Evaluation of the potential to produce Polyhydroxybutyrate -PHB on an industrial scale using mixed cultures from renewable sources through simulation.

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1. Introduction

Within the background of simulations through bioprocess modelling to produce PHA polymers using mixed microbial cultures and diluted raw materials rich in volatile fatty acids, Werker et al. (2022), stands out which simulated using a mathematical model based on oxygen mass balances, chemical oxygen demand, and the model parameters are determined by biomass characterizations and experiments. Experiments include laboratory and pilot-scale SBR operations, FTIR measurements on biomass samples, estimation of substrate uptake rates and other parameters using nonlinear least squares regression analysis. The equations used in the model are based on the exposure history of the dissolved substrate concentration, and the total substrate uptake rate of the process is determined by the sum of the uptake rates of all elements in the holding zone.

The mathematical model of PHA developed by Tamis et al. (2014), is based on the modelling of PHA production by microbial enrichment. The model predicts PHA production and microbial community dynamics in response to different operating conditions. The model was calibrated using experimental data from a PHA enrichment cycle and characteristic process parameters were used to estimate yields and specific biomass growth rates. This is a dynamic model that considers PHA production and microbial community dynamics in response to different operating conditions. It also considers PHA degradation and active biomass production.

According to the background, there are dynamic models to define the kinetics of the behaviour of PHA-producing bacteria when the processes are carried out with mixed cultures, which served as a theoretical basis to develop the modelling of the industrial scale-up of the production of PHB obtained from mixed cultures from the sludge of a wastewater treatment plant, analysing its costs and environmental impacts using specialised software for this purpose.

Key words: Simulation, Bioplastics, Mixed cultures, Polyhydroxybutyrate.

2. Materials and methods

2.1 Simulation of the PHB production process in Pilot

Initially, the analysis of the PHB production variables at pilot scale was carried out through simulation in the SuperPro Designer v13 software, to evaluate the whole process from a technical point of view.

2.2 PHB Production Simulation Methodology on an industrial scale

To model the industrial production of Polyhydroxybutyrate, a scaling of the process was designed using the SuperPro software. Designer v13, based on the capacity of the raw material that is generated by the El Salitre WWTP, therefore, the simulation transforms 700 m^3 of sludge per day.

Firstly, seven (7) acidogenic fermentation reactors were designed for the permanent production of volatile fatty acids, which will subsequently be decanted into two (2) tanks. The inoculation will be carried out with the WWTP's own sludge and residual water from the same plant streams will be used.

Subsequently, for the inoculation train phase, two (2) bioreactors were designed with a capacity of 50.67 m^3 and 505.21 m^3. For the fermentation of PHB production, three (3) bioreactors are required, one (1) with a capacity of 12,000 m^3 to which the VFAs from three reactors arrive and two (2) with a capacity of 8,000 m^3 to which the AGVs arrive. of two reactors.

For the polymer extraction phase with SDS, the modelling of the plant requires the separation of the supernatant with the use of one (1) centrifuge to perform double centrifugation of the fermented broth from the previous stage and to wash the biomass of excess impurities then it must go through digestion in one (1) tank with SDS for one hour and finally drying the material for one day.

The last phase involves the purification of the polymer, for which it was scaled using the acetic acid and methanol method. After mixing the methanol with the PHB, the use of one (1) filter is required to eliminate the still persistent impurities. It will be dried at 70°C in a tank and the remaining material will be mixed with methanol, then the separation will be carried out by centrifugation and finally it will be transferred to the drying tank for one day.

3. Results and discussion

3.3.1 Material balances

According to the simulation, the batch size is 21.3 MT, and the total number of batches per year is 35, leading to a Unit Production Rate of approximately 746 MT/year. Table 1 shows the raw material requirements in Tons/year and Tons/batch. It reveals that water, sludge and SDS are the materials used in large quantities in this process. It is interesting to note that the consumption of acetic acid is 16.26 Ton per batch, i.e. 0.76 Ton per Ton of PHB and that of methanol is 22.22 Ton per batch, corresponding to 1.04 Ton per Ton of PHB. Therefore, to reduce the large volumes of solvents in each batch, most of it can be recovered by distillation and recycled.

Table 1. Bulk materials from the entire process

Material	Ton/year	Ton/lot
Acetic acid	569.18	16.26
Residual water	71,184.11	2,033.83
Air	2,201,575.33	62,902.15
Ammonium sulphate	2,987.56	85.36
Ammonia	10.71	0.31
Monopotassium phosphate	2,827.33	80.78
Yeast	48.46	1.38
Primary Sludge	170,841.84	4,881.20
Magnesium sulphate	319.51	9.13
Methanol	777.85	22.22
Micronutrients	2,133.71	60.96
Disodium phosphate	3,616.90	103.34
SDS	6,491.22	185.46
Sodium chloride	96.92	2.77
sodium hydroxide	33.58	0.96
Saccharose	484.54	13.84
Tryptose	96.92	2.77
Water	966,289.89	27,608.28
Total	**1,228,810.21**	**35,108.86**

3.3.2 Industrial scale PHB production simulation results

The design of the PHB production plant at the El Salitre WWTP was simulated using SuperPro Designer v13, including all stages of the process, sectioned into Fermentation, Extraction and Purification. The designed plant requires the necessary facilities for the process. Table 2 shows the summary of the mandatory equipment for its construction by stages.

