, 10 wt % water, 2.8 wt % ashes, 2.6 wt % sodium methoxide and 1.9 wt % soaps) could have an inhibitory effect on biomass growth of the biopolymer producing microorganisms (Luo et al., 2016). Therefore, the possibility of a glycerol purification sector is included in the superstructure (Fig. 1).

In this process, the crude glycerol feed stream is flashed in unit FL1 and the resulting water-methanol top stream is fed to a distillation column (DS1). Methanol is recovered as top product from this column with 91.7 % purity (Posada et al., 2011) and sold as by-product. In the following step, the FL1 bottom stream is mixed with hydrochloric acid (HCl) 31 wt %, in a 0.355:1 HCl-to-glycerol ratio in reactor RC1, to neutralize the remnant catalyst (sodium methoxide) from the biodiesel production process. Additionally, soaps react with HCl to form free fatty acids (FFA) and sodium chloride (NaCl). After the neutralization reaction, traces of ashes are removed in a centrifuge unit (CN1). The resulting solid-free stream is sent to a decanter unit (DC1), where it is washed with water in a 2.4 kg of water per kg of glycerol (Posada et al., 2011), to obtain glycerol free of salts and solids. Finally, water and remaining amounts of methanol are removed by evaporation in a flash unit (FL2), followed by a distillation column (DS2) to obtain a 98 % glycerol stream as final product. At this point, two alternatives are included for purified glycerol: either its sale or its use as substrate in the biosynthesis section for PHA production.

2.1.1.2. Starch

Starch is one agro-industrial product that holds a promising outlook as substrate for biomaterial production. Three potential alternatives are considered for starch production...
in the superstructure presented in Fig. 1. The first one consists in including the process of starch production from corn. The second option involves the direct purchase of corn starch, while the third one contemplates the possibility of using cassava starch (Poomipuk et al., 2014). The superstructure also includes the possibility of producing glucose, through starch liquefaction and saccharification, to be used as substrate for the microbial biosynthesis in the PHA production stage.

Corn starch production process begins with stages related to grain handling. Particularly, a screw conveyor (CT) is used to transport the grain, considering a material loss of 2.4 % (Ramirez et al., 2009), to a tank (TK1) where it is soaked to enable starch granules release. Water content of the resulting stream is around 45 wt %. In the next step, the process stream is filtered with a mesh (ML1) for soluble solid recovery, while the excess water, containing traces of starch and dissolved proteins, is concentrated through evaporation (FL3 and SD1) to produce gluten feed, after mixing with corn fibers downstream in the process. Soluble solids are sent to a milling stage where oil-rich corn germ is separated from the rest of the starchy slurry. To accomplish this goal a mill is used (MO1), followed by an hydrocyclone (HC1), obtaining a lipid rich stream (36 wt % lipids, 20 wt % proteins, 18 wt % fibers, 12 wt % water, 12 wt % starch and 2 wt % ashes) (Ramirez et al., 2009). After lipid extraction, the filtered starch suspension (ML2) is sent to a second mill (MO2), and to a filtering stage (ML3) in order to remove part of the water and release starch from fibrous material. This fiber rich stream is mixed with the concentrated starch and proteins from the aforementioned soaking stage (TK1) and sent to a dryer (SD1) before being sold as gluten feed with the following weight composition: 52 % fibers, 19 % starch, 16 % proteins, 10 % water, 2 % lipids and 1 % ashes. In the next stage, protein-enriched gluten is separated from starch by the use of a hydrocyclone (HC2) and a tank (TA) in order to obtain a gluten meal by-product with the following weight composition: 65 % proteins, 15 % starch, 10
% water, 5% fibers, 3% lipids and 2% ashes. Finally, corn starch is washed with water in a tank (TK2) to reach 60% humidity with 2% impurities. Once the processing is over, the starch obtained from corn grain can be used as a substrate for the biotechnological production of PHAs.

The proposed superstructure also considers the possibility of buying corn starch or cassava starch. Another alternative included is the glucose production from starch. This process involves evaporation in a flash (FL4) up to a 69 wt% concentration of starch (Van der Veen et al., 2006), followed by preheating up to $110 \, ^\circ\mathrm{C}$ to hydrolyze starch using $\alpha$-amylase in a liquefaction reactor (RL). The enzyme is added in a 0.5 wt% solution, in a 1:1000 enzyme-to-starch ratio. The resulting mixture contains starch oligomers called maltodextrins, which are saccharified in a reactor (RS) that operates at $60 \, ^\circ\mathrm{C}$. The saccharification is accomplished by the utilization of glucoamylase, which is added in a 1:40 enzyme-to-starch ratio with a concentration of 0.9 wt%. The final stream is obtained through evaporation in a flash (FL5) up to a glucose concentration of 80 wt%.

### 2.1.1.3. Sugarcane

Argentina’s current sugarcane production is around 28 Mt/y, a volume that exceeds its domestic demand for refined sugar. Its use for ethanol production has increased following national regulations imposing an increase of ethanol concentration in blends for transportation fuel, from 10% in 2014, to 12% in 2016. Even though it is proposed to further increase this fraction to 15% in few years, the sugarcane installed capacity excess would even allow for such fraction to reach 17%. Therefore, it would be of interest to develop alternative sugarcane-based processes. One of these processes could be the use of sucrose from sugarcane (70 wt% water, 14 wt% sugars, 13.5 wt% fibers, 1.5 wt% ashes, 0.6 wt% others and 0.4 wt% proteins) as a carbon source for the production of biopolymers (Nonato et al., 2001). In this sense, such possibility is
embedded within the proposed superstructure for PHA production, including simplified models for sugarcane processing, as it is shown in Fig. 1.

