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INDUSTRIAL-SCALE BIOPROCESS SIMULATION OF POLYPHENOL PRODUCTION USING SUPERPRO DESIGNER

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Abstract

Polyphenols are molecules with antioxidant potential for several diseases. In this research, an analysis of polyphenols production costs from Theobroma cacao L suspension cell culture was evaluated. The latter proposing different scenarios based on a large-scale plant production using the SuperPro Designer software. Two strategies for bioprocess improvement were proposed based on information from a traditional suspension cell culture (Strategy I). These improvements are firstly based on adding elicitors (Strategy II) for increasing polyphenol production and secondly, energetic use of cane bagasse and rice bran (Strategy III) by adding a gasifier. Results showed that the percentage of losses decreases from 19 % to only 5.8 % applying a recirculation system in losses lines product regarding Strategy II. Also, adding Methyl Jasmonate (MJ) elicitor increased the Y p / s yield up to 0.08 kg polyphenols/kg glucose. Gasification proposed in Strategy III achieved supplying 37 % of energy requirements using only 1.14 % of bagasse. One essential finding refers to the decreased cost of producing a 100 mg tablet formulated at 50 % of the active substance. Therefore, the latter resulted in 0.23 USD / tablet: promising data compared to 1.0-2.0 USD / tablet corresponding to the average value in the market. Thus, this research demonstrates that the increase of polyphenols by adding elicitors positively influences the operating costs.

Keywords: Bioprocess simulation, Elicitation, Gasification, Phenolic compounds, Plant design, Software.

1. Introduction

Suspension plant cell cultures are considered as an alternative to increase and optimize the production of secondary metabolites. The majority of research has focused on species such as *Catharanthus roseus* in alkaloid production [1], *Medicago sativa* in isoflavonoids and saponins production [2], *Solanum tuberosum* for polyphenols, and recombinant protein production [3, 4]. Other studies report a presence of Phenolics compounds in *Prunus domestica L.* [5], phytoalexins in *Pyrus pyrifolia* cells suspension [6], and polyphenol production from *Theobroma cacao L.* cells [7].

Polyphenols are a group of secondary metabolites with antioxidant activity, including acid phenols and flavonoids. The importance of this heterogeneous group of bioactive molecules is its ability to prevent cardiovascular disease, sclerosis, neoplasm, or cancer [8]. Some of these molecules are metabolized to form ferulic acid and caffeic acid, whose primary function is to be precursors of more complex derivatives of coumarins, lignin, tannins, flavonoids, and isoflavonoids [9].

The antioxidant system of polyphenols, particularly flavonoids, delay or prevent oxidative stress, creating a pro-oxidant / antioxidant balance. For this reason, these are of vital importance in the pharmaceutical industry in the formulation and production of safe drugs and effective for the treatment or prevention of cardiovascular diseases and neoplasms [8]. Approximately every year, there are 12 million new cancer cases, about 5.4 million are in developed countries, and 6.7 million occur in developing countries. The most common diseases are cervical cancer or stomach cancer [10]. Therefore, knowledge and research are required for developing commercial drugs based on secondary metabolites in the pharmaceutical industry. Therefore, plant biotechnology provides suspension cell culture, focusing on the increase in heterogeneous bioactive compounds.

Elicitation is a strategy that allows increasing the secondary metabolites production. Elicitors activate cellular defense mechanisms because these substances generate cellular stress. Although the cell deflects nutrients and energy to the production of secondary metabolites, these substances are considered toxic to the cell. Thereby, its use requires the optimal concentration and adequate time for application [11]. Studies show that elicitors in plant species considerably increase the amount of secondary metabolite production belonging to the group of phenolic acids and monoterpenoids [12].

Research by Flórez et al. [7], on cells in suspension Theobroma cacao L., shows that the use of different concentrations of Methyl Jasmonate increased the polyphenol content. In the same way, using the MJ increases the biosynthesis of astragaloside, a saponin bioactive that inhibits replication of human adenovirus type 3 [13]. It is well known that there are significant challenges to carry metabolites obtained from the culture of suspended cells on an industrial scale. That is why the critical aspects of the economy of a bioprocess should be studied to find the balance point that allows the viability of a product. A detailed bioprocess model integrating the upstream and unit operations from the pre-treatment from raw materials to the metabolite purification can benefit economic-technical analysis on an industrial scale.

