Optimization on the use of crude glycerol from the biodiesel production to obtain poly-3-hydroxybutyrate

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Abstract: The biotechnological production of poly-3-hydroxybutyrate (PHB) from crude glycerol obtained during the biodiesel production was techno-economically assessed. For the fermentation process, two different strains, *Cupreavidus necator* and *Bacillus megaterium* were considered. Moreover, three downstream processes for PHB separation and purification were analyzed. Thus, in total six biotechnological schemes to transform the crude glycerol obtained in the biodiesel industry were compared. Each biotechnological scheme considered five main process stages namely: (*i*) glycerol purification, (*ii*) glycerol fermentation to PHB, (*iii*) mass cell pretreatment, (*iv*) PHB isolation, and (*v*) PHB purification. Aspen Plus and Aspen Icarus were used for the processes simulation and for the economic assessment, respectively. During the processes simulation the crude glycerol stream was purified from 60 to 98 wt %, and the fermentation process was considered in two continuous stages where mass cell growth and PHB accumulation occurred, respectively. Also, the three downstream processes were based on: (*i*) heat pretreatment and chemical-enzymatic digestion, (*ii*) high pressure homogenizer and solvent extraction, and (*iii*) alkaline pretreatment and chemical-enzymatic digestion. Economic results showed that the best technological scheme uses *C. necator* for the fermentation stage, with a heat pretreatment and enzymatic-alkaline digestion for the downstream process.

Keywords: *Glycerol fermentation, PHB production, Process simulation, Process assessment.*

1. Introduction

Glycerol as a by-product on biodiesel production is obtained at high concentration in a weight ratio of 1/10 (glycerol/biodiesel) [1]. Moreover, the growing market of biodiesel has generated a glycerol oversupply, where its production increased 400% in a two-year period and consequently the commercial price of glycerol fell down about 10 times. As a result of the low prices of glycerol, traditional producers such as Dow Chemical and Procter and Gamble Chemicals, stopped the glycerol production [2].

Since glycerol sales have represented an important profitability for the biodiesel industry, it is reasonable to think that low prices of glycerol could affect negatively the economy of biodiesel producers. For this reason, the correct exploitation of glycerol as raw material should be focused on its transformation to added-value products [3]. Thus, the use of glycerol is a high-priority topic for managers and researchers related to the production of biodiesel. In this sense, the establishment of glycerol's biorefineries capable to co-generate added-value products is an excellent opportunity not only to increase the biodiesel profitability, but also to produce high-demanded chemicals from a biobased raw material [4].

Glycerol as the structural component of many lipids is abundant in nature. It is produced by yeasts during osmoregulation to decrease extracellular water activity due to its compatible solubility [5]. Wide glycerol occurrence in nature allows different kinds of microorganisms to metabolize it as a sole carbon and energy source. Thus, in some industrial fermentation processes, glycerol can substitute traditional carbohydrates, such as sucrose, glucose, and starch [5]. In this way, one of many promising applications to take advantage of the glycerol surplus is its bioconversion to high value compounds through microbial fermentation. An interesting application is to transform glycerol to poly-3-hydoroxybutyrate (PHB), which has similar properties to conventional plastics such as polypropylene or polyethylene. Also, PHB

can be extruded, molded, spun into fibers, made into films, and used to make heteropolymers with other synthetic polymers [6].

Here, the biotechnological production of PHB from crude glycerol obtained during the biodiesel production was techno-economically assessed. For the fermentation process, two different strains, *Cupreavidus necator* and *Bacillus megaterium* were considered. Moreover, three downstream processes for PHB separation and purification were analyzed. Thus, six biotechnological schemes to transform the crude glycerol obtained in the biodiesel industry were compared.

2. Methodology

The approach used for the processes design was a knowledge-based strategy that considers both heuristic rules and researcher's experience. Process simulation and economic assessment were carried out utilizing the Aspen Plus software and the Aspen Icarus package, respectively. Material and energy balances were obtained as simulation results for each technological scheme. As a result, requirements of raw materials, services, and energy were obtained. Also, estimation of energy consumption and related costs were calculated based on the simulation results for thermal units such as heat exchangers, reboilers, and evaporators. This methodology has been previously presented by Cardona et al. [5-10].