This process begins with raw material washing in tank TK3, the process stream is then processed in a mill (MO3) for the production of sugarcane juice and bagasse. Sugarcane bagasse constitute an important residue from the sugar industry, which can be processed for the production of thermal and electrical energy. In the proposed superstructure, a typical electrical energy production system is considered as a destination for this sub-product (Rincón et al., 2014). It consists in moisture elimination by a dryer (DR) to a 48 wt % of water stream. Then, it goes through in a combustion chamber (FR), with excess air. Released energy is used to generate high pressure steam, which is then circulated through a turbine (TB), producing electricity and low-pressure steam, which can be used to satisfy the biorefinery energy requirements. Sugarcane juice obtained from the milling stage needs to be treated to obtain high quality sugars due to the presence of a complex mixture of organic and inorganic contaminants. This process takes part in a clarifier (CL), at 65 ºC, through the incorporation of sulfuric acid (H₂SO₄) and calcium hydroxide (Ca(OH)₂), to promote impurity precipitation (Amores et al., 2013). In the case of H₂SO₄, 1.4 g of acid is used for each kg of sugars in sugar juice, added in a water solution of 4 wt %. For Ca(OH)₂, 9.32 g are used per kg of sugar (Amores et al., 2013). The waste stream from the clarification stage is sent to a rotating drum filter (RD) for cachaza production. This residue, obtained from the solids suspended in the juice can be commercialized as compost or animal feed (Moncada et al., 2013). After the clarification process, sugarcane juice can be used as carbon source for PHA producing microorganism growth. As alternatives to the afore described process, the possibility of directly buying processed sucrose or sugarcane molasses (Moncada et al., 2013) is included in the superstructure.

2.1.2. Biosynthesis
PHA production takes place in the biosynthesis stage by the biopolymer intracellular accumulation in a microbial strain (Fig. 2), through an excess supply of a carbon source and the limitation of another growth essential nutrient like nitrogen or phosphorus.

The first step in this process stage involves a sterilization of the carbon source (ST1, ST2 or ST3), where temperature and pressure are drastically increased to reduce the possibility of other microorganisms growth that might compete with the one producing the biopolymer. According to the selected carbon source, several operational alternatives are presented for the bioreactors used in this stage of the production process. Depending on the selected substrate, technologies involving two fermentation stages are presented. The first stage is included for biomass growth (BR1, BR3, BR5, BR7, BR9, BR11, and BR13), therefore no nutrient limitation is imposed. In the second bioreactor (BR2, BR4, BR6, BR8, BR10, BR12, and BR14), biopolymer production takes place and its accumulation is triggered by the limitation of essential nutrient sources. Table 1 shows the main parameters for the bioreactors employed for PHA production, which have been taken from the literature and are used as parameters for the model.

2.1.3. Upstream processing

Biopolymer extraction from the microorganism cytoplasm is a crucial step in the productive process, because its quality sets the product final price. Therefore, an appropriate selection of the extraction technology is necessary to achieve an economically viable process. As shown in Fig. 3, four extraction alternatives are included in the PHA production process superstructure, the use of enzymes, solvent, surfactant-NaOCl or surfactant-chelate.

In the enzymatic extraction alternative, the process stream is heated up to 85 °C (HX8) (Posada et al., 2011) before entering the digester (DG1), where the cell membrane lysis
is carried out by the use of enzyme pancreatin (Kapritchkoff et al., 2006) in a 2 wt % concentration. Simultaneously, to improve the dissolution of the cell membrane, which will be afterwards removed from the process, 0.5 kg of a 30 wt % NaOCl solution is added for each kg of total biomass. It is assumed that this process is able to extract 90 % of biopolymer within the microorganism biomass. Residual biomass, dissolved in the NaOCl solution is withdrawn through centrifugation (CN3). The re-suspended PHA stream is treated in a tank (TK4) with a 1.73 wt % \( \text{H}_2\text{O}_2 \) concentrated solution, with a 3.05:1 solution-to-PHA wt ratio, to bleach the polymer (Jacquel et al., 2008). Finally, water is partially eliminated through flash evaporation (FL6), to obtain a resulting 53 wt % PHA stream (Posada et al., 2011).

The process that employs solvent for PHA extraction uses diethyl-succinate (DES) and includes a homogenizer (TK5), which operates at 700 bar to enable cell lysis (Posada et al., 2011). This unit is assumed to have a retention time of 45 min. Part of the excess water contained in the process stream is removed in a centrifuge (CN4). Afterwards, both the process and the solvent streams are preheated (HX10 and HX11), up to 110 °C, and then mixed in an extractor (EX). In this unit, the solvent stream is fed in a solvent-to-total biomass ratio of 20:1, and the biopolymer recovery is assumed to be 95 % of the total cell weight. Residual biomass is withdrawn by centrifugation (CN5). Solvent recovery is performed through decantation (DC2) at 25 °C, with an assumed recovery of 93 % of the solvent mass, which is recycled to the extractor.