Polyphenols production from plans cultures is costly due to its low yield and requires excessive processing times [7], hindering its technical-economic viability. In addition, the production of these molecules requires excessive time to meet current market demand. Therefore, its traditional production is limited to

polyphenols extraction directly from plants crops. For these reasons, a strategy to increase its yield and reduce process time consists of using elicitors in suspension cell cultures. However, the feasibility of obtaining polyphenols is currently questioned since, according to the authors' knowledge, there are no reports of bioproduct costs on an industrial scale to define its technical-economic feasibility from *Theobroma cacao* suspension cell type cultures at a large scale.

SuperPro Designer has become an essential tool for bioprocess simulation. The software has been used in several studies based on bioprocess simulations for obtaining various products. One of them is biodiesel, a bioprocess that has a problem due to its high production and operation costs but is considered an alternative that is contributing to sustainable development [14]. Research by Villamizar and Lopez [15] shows a solution to a phytosanitary problem regarding plant residues, using simulations for obtaining polyphenols from the waste cocoa shell. Likewise, simulations have been performed to obtain tissue plasminogen activators (t-PA) from the Chinese hamster ovary [16]. However, the software allows the simulation of a bioprocess for obtaining a product and has been used in environmental studies, as evidenced by Ontiveros [17]. In his study, he showed an alternative for reducing toxic components regarding wastewater treatment in sewage treatment plants. Therefore, SuperPro Designer is considered a primary tool used in the simulation of bioprocesses.

Based on the precedents, an evaluation of polyphenols production from simulation data is done. The latter focuses on providing a low-cost alternative at large-scale production applied to the pharmaceutical industry. This research relates to the simulation in SuperPro Designer to produce polyphenols from suspended plant cells of *Theobroma cacao L.* Design parameters required for the process are set up based on references. Two strategies for bioprocess improvement were proposed based on information from a traditional suspension cell culture (Strategy I). These improvements are firstly based on adding elicitors (Strategy II) for increasing polyphenol production and secondly, energetic use of cane bagasse and rice bran (Strategy III) by adding a gasifier.

2. Experimental methodological design

2.1. Basic characteristics of simulations

The SuperPro Designer V 10 (Intelligent, Inc., USA) software was used for running simulation. *Theobroma cacao L* suspended plant cells were used as a biocalatizer for the large-scale polyphenols bioprocess simulation. Based on the latter, a 10 % production volume was set up as inoculum. Culture media mainly consists of glucose as a limiting substrate fed at 30-50 g / L and enriched with MS culture medium. Based on references, glucose is obtained from cellulose enzymatic hydrolysis from cane bagasse. The required oxygen supply was set up at 8-10 mmol / L h with a stirring velocity range of 80-300 rpm [18].

2.1.1. Plant design

The design of the plant is divided into three sections: (a) Pre-treatment section. (b) Fermentation section and (c) Purification section of the product. Table 1 shows details of each unit operation proposed for the bioprocess simulation.

Table 1. Sections of the plant design for the simulation of the bioprocess of polyphenol production.

Pre-treatment section	
Washing	Performed by washing to bagasse to remove solid residues
Drying	Drying of the cane bagasse to reduce humidity.
Grinding	Reducing the size of the cane bagasse.
Bioreactor:	Enzymatic hydrolysis was performed for glucose conversion from sugarcane bagasse.
Microfiltration	Glucose separation from enzymatic hydrolysis of cellulose and hemicellulose. Glucose particle size 0.03 µm
Blender	In this unit operation, the culture medium was prepared.
Sterilization	It is carried out for the culture media sterilization 140 °C.
Fermentation section	
Comprised of 5 small fermenters and five production fermenters with their respective compressors for oxygen supply. Simulations studies are shown in Figs. 1, 2, and 3. In the fermentation section, three studies were performed using two working volume levels: VP 15000-15400 L and VP 24000-25000 L. Therefore, a required impeller power input was set up at ranges of 43.4-100 kW. Fermentation was carried out at a temperature of 30 °C, with an oxygen supply of 8.5 mmol / Lh and a residence time of 5 days.	
Purification section	
Centrifugation	Separation by difference of densities for recovery of biomass.
Cell disruption	Cell membrane rupture.
Centrifugation	Intracellular waste products resulting from cell lysis are separated.
Microfiltration	Product recovery. Particle size larger than 0.2 µm is separated.
Chromatography	Separation of the secondary metabolite (polyphenols).
Crystallization	Polyphenol crystal formation. Melting temperature: 172 °C.
Drying	Reducing the moisture percentage.
Formulation	A solid presentation (tablets) was created. Composition: 50 % polyphenols and 50 % excipients (25 % lactose, 15 % microcrystalline cellulose, 5 % magnesium stearate and 5 % polyvinylpyrrolidone)