2.1. Process description

The PHB production process from crude glycerol requires five main stages [11], namely: glycerol purification, glycerol fermentation (cell growth and PHB accumulation), mass cell pretreatment, PHB isolation, and PHB purification. *Cupreavidus necator* and *Bacillus megaterium* were considered as the bacterial strains in order to compare the fermentation process. Glycerol purified at 98 wt % was considered as substrate in all cases and the three different downstream processes were analyzed with each strain, as shown in Fig. 1.

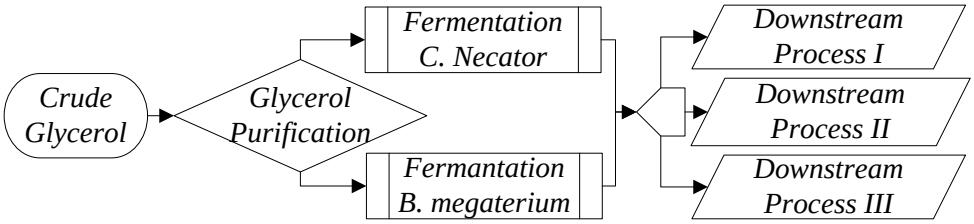

Fig. 1. Schematic representation of the PHB production from crude glycerol by C. necator and B. megaterium.

2.1.1. Glycerol purification

A typical composition for a crude glycerol stream obtained from the biodiesel production process is as follows: 32.59 wt % methanol, 60.05 wt % glycerol, 2.62 wt % $NaOCH_3$, 1.94 wt % fats, and 2.8 wt % ash [3].

Fig. 2 shows the flowsheet for crude glycerol purification up to 98 wt % [4]. The crude glycerol is initially evaporated and 90 % of the methanol is recovered at 99 wt %. Also, this obtained stream meets the necessary conditions to be reused in the transesterification process. The resulting bottom stream from the evaporation stage is neutralized using an acid solution where both the salts produced during the neutralization and the remaining ashes are removed by centrifugation. The fluid stream is washed with water using a weight ratio of 2.4 (water/glycerol stream), and thus an aqueous glycerol free of salts and solids, with a low

concentration of methanol and triglycerides is obtained. More than 90 % of water and the remaining methanol are then removed by evaporation. At this point the purity of the obtained glycerol is 80 wt %, and the glycerol stream is finally purified through a distillation column up to 98 wt %.

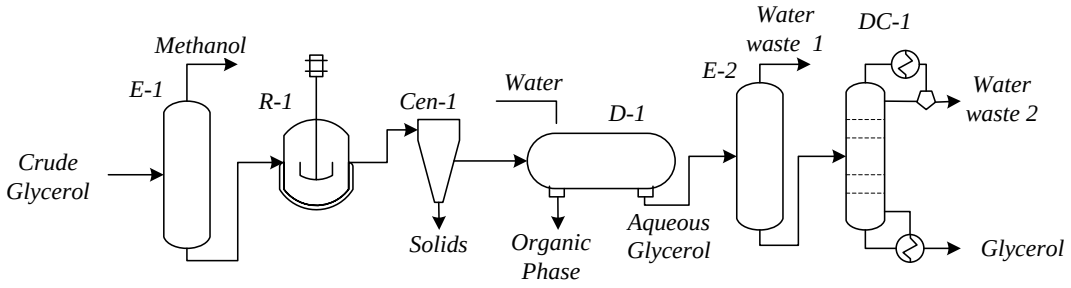

Fig. 2. Simplified flowsheet of the glycerol purification process up to 98 wt%.E-1: Evaporator I, R-1, Neutralizing Reactor I, Cen-1: Centrifuge I, D-1: Decanter I, E-2: Evaporator II, DC-1: Distillation Column I.