The third option involves the use of NaOCl and a surfactant for biopolymer extraction. In the first step, the process stream is mixed with an 11 wt % NaOH solution in a solution-to-total biomass weight ratio of 0.4:1 and preheated up to 35 °C in tank TK6. Then, a chemical digestion takes place in a digester (DG2), which operates at 55 °C, with 20 min retention time, by adding a surfactant (Sodium dodecyl sulfate, SDS) and NaOCl (32 wt %) in the same weight proportions of 1:3 SDS-to-total biomass, and
NaOCl-to-total biomass (Dong et al., 2000). In a similar way to that of the enzyme-based extraction process, the stream is centrifuged (CN6) to remove residual biomass, and it is washed with \( \text{H}_2\text{O}_2 \) in tank TK7, to achieve biopolymer bleaching. Finally, the volatile components are evaporated in flash FL7, to achieve a 25 wt % biopolymer stream.

The last technological route embedded in the superstructure involves surfactant and chelate as extraction agents. It is based on the process proposed by Chen et al. (2001), which pursues the maximization of water reuse in the extraction process. This method is presented as a promising alternative because it is environmentally friendly, having a higher quality of the final product and minimizing the requirement of chemicals. This process consists of several operations and begins with a digester (DG3) that operates at 50 \(^\circ\)C, where biomass is treated with surfactant (betaine) and water dissolved chelate (disodium salt of ethylenediaminetetraacetic acid). Chelate and surfactant addition produce internal and external cell membrane destabilization, via the formation of divalent cationic complexes (Jacquel et al., 2008). These changes lead to a disruption of the microorganism and enable a subsequent high purity biopolymer extraction. In this operation, mass ratio of surfactant-to-total biomass and chelate-to-total biomass is 0.12:1 and 0.08:1, respectively. Water is added to obtain a 0.8 wt % water concentrated stream. The second operation consists in reusing treated water from the process downstream. For this purpose, a second digester (DG4) is used after centrifugation (CN7), in which surfactant and chelate are added to the process stream in 0.0075:1 and 0.01:1 ratios, respectively. At this point, the addition of a 5 M NaOH solution intends to regulate the pH of the stream to keep it close to 13. Water purification is carried out in reactor RC2, through the treatment with a 4 M HCl solution and a pH value of 3, and a further solid content extraction through centrifugation (CN9). At the same time, water is treated in reactor RC3 with the addition of 0.5 kg of activated coal per kg of water,
considering a water purge of 10% of the water inflow. Finally, the biopolymer is washed in tank RC4 with water and acetone in a water-to-PHA and acetone-to-PHA mass ratio of 1:1 and 4:1, respectively. The biopolymer is recovered through centrifugation (CN10), considering a 2% product loss. Acetone recovery is 50% through flash distillation (FL8), to be recycled to the aforementioned washing step.

2.2. Mathematical model

The proposed superstructure is formulated as an MINLP problem, implemented in GAMS (McCarl et al., 2017) to determine the optimal design of a biorefinery for the production of 10,000 t/y of PHA. The economic objective function to maximize is the project net present value (NPV), subject to constraints that include process mass and energy balances, detailed equipment design equations and capital cost correlations. Integer variables and constraints are used to formulate the selection between alternative technologies.

2.2.1. Mass balances

The mass balances for the non-reactive units ($\theta$) of the proposed superstructure (Fig. 1-3) are formulated as follows:

$$\sum_{k \in K} f_{\theta, j}^k = \sum_{r \in R} f_{\theta, j}^r \quad \forall j \in J$$

(1)

where $f_{\theta, j}^k$ is the mass flowrate of component $j$ from inlet stream $k$ to unit $\theta$, in kg/h. $f_{\theta, j}^r$ is the mass flowrate of component $j$ from non-reactive unit $\theta$ to outlet stream $r$, in kg/h.

Similarly, mass balances for reactive units ($\theta'$) are described by Eq. (2), through the use of yield parameters based on the inflows to the processing units. This is done due to the partial absence of stoichiometric information of the chemical reactions involved in the described processes.
\[
\dot{m}_{\theta',r,j} = \sum_{k \in K} \dot{m}_{\theta',k,j} + \sum_{h \in H} \xi_{j,h} \cdot C_h \cdot \sum_{k \in K} \dot{m}_{\theta',s_h} \quad \forall j \in J
\]

where \( \dot{m}_{\theta',r,j} \) is the mass flowrate of component \( j \) from reactive unit \( \theta' \) to outlet stream \( r \), in kg/h. \( \dot{m}_{\theta',k,j} \) is the mass flowrate of component \( j \) from inlet stream \( k \) to non-reactive unit \( \theta' \), in kg/h. \( s_h \) denotes the limiting reactant for reaction \( h \). \( \xi_{j,h} \) is the mass coefficient between component \( j \) and component \( s_h \), in kg/kg, in reaction \( h \). \( C_h \) is the limiting reactant conversion for reaction \( h \). \( \dot{m}_{\theta',s_h} \) is the mass flowrate of component \( s_h \) from inlet stream \( k \) to unit \( \theta' \), in kg/h.