2.1.2. Design of proposals to improve bioprocess

The proposals for improvement were worked based on Strategy I to mitigate the deficiencies or losses in the bioprocess.

Strategy I: Standard process implementation described in the plant design with the essential characteristics required for the simulation.

Strategy II: Recirculation was implemented in the product loss lines from DE2, DE3, C1, and C2 equipment's from the purification zone of Strategy I simulation observed in Fig. 1, and the MJ was added to the culture medium at a concentration of 25 µM [7].

Strategy III: Sugarcane bagasse and rice husk were connected to a drying equipment to reduce the percentage of moisture to 20 % followed by a fixed flow bed gasifier (GSF) added to the plant design to supply the energy needs required by the process (Fig. 3).

2.1.3. Design parameters

Operating parameters and setup conditions are shown in Table 2 according to the refereed data.

Table 2. Sections of the plant design for the simulation of the bioprocess of polyphenol production.

Design parameters		
Parameter	**Parameter reference**	**Used range**
Inoculum	*Theobroma cacao L*. plant cells in suspension [19]	Cells inoculated at 10% of their VP
Culture medium	A culture medium with ½ MS of glucose at 20-50 g / L is proposed [20].	Culture medium with ½ MS of glucose at a concentration of 30 g / L
Temperature	Ranges of between 15-35 °C. [11, 21].	Temperature of 30 °C is considered the optimum temperature for cell growth and production of secondary metabolites [11]
Elicitation	Previous researches suggest an increase in polyphenol production by supplying 25 μM of MJ [7].	25 μM of MJ
Tablet composition	50% polyphenols and 50% excipients [22].	microcrystalline cellulose 15% magnesium stearate 5% lactose 25% polyvinyl pyrrolidone 5% polyphenols 50%
Presentation of the product	Solid (tablets).	100 mg

2.2. Economic analysis

The economic analysis was determined by the SuperPro Designer software, which uses a tool database cost, estimating detailed economic evaluation reports (EER). The

program also includes annual operating costs, raw materials, consumables costs, utilities (power, heating, or air services), transportation, labor, cost and waste disposal, advertising cost, laboratory cost, and royalty execution, among others [23].

3. Results and Discussions

The Strategy I identified a 19 % product loss percentage in outputs lines from equipment DE2, DE3, C1, and C2 located in the purification zone (Fig. 1). To decrease the percentage of losses, implemented processes (Strategy II and III) were carried out based on recirculation lines, followed by the process of crystallization and chromatography. The latter successfully reduced the percentage loss of the product to 5.88% (Figs. 2 and 3).