2.1.2. Fermentation process

Biotechnological production of PHB from glycerol requires a limitation of an essential nutrient such as: N, P, Mg, K, O or S, and an excess of a carbon source. Some of the bacterial strains capable of doing this transformation are *Bacillus megaterium*, *Cupriavidus necator*, *Alcaligenes eutrophus*, *Pseudomonas extorquens, and Pseudomonas oleovoran,* among others. Also, during the fermentation process the PHB accumulation could reach values between 40 to 70 wt %, with productivities up to 1.5 g/(L h) [6].

For the fermentation process two strains were compared, *C. necator* and *B. megaterium*, using a two stages fermentation process. In the first stage the cell growth occurs, while in the second stage the PHB accumulation takes place. Since the dissolved oxygen in the cell growth media must be between 15 and 20 %, air and oxygen should be fed in the first fermentation stage. Prior to the fermentation process, the diluted glycerol should be sterilized at 25 atm and 139 °C, as shown in Fig. 3.

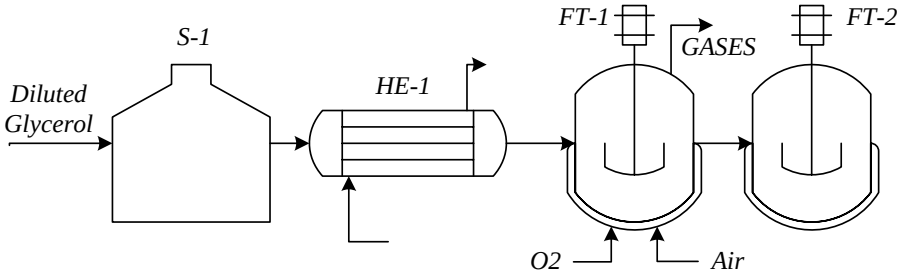

Fig. 3. Simplified flowsheet for the glycerol fermentation to PHB. S-1: Sterilizator I, HE-1: Heat Exchanger I, FT-1: Fermentation Tank I, FT-2: Fermentation Tank II

When the fermentation is carried out by *C. necator* the glycerol stream is diluted at 249 g/L [12], and the residence time for both fermentation stages are 21 and 22.5 h. In the case of *B. megaterium*, the glycerol stream is diluted at 50 g/L [13] with the respective residence times of 75 and 21 h.

2.1.3. Downstream processes

The downstream process for PHB purification from a fermentation broth can be divided in three parts: pretreatment, extraction, and purification. In the pretreatment step, cell disruption can be carried out by the action of heat, alkaline media, salty media, or freezing. In the case of extraction, some alternatives such as: solvent extraction, chemical digestion, enzymatic digestion, mechanical cell disruption, supercritical fluid extraction, cell fragility, and spontaneous liberation can be used. Finally, the purification methods involve a hydrogen peroxide treatment combined with the action of enzymes or chelating agents. [11].

The first considered downstream process starts with a heat pretreatment stage at 85 °C, followed by a simultaneous chemical-enzymatic digestion with both NaOCl (30 wt %) and the enzyme *Burkholdeira sp. PTU9* (2 wt %), at 50 °C and pH 9. Then the disrupted mass cell is discarded by a centrifugation process. The suspended PHB stream is washed with a H_2O_2 diluted stream (1.2 v/v %). Finally, the PHB is purified up to 99.9 wt % by evaporation and spray drying, as shown in Fig. 4.