Detailed mass balances for each process unit in the superstructure are presented as Supplementary material.

2.2.2. Energy balances

Energy balances take into account the required energy for each process unit operation in the proposed superstructure. These requirements can be satisfied through electrical (motors, agitation) or thermal (exchange between process streams) energy (Ramos et al., 2017). For heat exchangers, a general, steady state energy balance is formulated for cold and hot streams, considering negligible heat transfer between the exchanger and its surroundings, and negligible potential and kinetic energy changes. Then, the difference between the entrance and exit enthalpies of process stream \( k \) (\( \sum_{j=1}^{39} \dot{m}_{\theta',j} \)), denoted by \( h_i \) and \( h_o \), respectively, corresponds to the heat exchanged in each unit \( \theta \), denoted by \( q_\theta \).

This relation is expressed by Eq. (3).

\[
q_\theta = \sum_{j=1}^{39} \dot{m}_{\theta',j} \cdot (h_o - h_i)
\]
Furthermore, electric energy required by centrifuges, reactors, digesters, homogenizers and bioreactors are calculated using nonlinear correlations proposed by Ulrich and Vasudevan (2004), as follows:

\[ EC_\theta = ECR_\theta \cdot \left( \sum_{j=1}^{f_{\theta,j}} \right) \]

where \( EC_\theta \) corresponds to energy consumption in unit \( \theta \), in kJ/h, \( ECR_\theta \) is energy consumption ratio per unit of mass flowrate relative to unit \( \theta \), in kJ/kg, and \( n \) is an exponential factor tabulated for each process unit.

### 2.2.3. Integer and mixed integer constraints

Potential units proposed in the superstructure (Fig. 1-3) are associated to binary variables, which are the main decision variables for determining the optimal technological pathway. The integer and mixed integer constraints are formulated using propositional logic and represented in terms of linear inequalities involving 0-1 variables (Raman and Grossmann, 1994), and are given in the Supplementary material.

Binary variables are also included in the mass balances by big M formulations (Grossmann and Ruiz, 2012) for potential units in the superstructure, as follows.

\[ F_{\theta,j}^k - M y_i \leq 0 \quad \forall j \in J, i \in I, k \in K, \theta \in \Theta \]

where \( F_{\theta,j}^k \) corresponds to the mass flowrate of component \( j \) in stream \( k \) fed to equipment \( \theta \) and \( M \) is a number large enough that when \( y_i=1 \), the constraints over the flowrate become redundant, otherwise, if \( y_i=0 \), the mass flowrate is enforced to be null.

### 2.2.4. Design and economic constraints

The model includes detailed sizing equations for each process unit embedded in the superstructure and nonlinear capital cost functions (Ulrich and Vasudevan, 2004; Echarte 2010), which are given in the Supplementary material section.
2.2.5. Objective function

The objective function to be maximized is the project net present value ($NPV$) given by Eq. (6).

$$NPV = -I + UT a^{-1} + V_{rec} f_{ut}^{-1}$$

(6)

where $a$ is an annuity factor, and $f_{ut}$ is an update factor to bring a cash flow at the end of the project lifetime ($n$) to the present. They are both calculated for a project lifespan of 15 years, and an interest rate ($i$) of 0.1, as follows:

$$a = \frac{i(1 + i)^n}{(1 + i)^n - 1}$$

(7)

$$f_{ut} = (1 + i)^n$$

(8)

In $NPV$ definition, Eq. (6), $I$ is the total investment cost. This variable is defined by Eq. (9) as the sum of the fixed capital ($C_{fixed}$) and the working capital ($C_{working}$).

$$I = C_{fixed} + C_{working}$$

(9)

Fixed capital ($C_{fixed}$) is calculated by Eq. (10) as the sum of the equipment cost ($C_{equip}$), land cost ($C_{land}$), and piping and instrumentation cost ($C_{piping/instrument}$). It is worth mentioning that a tax contingency factor ($\alpha = 1.18$) and a grass-root factor ($\beta = 1.3$) are taken into account, as we address a totally new plant.

$$C_{fixed} = C_{equip}(\alpha + \beta) + C_{land} + C_{piping/instrument}$$

(10)

Working capital is assumed to be 10% of the fixed capital, according to the recommendation of Ulrich and Vasudevan (2004).

In Eq. (6), variable $UT$ corresponds to the net annual profit expected from the project. This variable involves revenues from products and sub-products sales ($IT$), manufacturing, both direct and indirect, costs ($C_{manu}$), general costs ($C_{general}$) and income tax ($T_{income}$).
\[ UT = IT - (C_{\text{manu}} + C_{\text{general}} + T_{\text{income}}) \] (11)

The recovery value \( V_{\text{rec}} \) is the estimated income from selling the equipment (assumed to be 20 % of total investment cost), and the working capital recovery at the end of the project lifespan.

\[ V_{\text{rec}} = C_{\text{working}} + 0.2 \cdot I \] (12)

2.2.6. Sensitivity analysis

A sensitivity analysis is performed in order to evaluate the impact of uncertainty in model parameter values over the objective function. Furthermore, this study provides valuable information regarding potential technological and marketing improvements, which can be implemented to accomplish a higher profit.