Table 2. Equipment for industrial production of PHB

Section	Fundamental Team	Ability	Unit
VFA Fermentation	VFAs Anaerobic Bioreactor	1411.36 m^3	7
PHB fermentation	Decanter Tank	19449.34 m^3	2
PHB fermentation	Pre-inoculum bioreactor	50.67 m^3	1
PHB fermentation	Bioreactor Inoculum	505.21 m^3	1
PHB fermentation	PHB Fermentation Bioreactor	12000 m^3	1
PHB fermentation	PHB Fermentation Bioreactor	8000 m^3	2
Extraction with SDS	Centrifuge	5997.33 m^3	2
Extraction with SDS	Digestion Tank	138.87 m^3	1
Extraction with SDS	Drying Tank	10 m^3	1
PHB Purification	Filter	16 m^3	1
PHB Purification	Drying Tank	10 m^3	1

3.3.3 Cost analysis of PHB production on an industrial scale

As part of the analysis of the PHB production variables, cost analysis was performed in SuperPro software. They were estimated by differentiating the fermentation processes, PHB extraction with SDS and the purification step with acetic acid and methanol. Tables 3 detail the associated costs. The values are in US dollars (US$), the analysis is annualised, and 45 batches would be produced in one year under pilot conditions.

Table 3. Total investment cost

Description	US$ value	%
Direct fixed capital	34,831,429.08	91.99
Working capital	1,292,246.02	3.41
Startup cost	1,741,571.45	4.6
Total inversion	37,865,246.55	100

According to the cost analysis, an investment of US$37,865,246.55 is required to start up the plant.

3.3.4 Analysis of environmental impacts of PHB production on an industrial scale

Complementary to the technical and economic analysis of industrial simulation in SuperPro Software Designer, the results of the waste generated for the fermentation, extraction, and purification process of PHB are presented. Table 4 shows the production waste.

Table 4. Total Waste Generated in the process.

Component	kg/process	kg/Year	%
Ammonium	0.36	13	0
Biomass	23,413.70	819,480	0.03
Carbon dioxide	0.38	13	0
Sulfuric acid	0.08	3	0
Methane	0.99	35	0
Methanol	22,224.14	777,845	0.02
Nitrogen	48,286,501.85	1,690,027,565	52.99
Oxygen	14,658,841.85	513,059,465	16.09
Waste	27,673,490.43	968.572.165	30.37
SDS	185,463.48	6,491,222	0.2
Water	278,926.21	9,762,417	0.31
Total	91,128,863.49	3,189,510,222	100

According to this analysis, it is possible to identify that the largest amount of waste generated corresponds to residual nitrogen waste with 53%, as well as 30.4% are waste from the centrifugation process of extraction with SDS and emissions of oxygen during fermentations.

4. Conclusions

The industrial-scale simulation allowed us to establish a production potential of 746 tons of PHB per year, under the tactical conditions established in this research, with operational costs of $US 30,153,035.48, the sales price to recover the investment at the 12 years of implementation of the project is $US 45 per kg, which is a high price compared to the prices of petrochemical plastic, from which it is inferred that it is necessary to deepen the reduction of operational costs through energy integration to reduce electrical consumption during the process, as well as research into the optimization of the production process, considering alternatives to the use of other solvents and other operational issues that allow increasing the amount of material produced in the shortest possible time, since currently the process takes around of 11 days of production represented in 35 lots. The main impacts of the process are produced by aqueous waste and emissions into the atmosphere.

References

Werker, A., Lorini, L., Villano, M., Valentino, F. & Majone, M. (2022). Modelling Mixed Microbial Culture Polyhydroxyalkanoate Accumulation Bioprocess towards Novel Methods for Polymer Production Using Dilute Volatile Fatty Acid Rich Feedstocks. *Bioengineering*, 9(3) 125. https://doi.org/10.3390/bioengineering9030125

Tamis, J., Lužkov, K., Jiang, Y., Loosdrecht, M., & Kleerebezem, R. (2014). Enrichment of Plasticicumulans acidivorans at pilot-scale for PHA production on industrial wastewater. *Journal of Biotechnology*, 192, 161–169. doi:10.1016/j.jbiotec.2014.10.022