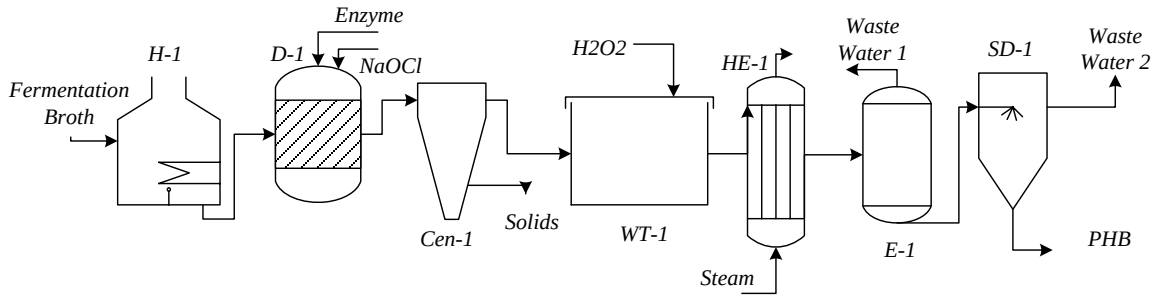

Fig. 4. Simplified flowsheet for the first downstream process. H-1: Heater I, D-1: Digestor I, Cen-1: Centrifuge I, WT-1: Washer Tank I, HE-1: Heat Exchanger I, E-1: Evaporator I, SD-1: Spray Drier I.

The second downstream process starts with a high pressure homogenizer at 70 MPa and 110 °C, where the cell mass is disrupted. Then, the centrifugation is carried out prior to the solvent extraction. In this extraction diethyl-succinate (DES) at 110 °C in a mass ratio of 1/20 (PHB/solvent) is used. A second centrifugation stage is employed in order to withdraw the residual cell mass. Thus, a mixture of PHB-water is gelled by cooling and the DES is recovered. Finally, the PHB at 99.9 wt % is obtained by spray drying, as shown in Fig. 5.

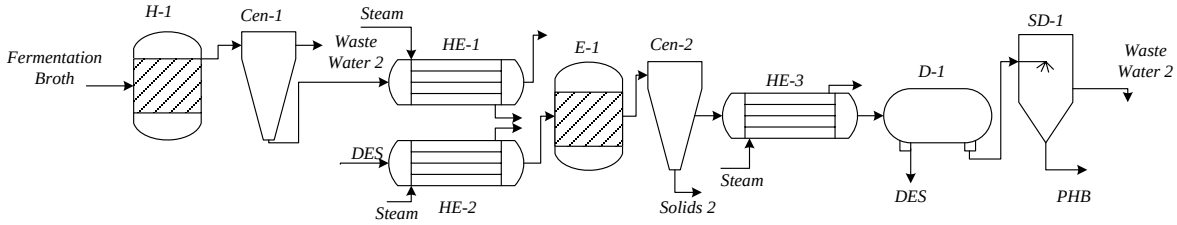

Fig. 5. Simplified flowsheet for the second downstream process. H-1: Homogenizer I, Cen-1: Centrifuge I, HE-1: Heat Exchanger I, HE-2: Heat Exchanger II, E-1: Extractor I, Cen-2: Centrifuge II, HE-3: Heat Exchanger III, D-1: Decanter I, SD-1: Spray Drier I.

In the third downstream process, the fermentation broth is pretreated with an alkaline solution of NaOH at 70 MPa and 110 °C. Then, a digestion process is carried out using NaOCl and sodium dodecylsulfate (SDS) at 55 °C. The disrupted cells are centrifuged and the PHB is washed with H_2O_2 (1.2 v/v %). The obtained mixture is subjected to an evaporation process

and most of the water content is discarded. Finally, PHB at 99.9 wt % is obtained by spray drying, as shown in Fig. 6.

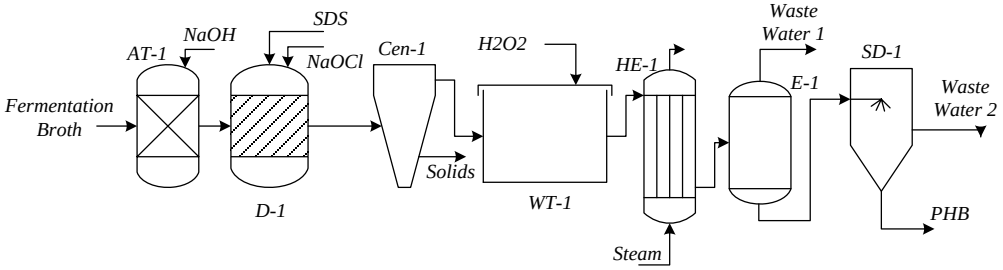

Fig. 6. Simplified flowsheet for the third downstream process. AT-1: Alkaline Tank I, D-1: Digester I, Cen-1: Centrifuge I; WT-1: Washer Tank I, HE-I: Heat Exchanger I, E-1: Evaporator I, SD-1: Spray Drier I.