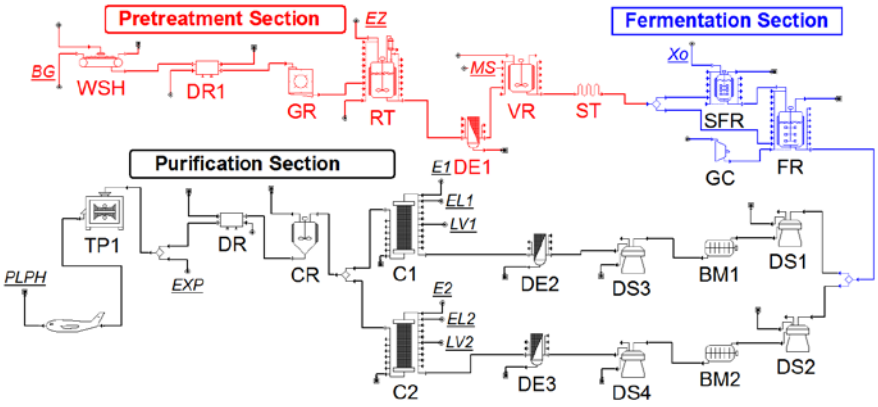

Fig. 1. Diagram simulation strategy I: The production of polyphenols from cells in the suspension of *Theobroma cacao L.* Superpro Designer V8.5.

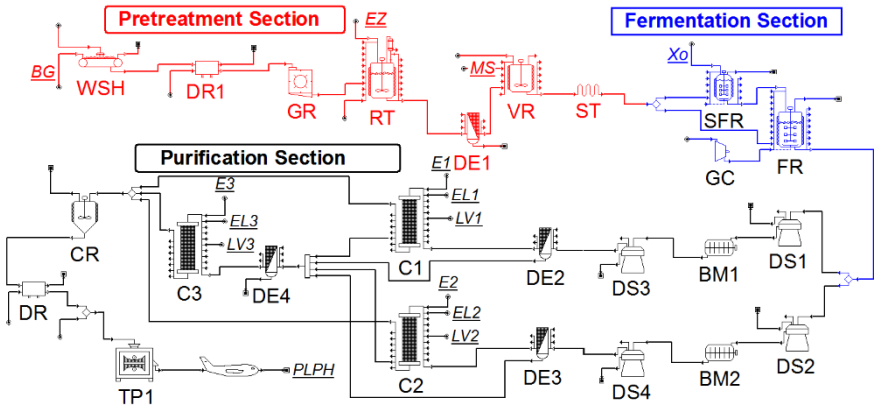

Fig. 2. Diagram simulation strategy II, corresponding to the first improvement to the design of the plant to produce polyphenols from cells in suspension in *Theobroma cacao L.* Superpro DesignerV8.5.

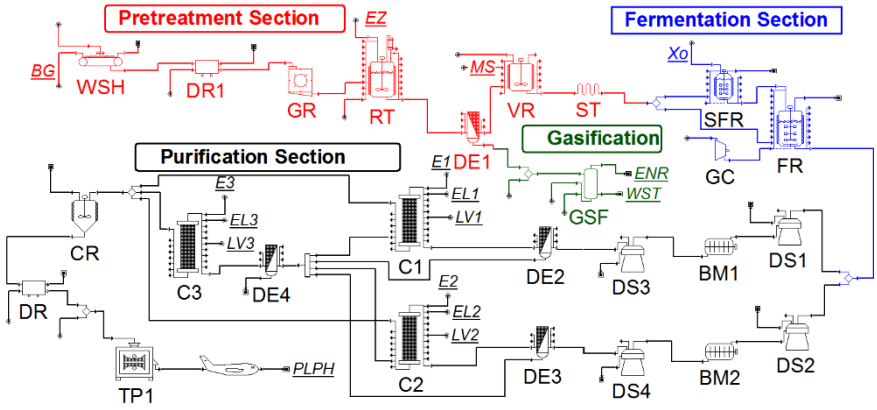

Fig. 3. Diagram simulation strategy III correspondings to the second improvement to the design of the plant to produce polyphenols from cells in suspension in *Theobroma cacao L*. Superpro designer V8.5.

Strategy III was proposed to use waste sugarcane bagasse and rice husk as residual biomass in the gasification process to supply the energy requirements required. In Colombia, 828.94 Ton / h of sugarcane bagasse and 6 Ton / h of rice husk are produced. The 1.94% of sugarcane bagasse generated daily and 8,33% rice husk was used as a basis for simulation considering the Ton / h produced in Colombia (Fig. 3), determining a viable alternative to the use of waste from other industries. Gasification produced combustible gases (syngas) with energy of 49'825.966,01 KW-h / year, making supply 37.8% of the energy required during operation. The By-products generated during gasification at a production rate of 6354200.707 kg/year carbon and 3265888.96 kg/year ashes generate a profit of 3'948.359,619 $ / year (Table 3).