3. Results and discussion

The MINLP model for the production of 10,000 t/y of PHA is formulated in an equation oriented framework in GAMS 24.2.3 (McCarl et al., 2017). The model is implemented in a personal computer with an Intel® Core™ i7-3770K processor, operating at a CPU frequency of 3.5 GHz, and with 8 Gb RAM. The formulated model includes 8,249 continuous variables, 25 discrete variables and 7,456 constraints, and is solved using DICOPT, with CONOPT and CPLEX as nonlinear and linear sub solvers, respectively (Grossmann et al., 2003). The model is solved to an objective function value of \( NPV = 75.01 \) million USD, in a CPU time of 14.62 s. The model is also solved with a global optimization solver, BARON (Tawarmalani and Sahinidis, 2005), providing the same optimal alternative and objective function. CPU time is largely increased, taking 2,104 s to reach relative and absolute gaps of 0.001 and 0.246.10^6, respectively.

The optimal flowsheet corresponding to \( NPV \) maximization includes the use of sugarcane as carbon source for biopolymer production, selecting an enzymatic technology as the PHA extraction method. This scheme, together with the optimal mass
flowrates of the main process streams are presented in Fig. 4. A PHA producing capacity of 1,142 kg/h (10,000 t/y) is similar to numerous currently industrial production plants, like Metabolix, TianAn Biologic Material Co, Tianjin GreenBio and Bio-on (Levett et al., 2016). The required amount of sugarcane in the optimal configuration for PHA production is 23,731 kg/h (0.208 Mt/y). For comparison purposes, it corresponds to approximately 1 % of the total production estimated for Argentina during 2017/2018 season, which is 22.5 Mt/y. This is considered to be an indication towards the feasibility of this process from sugarcane.

Based on the international market prices for raw materials and products, the maximum NPV attainable in the optimal configuration is 75.01 million USD. In this case, PHA production cost is 3.02 US$/kg, which is comparable to PHA production costs throughout the world, which vary around 4 US$/kg (Koller et al., 2017). Furthermore, taking into account that PHA is a biodegradable biopolymer, incentives could be provided to enhance its production, rendering a lower production cost.

Fig. 5a shows the PHA production cost breakdown for the optimal process configuration. The main contribution is related to raw materials and other supplies, representing 75.9 % of the total production cost. In particular, the carbon source cost turns out to be 26 % of the biopolymer production cost, as shown in Fig. 5b. These results are in agreement with the published literature (Posada et al., 2011; Levett et al., 2016; Koller et al., 2017), which mention that the carbon source can represent up to 45 % of the production cost. From cost breakdown analysis, it can be seen that one strategy to lower PHA production cost could be to reduce costs associated to enzymes used in the biopolymer extraction section, which are around 28 US$/kg (Kapritchkoff et al., 2006). In this sense, a contribution could be made by exploring the use of lower cost enzymes in the biopolymer extraction process. Energy consumption for the optimal PHA production scheme is estimated to be 22.56 MJ/kg PHA. This value is comparable
with values available in the literature. For instance, Akiyama et al. (2003) report an energy consumption of 41.88 MJ/kg PHA for a 5,095 t/y plant capacity, where the biopolymer is produced from soybean oil. López-Arenas et al. (2017) obtained energy consumptions of 26.6 MJ/kg PHB in a fed-batch bioreactor and 29.2 MJ/kg PHB in a batch bioreactor, as result of the economic assessment for a PHB production plant using sucrose as carbon source. Fig. 5c presents production process energy consumption breakdown, considering process stage and type of energy (electrical or thermal). It can be seen that the main energy consumption is associated to the extraction and purification section, 12.80 GJ/h, 96 % of which is thermal energy. A deeper energy consumption assessment of the extraction and purification section is performed and shown in Fig. 5d. It can be noted that the heat exchanger (HX9), used to evaporate the water contained in the process stream, is the equipment which has the major energy requirement (66.94 %), followed by the spray drier (SD2), used to purify the final product (26.58 %). Therefore, these units are candidates for the implementation of an energy integration scheme, which could have a positive effect on the biorefinery by lowering total energy consumption. Also, it is worth mentioning that studies such as the one presented by Shahzad et al. (2013), highlight the importance of energy aspects not only for economics purposes but also for achieving a sustainable PHA production.

Since NPV does not provide on its own the complete information to perform a project assessment, additional profitability indexes are calculated (Table 2). For these estimations, an interest rate (i) of 10 % and a project lifetime of 15 years were used. The internal rate of return (IRR) represents the maximum value that i could have before the NPV becomes negative. For the optimal flowsheet scheme, IRR has a value of 52.53 %. The large difference between IRR and i can be considered as an indication of the high project profitability. On the other hand, the return on investment (ROI_A), which takes into account time value of money and represents the return that is expected from the
total capital investment, is 22.36% for the optimal configuration. Finally, the
annualized payback period ($PBP_A$), which is a measure of the time required to recover
capital investment, takes a value of 3 years. It resembles the investment payback time
reported by Shahzad et al. (2017), which varies from 3.25 to 4.5 years depending on
market fluctuations. According to Lopez-Arenas et al. (2017), as long as the PHA cost
of production is lower than its selling price, a biopolymer production process can be
considered profitable if $ROI_A$ is greater than 20%. This condition is fulfilled by the
optimal technological configuration, as it can be seen in Table 2.