2.2. Simulation procedure

The PHB production processes were simulated using Aspen Plus (Aspen Technologies Inc., USA). Thus, the design of the distillation columns in all cases required the definition of the preliminary specifications using the DSTWU shortcut method included in Aspen Plus. This method uses the Winn–Underwood–Gilliland procedure providing an initial estimate of the minimum number of theoretical stages, the minimum reflux ratio, the localization of the feed stage, and the products split. To perform the rigorous calculation of the distillation columns, the Rad-Frac module (based on the MESH equations) was used. Also, in order to study the effect of the main operation variables (e.g., reflux ratio, feed temperature, number of stages, etc.) on the glycerol composition, a sequential design procedure including a sensitivity analysis was performed.

On the other hand, *C. necator* and *B. megaterium* were simulated as solid compounds while the enzymes were simulated as non-conventional compounds. For the thermodynamic analysis, the UNIFAC model was used. The fermentation process was simulated based on a yielding approach where glycerol is completely consumed in two continuous fermentation stages. The first fermentation stage is governed by mass cell growth and the second fermentation stage is governed by PHB accumulation. The enzymatic digestion was simulated based on a stoichiometric approach. Calculation of energy consumption was based on the thermal energy required by heat exchangers, reboilers, flash drier units and the power supply required by the pumps.

The economic analysis was performed using the Aspen Icarus (Aspen Technology, Inc., USA) package. This software estimates the capital costs of process units as well as the operating costs, among other valuable data, utilizing the design information provided by Aspen Plus and the data introduced by the user for specific conditions such as project location among others. This analysis was estimated in US dollars for a 10-year period at an annual interest rate of 16 %, considering the straight-line depreciation method and a 33 % income tax. The cost of crude glycerol was USD$ 0.0554/L [3]. The labor cost used for operatives and supervisors was USD$ 2.14/h and USD$ 4.29/h, respectively. Also, the prices used for electricity, water and low pressure vapor were USD$ 0.03044/kWh, USD$ 1.252/m^3 and USD$ 8.18/ton, respectively [5]. All of these values are based on Colombian conditions.

3. Results and Discussions

The PHB production processes from crude glycerol require the glycerol purification up to 98 wt %. During this purification process, methanol at 99 wt % is recovered and thus for the economic assessment, two scenarios can be analyzed. In the first scenario, the obtained methanol is considered as a process waste. In the second scenario the methanol is considered as a co-product which could be recycled to the transesterification process and an economic value is given to this stream. The lowest cost for glycerol purification was obtained under the second scenario conditions (0.149 USD$/kg). This value was used as the raw material cost in all cases, thus only the purification cost was considered since the purification process was assumed to be adjacent to the biodiesel production process.

Glycerol fermentation was analyzed using two different strains and two glycerol concentrations in the fermentation media were considered (249g/L and 50 g/L). The cell mass values were 81.6 and 15.8 g/L and the PHB concentrations were and 57.1 and 8.8 g/L when *C. necator* and *B. megaterium* were used respectively. Thus, the reached yields to biomass and PHB were 5.16 and 6.49 fold higher when *C. necator* was used. These differences may be due to the fact that the first strain is a well adapted bacterium meanwhile the second one is still under adaptation to the fermenting conditions used in this work.

The fermentation broth is mainly a mixture of incorporated PHB in the cell mass and water. In order to recover the PHB from this broth, three different downstream processes were compared. The total production costs of PHB at 99.9 wt % from crude glycerol using *C. necator* and *B. megaterium* are shown in Table 1, where the costs were discriminated by raw material, services, operatives, maintenance, administration, and depreciation.

Table 1. Total PHB production cost from crude glycerol using C. necator and B. megaterium.