Table 3. Gasification products of strategy III.

Products	Unit of measure/year	USD / unit of measure	Utility / year
Carbon	6'354.200,70 kg / year	0,35 $ / kg	2'223.970,24 $ / year
Ashes	3'265.888,96 kg / year	0,528 $ / kg	1'724.389,37 $ / year
Syngas (Combustible Gases)	49'825.966,01 KW-h / year	0,1 $ / KW-h	4'982.596,60 $ / year

The amount of polyphenols produced by *Theobroma cocoa L* in the absence of elicitors is 5.7-6.0 mg / g of GAE [24] (input parameter required in Strategy I), and considering the MJ elicitor, the number of polyphenols increases to 12.828 mg / g GAE [7], (input parameter used in Strategy II and III). The performance of the production of polyphenols by glucose consumed (Y p / s) in Studies II and III is increased compared to the results obtained in Strategy I (Table 4). The latter is attributed to MJ supplying due to its well-known documented capacity to activating cellular defense mechanisms and generating cellular stress. Consequently, the cell

deflects nutrients and energy to the production of polyphenols, which leads to increased yield in terms of the production of secondary metabolites.

Table 4. Biomass and polyphenol production performance analysis.

Performance	Strategy I	Strategy II	Strategy III
Y x / s (small fermenters)	0,6080	0,5999	0,6000
Y x / s (production fermenters)	0,6999	0,6999	0,6981
Y p / s (production fermenters)	0,0379	0,814	0,814

As observed in Table 5 the annual production in Strategy I is of 73’209.891 Tablet / year in the simulation without elicitors. However, in Strategies II and III, the annual production rate increases to 198.280.021-202.408.836 tablet/year due to the change in the input parameter of the elicitor agent.

Table 5. Results of total annual costs, operating costs per tablet and total annual production.

Costs and productivity (15000 L)			
Bioprocess simulation strategy	**Annual production (Tablet / year)**	**Process cost ($ / year)**	**Unit cost ($ / Tablet)**
Strategy I	73’209.891	58’377.000	0,79
Strategy II	198’280.021	74’445.000	0,37
Strategy III	202’408.836	77’860.000	0,36
Costs and productivity (24000 L)			
Bioprocess simulation strategy	**Annual production (Tablet / year)**	**Process cost ($ / year)**	**Unit cost ($ / Tablet)**
Strategy I	156’151.659	87’589.000	0,56
Strategy II	406’145.642	95’184.000	0,23
Strategy III	414’663.603	105’325.000	0,23

Figures 4 to 6 show the operating cost of each simulated strategy. Operating cost are distributed in different percentages based on production volumes (VP 15000-15400 L, VP 24000-25000 L). It is evident in strategy II and III that consumable costs increased due to the increased demand for DFT PBA chromium resin used in the ion-exchange chromatography. The latter is related to (i) the increased polyphenols production and (ii) the addition of a unit operation. Therefore, using these improvements (Strategy II and III) consumable costs represent a level between 21-28% of production costs (Figs. 5 and 6). The energy costs (utilities) in diagrams production volumes ranging between 24,000 to

25,000 L are higher than those set up for 15000-15400 L, due to the required impeller power input is proportional to working volume. Therefore 100 kW is required for stirring 24,000-25,000 L. The latter is higher compared to values required for mixing 15000-15400 L (43.4 kW). The installation and plant equipment operation costs are between 26-43 % for all strategies evaluated in this research, representing the most significant production costs. The raw material, transport and labor generate the remaining percentage between 29-34 % at different production volumes.