The total capital investment cost ($I$) takes into account the following: required capital
for plant construction (piping and instrumentation, land acquisition, contingencies,
construction labor), biorefinery starting up, working capital and equipment costs. Total
investment for the PHA production plant from sugarcane is 21.335 million USD and its
breakdown is presented in Fig. 5e.

Sensitivity analysis is performed on the optimal technological route (sugarcane and
enzymatic extraction) in order to evaluate the influence of different parameter values
over the economic objective value ($NPV$). As it can be seen in Fig. 6, the most
significant parameters are biopolymer selling price, enzyme concentration in the
digester (DG1) of the extraction section, and electrical energy price. Additionally, $NPV$
is considerably sensitive to changes in thermal energy cost and the biopolymer yield of
bioreactors in the biosynthesis section. On the other hand, parameters like the price of
the generated cachaza have very little influence on the objective function.

This analysis can help to identify aspects of the process where there are potential
enhancements, and therefore lead research efforts to improve PHA technologies.
Although some of the aforementioned parameters are linked to the market situation (raw
materials, energy and product prices), some other are process related and could generate
an improvement in the economic assessment of the biorefinery. For instance, some
biological parameters have a strong influence in \( NPV \), such as the efficiency of the enzymes considered and the microorganism biosynthesis yield.

The implementation of an algebraic model as an MINLP based on a superstructure allows enumerating the different sub-optimal technological routes that could also have an economic potential. This is achieved by the addition of integer cuts which make infeasible previous optimal solutions (Rizwan et al., 2015). The iteration of this process (solving the model and adding constraints to exclude the last best solution), allows the generation of a list that includes the sub-optimal pathways, which can also be considered attractive and worth of future technological improvements studies. This process is described in detail by Eq. (13).

\[
\sum_{i \in A^n} y_i - \sum_{i \in B^n} y_i \leq |A^n| - 1
\]

where \( A^n = \{i | y_i^k = 1\} \), \( B^n = \{i | y_i^k = 0\} \) and \( n \) is the number of integer cuts.

The first four technological alternatives are presented in Table 3. The results show that sugarcane remains the most profitable feedstock, placing sugarcane molasses secondly as carbon source for PHA producing microorganisms. Regarding extraction technologies, the most profitable is enzymatic extraction, followed by the extraction using surfactant-chelate, and surfactant-NaOCl placed in third position.

4. Conclusions

This article presents an MINLP algebraic model representing a superstructure of technologies for the industrial production of PHAs. Optimization results point out that the optimal technological route is the one that employs sugarcane as a carbon source for PHAs production and enzymes for PHAs extraction. \( NPV \) for this configuration is 75.01 million USD, rendering a biopolymer production cost of 3.02 US$/kg and an energy requirement of 22.56 MJ/kg PHA. Additional profitability indexes calculation enhances
the selection of this bioprocess technology. Furthermore, sensitivity analysis shows the potential aspects that should be taken into account to increase the process profit.

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Appendix A.

E-supplementary data of this work can be found in online version of the paper.

References

1. Akiyama, M., Tsuge, T., Doi, Y., 2003. Environmental life cycle comparison of polyhydroxyalkanoates produced from renewable carbon resources by bacterial fermentation. Polym. Degrad. Stab. 80, 183-194.

2. Amores, M. J., Mele, F. D., Jimenez, L., Castells, F., 2013. Life cycle assessment of fuel ethanol from sugarcane in Argentina. Int. J. Life Cycle Assess. 18, 1344-1357.

3. Biegler, L. T., Grossmann, I. E., Westerberg, A. W., 1997. Systematic methods of chemical process design, first ed. Prentice Hall, New Jersey.

4. Cavalheiro, J., De Almeida, A., Grandfils, C., Da Fonseca, M. M. R., 2009. Poly(3-hydroxybutyrate) production by *Cupriavidus necator* using waste glycerol. Process Biochem. 44, 509-515.

5. Chen, Y.; Yang, H., Zhou, Q., Chen, J., Gu, G., 2001. Cleaner recovery of poly(3-hydroxybutyric acid) synthesized in *Alcaligenes eutrophus*. Process Biochem. 36, 501-506.

6. Dheskali, E., Michailidi, K., Machado de Castro, A., Koutinas, A. A., Kookos, I. K., 2017. Optimal design of upstream processes in biotransformation technologies. Bioresource Technol. 224, 509-514.
7. Dietrich, K., Dumont, M. J., Del Rio, L. F., Orsat, V., 2017. Producing PHAs in the bioeconomy-Towards a sustainable bioplastic. Sustainable Prod. Consumption. 9, 58-70.

8. Dong, Z., Sun, X., 2000. A new method of recovering polyhydroxyalkanoate from *Azotobacter chroococcum*. Chinese Sci. Bull. 45, 252-256.

9. Douglas, J. M., 1988. Conceptual design of chemical processes. McGraw-Hill, New York.

10. Echarte, R., 2010. Equipos para procesos químicos. EdiUNS, Bahía Blanca.

11. Gong, J., You, F., 2014. Global optimization for sustainable design and synthesis of algae processing network for CO2 mitigation and biofuel production using life cycle optimization. Aiche J. 60, 3195-3210.