Item	***Cost (US$/kg) and Share (%)***	***Downstream Process I***		***Downstream Process II***		***Downstream Process III***	
		B. megat.	*C. necator*	*B. megat.*	*C. necator*	*B. megat*	*C. necator*
Raw material	***Cost***	*0.149*	*0.149*	*0.149*	*0.149*	*0.149*	*0.149*
	Share	*3.80*	*7.71*	*3.15*	*6.27*	*3.60*	*7.07*
Utilities	***Cost***	*1.358*	*0.658*	*1.908*	*0.953*	*1.691*	*0.841*
	Share	*34.64*	*33.96*	*40.36*	*39.97*	*40.88*	*39.77*
Operating labor	***Cost***	*0.151*	*0.066*	*0.156*	*0.068*	*0.146*	*0.064*
	Share	*3.85*	*3.41*	*3.30*	*2.85*	*3.53*	*3.03*
Maintenance and opera. charges	***Cost***	*0.479*	*0.210*	*0.492*	*0.236*	*0.485*	*0.223*
	Share	*12.22*	*10.84*	*10.41*	*9.90*	*11.73*	*10.55*
Plant overhead and admin. costs	***Cost***	*0.407*	*0.186*	*0.427*	*0.218*	*0.414*	*0.191*
	Share	*10.38*	*9.6*	*9.03*	*9.14*	*10.01*	*9.03*
Depreciation of capital	***Cost***	*1.376*	*0.668*	*1.595*	*0.76*	*1.251*	*0.646*
	Share	*35.10*	*34.48*	*33.74*	*31.87*	*30.25*	*30.55*
Total	***Cost***	***3.920***	***1.937***	***4.727***	***2.384***	***4.136***	***2.114***
	Share	***100***	***100***	***100***	***100***	***100***	***100***

The glycerol purification process represents only between 3.1 and 3.8 % of the total PHB production cost when *B. megaterium* is used. These values are higher, between 6.3 and 7.7%, when *C. necator* is used. In all cases, the raw material cost is lower than the obtained value

for traditional chemical processes considering that for most industrial processes the cost of raw material represents near 50% of the total production cost. In this way, the use of crude glycerol represents a significant decrease in the cost for raw material.

In general terms, due to the higher PHB yield, substrate tolerance, and lower energy requirements in the downstream processes, the lower production costs are obtained when *C. necator* is used for the fermentation process. Also, for both strains, the higher value for the total production cost was obtained in the second downstream process, which uses a solvent extraction stage. This extraction requires heating the expensive solvent DES up to 110 °C which increases the utility costs.

The total PHB production costs were between 3.92 and 4.73 US$/kg when *B. megaterium* was considered in the fermentation process, and between 1.94 and 2.38 US$/kg when *C. necator* was considered. These production costs were close to the sale prices of PHB reported in the literature for other substrates (i.e., 2.75-6.27 USD/Kg) [6].

Economically wise, the first downstream process was the most appropriate since the total production costs were the lowest obtained for both strains. This process was based on the BIOPOL flowsheet [14]. Moreover, the total production cost was almost twice as higher when *B. megaterium* was used for the fermentation stage compared to *C. necator*.

Generally, it has been suggested that the higher share of the total PHB production cost from other raw materials is the substrate cost [6]. Meanwhile, if crude glycerol is used as feedstock this share is lower than 8 %. These results indicate that using crude glycerol as feedstock to produce PHB could be a profitable alternative to develop biorefineries in the biodiesel industry.

4. Conclusions

Three downstream processes and two strains, *C. necator* and *B. megaterium,* were techno-economically compared to produce PHB from crude glycerol. Although in all the simultaed cases it was possible to obtain PHB at 99.9 wt %, the total production costs were twice as higher when *B. megaterium* was used compared to *C. necator*. This result is explained by the fact that *C. necator* is capable to consume glycerol at a higher concentration and yield than *B. megaterium*. The comparison showed here is important for the industrial production of PHB using crude glycerol since not only a profitable alternative was designed but also the fermentation conditions that take to a profitable process were clarified.

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