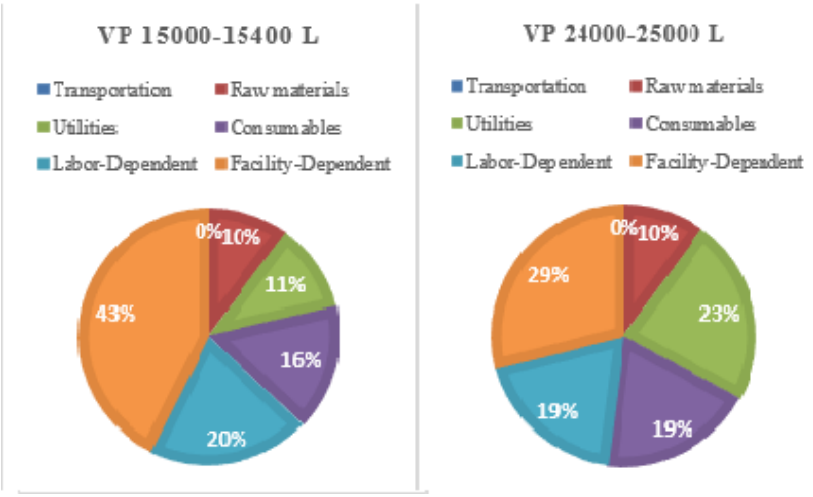

Fig. 4. Strategy I. (VP 15000-15400 L, VP 24000-25000 L) Annual operating costs to produce polyphenols from plant cell suspensions of *Theobroma cacao L.*

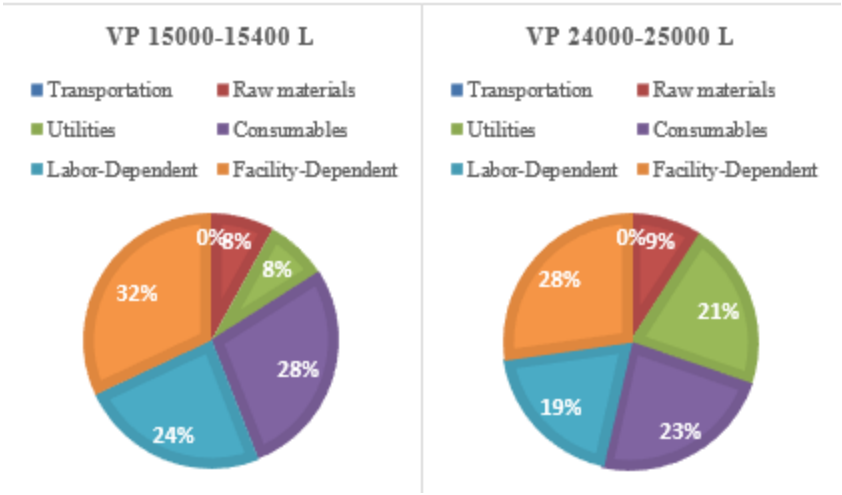

Fig. 5. Strategy II. (VP 15000-15400 L; VP 24000-25000 L) Annual operating costs to produce polyphenols from plant cell suspensions of *Theobroma cacao L.*

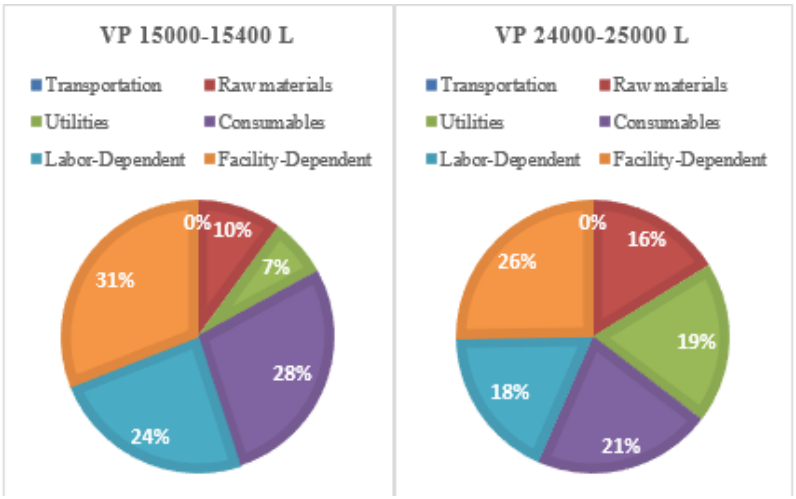

Fig. 6. Strategy III. (VP 15000-15400 L; VP 24000-25000 L) Annual operating costs to produce polyphenols from plant cell suspensions of *Theobroma cacao L.*