12. Grossmann, I. E., 1996. Mixed-integer Optimization Techniques for Algorithmic Process Synthesis. Advances. 23.

13. Grossmann, I. E., Viswanathan, J., Vecchietti, A., Raman, R., Kalvelagen, E., 2003. GAMS/DICOPT: A discrete continuous optimization package. Washington, DC, USA.

14. Grossmann, I. E., Ruiz, J. P., 2012. Generalized disjunctive programming: A framework for a formulation and alternative algorithms for MINLP optimization. in: Lee, J., Leyffer, S. (Eds.), Mixed Integer Nonlinear Programming, Springer, New York., 154, pp. 93-115.

15. Grossmann, I. E., Harjunkoski, I., 2019. Process systems Engineering: Academic and industrial perspectives. Comput. Chem. Eng. 126, 474-484.

16. 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.

17. Kapritchkov, F. M., Viotti, A. P., Alli, R. C. P., Zuccolo, M., Pradella, J. G. C., Maiorano, A. E., Miranda, E. A., Bonomi, A., 2006. Enzymatic recovery and
purification of polyhydroxybutyrate produced by *Ralstonia eutropha*. J. Biotechnol. 122, 453-462.

18. Koller, M., Marsálek, L., Miranda de Sousa Diaz, M., Braunegg, G., 2017. Producing microbial polyhydroxyalkanoate (PHA) biopolymers in a sustainable manner. New Biotechnol. 37, 24-38.

19. 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.

20. López, J. A., Naranjo, J. M., Higuita, J. C., Cubitto, M. A., Cardona, C. A., Villar, M. A., 2012. Biosynthesis of PHB from a new isolated *Bacillus megaterium* strain: Outlook on future developments with endospore forming bacteria. Biotechnol. Bioproc. E. 17, 250-258.

21. Lopez-Arenas, T., Gonzalez-Contreras, M., Anaya-Reza, O., Sales-Cruz, M., 2017. Analysis of the fermentation strategy and its impact on the economics of the production process of PHB (polyhydroxybutyrate). Comput. Chem. Eng. 107, 140-150.

22. Luo, X., Ge, X., Cui, S., Li, Y., 2016. Value-added processing of crude glycerol into chemicals and polymers. Bioresource Technol. 215, 144-154.

23. Mannina, G., Presti, D., Mntriel-Jarillo, G., Suárez-Ojeda, M. E., 2019. Bioplastic recovery from wastewater: A new protocol for polyhydroxyalkanoates (PHA) extraction from mixed microbial cultures. Bioresource Technol. 282, 361-369.

24. Martin, M., Grossmann, I. E., 2012. Simultaneous optimization of heat integration for biodiesel production from cooking oil and algae. Ind. Eng. Chem. Res. 51, 7998-8014.

25. McCarl, B. A., Meeraus A., van der Eijk, P., Bussieck, M., Dirkse, S., Steacey, P., Nelissen, F. 2017. McCarl GAMS User Guide, Washington, DC, USA.
26. Mohapatra, S., Sarkar, B., Samantaray, D. P., Daware, A., Maity, S., Pattanaik, S., Bhattacharjee, S., 2017. Bioconversion of fish solid waste into PHB using *Bacillus subtilis* based submerged fermentation process. Environ. Technol. 38, 3201-3208.

27. Moncada, J., El-Halwagi, M. M., Cardona, C. A., 2013. Techno-economic analysis for a sugarcane biorefinery: Colombian case. Bioresource Technol. 135, 533-543.

28. Nonato, R., Mantelatto, P., Rosell, C., 2001. Integrated production of biodegradable plastic, sugar and ethanol. Appl. Microbiol. Biotechnol. 57 (1-2), 1-5.

29. Poomipuk, N., Reungsang, A., Plangklang, P., 2014. Poly-β-hydroxyalkanoates production from cassava starch hydrolysate by *Cupriavidus* sp. KKV38. Int. J. Biol. Macromol. 65, 51-64.

30. Porras, M. A., Ramos, F. D., Diaz, M. S., Cubitto, M. A., Villar, M. A., 2019. Modeling the bioconversion of starch to PHB-co-HV) optimized by experimental design using *Bacillus megaterium* BBST4 strain. Environ. Technol. 40, 1185-1202.

31. Posada, J. A., Naranjo, J. M., López, J. A., Higuita, J. C., Cardona, C. A., 2011. Design and analysis of PHB production processes from crude glycerol. Process Biochem. 46, 310-317.

32. Raman, R., Grossmann, I. E., 1994. Modelling and computational techniques for logic based integer programming. Comput. Chem. Eng. 18, 563–578.

33. Ramirez, E. C., Johnston, D. B., McAloon, A. J., Singh, V., 2009. Enzymatic corn wet milling: engineering process and cost model. Biotechnol. Biofuels. 2, 2.

34. Ramos, F. D., Villar, M. A., Diaz, M. S., 2017. Optimal Design of poly(3-hydroxybutyrate) production using alternative carbon sources. Comput. Aided Chem. En. 40, 877-882.

35. Rincón, L. E., Becerra, L. A., Moncada, J. Cardona, C. A., 2014. Techno-economic analysis of the use of fired cogeneration systems based on sugar cane bagasse in south eastern and mid-western regions of Mexico. Waste Biomass Valori. 5, 189-198.
36. Rizwan, M., Lee, J. H., Gani, R., 2015. Optimal design of microalgae-based biorefinery: Economics, opportunities and challenges. Appl. Energ. 150, 69-79.

37. Rossell, C. E. V., Mantelatto, P. E., Agnelli, J. A. M., Nascimento, J., 2010. Sugar based biorefinery- Technology for integrated production of poly(3-hydroxybutyrate), sugar and ethanol, in: Kamm, B., Gruber, P. R., Kamm, M. (Eds.), Biorefineries-Industrial processes and products: status quo and future directions, Wiley VCH, Weinheim.

38. Shahzad, K., Kettl, K.-H., Titz, M., Koller, M., Schnitzer, H., Narodoslawsky, M., 2013. Comparison of ecological footprint for biobased PHA production from animal residues utilizing different energy resources. Clean Techn. Environ. Policy. 15, 525-536.

39. Shahzad, K., Narodoslawsky, M., Sagir, M., Ali, N., Ali, S., Rashid, M. I., Ismail, I. M. I., Koller, M., 2017. Techno-economic feasibility of waste biorefinery: Using slaughtering waste streams as starting material for biopolyester production. Waste Manage. 67, 73-85.

40. Tawarmalani, M., Sahinidis, N. V., 2005. A polyhedral branch and cut approach to global optimization. Math. Program. 103, 225-249.

41. Tripathi, A. D., Yadav, A., Jha, A., Srivastava, S. K., 2012. Utilizing of sugar refinery waste (cane molasses) for production of bio-plastic under submerged fermentation Process. J. Polym. Environ. 20, 446-453.

42. Ulrich, G. D., Vasudevan, P. T., 2004. Chemical engineering process design and economics: A practical guide. Process Publishing, Durham, New Hampshire.

43. Van der Veen, M. E., Veelaert, S., Van der Goot, A. J., Boom, R. M., 2006. Starch hydrolysis under low water conditions: A conceptual process design. J. Food Eng. 75, 178-186.
Figure captions

Figure 1. Superstructure of the raw materials purification section for the PHAs production biorefinery.

Figure 2. Superstructure of the biosynthesis section for the PHAs production biorefinery.

Figure 3. Superstructure of the extraction and purification section for the PHAs production biorefinery.

Figure 4. PHA biorefinery optimal configuration.

Figure 5. PHA biorefinery optimization results a) PHA production costs breakdown b) Raw materials and supplies costs breakdown c) Energy consumption distribution of the PHA production biorefinery d) Energy consumption distribution of the extraction and purification equipment e) Total capital investment cost breakdown.

Figure 6. Sensitivity analysis of the PHA production biorefinery.
Table 1. Biosynthesis section main parameters.

| Carbon source                  | $Y_{P/S}$ (kg/kg) | $Y_{X/S}$ (kg/kg) | PHA % | Retention time ($1^{st}$BR- $2^{nd}$BR h) | Carbon source consumption (%) | References                      |
|-------------------------------|------------------|------------------|-------|----------------------------------------|-------------------------------|-------------------------------|
| Purified glycerol             | 0.215            | 0.370            | 58    | 22-20.5                                | 77                            | (Cavalheiro et al., 2009)  |
| Crude glycerol                | 0.190            | 0.380            | 50    | 22-20.5                                | 71                            | (Cavalheiro et al., 2009)  |
| Corn starch                   | 0.138            | 0.212            | 65    | 20-58                                  | 93                            | (Porras et al., 2019)     |
| Cassava starch                | 0.15             | 0.340            | 44    | 36-60                                  | 85                            | (Poomipuk et al., 2014)  |
| Glucose                       | 0.38             | 0.475            | 80    | 12-22                                  | 60                            | (López et al., 2012)     |
| Sucrose                       | 0.4              | 0.533            | 75    | 24-30                                  | 97                            | (Rossell et al., 2010)   |
| Sugarcane molasses            | 0.24             | 0.32             | 75    | 24-36                                  | 97                            | (Tripathi et al., 2012) |
Table 2. Profitability indexes considered in the techno-economic assessment.

| Profitability indexes | Values | Units     |
|-----------------------|--------|-----------|
| NPV                   | 75.01  | million USD |
| $\text{IRR}$         | 52.53  | %         |
| $\text{ROI}_A$       | 22.36  | %         |
| $\text{PBP}_A$       | 3      | years     |
Table 3. Potential technological pathways for PHAs production.

|                | NPV (million USD) | Carbon source | Extraction and purification technology |
|----------------|-------------------|---------------|----------------------------------------|
| Global optimum | 75.01             | Sugarcane     | Enzymatic                              |
| Alternative 1  | 60.27             | Sugarcane     | Surfactant-chelate                      |
| Alternative 2  | 58.16             | Sugarcane     | Surfactant-NaOCl                        |
| Alternative 3  | 0.57              | Sugarcane molasses | Enzymatic                              |
| Alternative 4  | -21.39            | Sugarcane molasses | Surfactant-chelate                      |
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Highlights

- Design and optimization of a poly(hydroxyalkanoate) production plant.
- Mass and energy balances, design, sizing and cost equations.
- Polymer biosynthesis using economical carbon sources.
- Techno-economic assessment of the biopolymer process.
- Economic sensitivity analysis reveals potential improvements to the bioprocess.