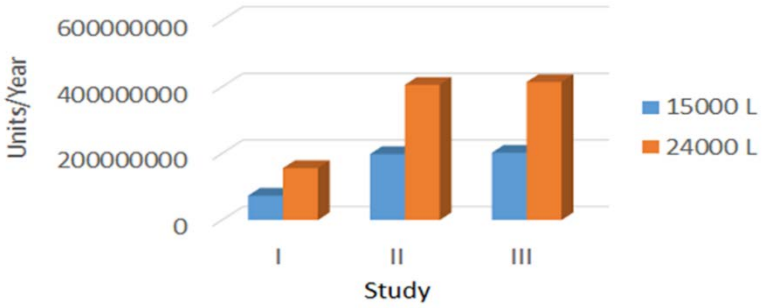

Fig. 7. Total annual production of polyphenols (tablets/year) from plant cell suspensions of *Theobroma cacao L.*

The Total annual production of polyphenol shown in Fig.7 is used to calculate the operating costs (Fig. 8). This latter is increased using a volume of VP 24000-25000 L, due to the increased demand of variables such as raw material and higher power requirements (Utilities). The increase in production costs related to strategies II and III compared to Strategy I is due to the implementation of more equipment in the plant's design and increased consumable costs and costs or operating machinery. Studies I, II and III; to VP 15000-15400 L generated unit costs from 0.36 to 0.79 USD / tablet. Unlike VP 24000-25000 L values between 0.23 to 0.56 USD / tablet, as evidenced in Fig. 9, the costs of installation and operation equipment remain constant and the increase in VP leads to the lower unit cost. Considering the annual operating costs and annual production of tablets, in Strategy III, the unit production cost is 0.23 USD/tablet with a production rate of 414,663,603 units of 100 mg per year, simulation that shows, as a result, the lower production cost per tablet.

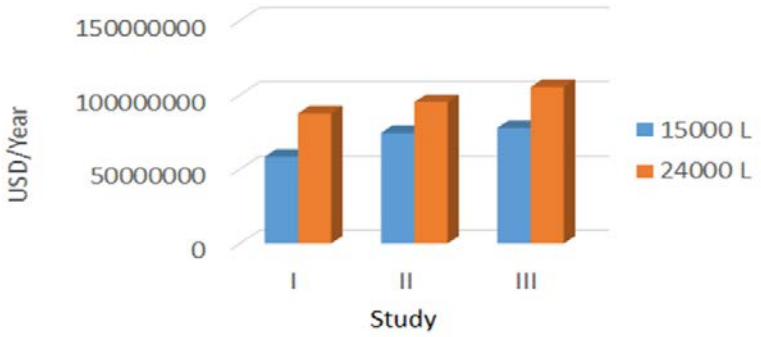

Fig. 8. Total annual production costs of polyphenols from plant cell suspensions of *Theobroma cacao L.*

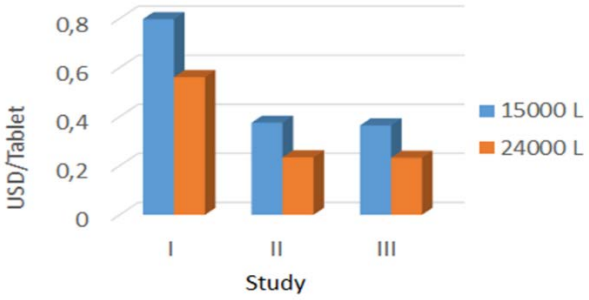

Fig. 9. Operating costs per tablet (USD / tab) to produce 100 mg of polyphenols.

4. Conclusions

In the simulation in SuperPro Designer V 8.5 of Strategy I (VP: 24000-25000 L) the total annual operating cost was 87.589.000 USD / year, compared to the total annual operating costs in strategy II and III (95.184.000-105.325.000 USD / year) which increases due to the implementation of more unit operations for the design and simulation of the polyphenol production, but decrease the production cost of a tablet in the first design from 0.560 USD / tablet to 0.232-0,232 USD / tablet.

The values for the unit production cost in Strategy II and III comply with improving process efficiency and reducing the production cost of a tablet of polyphenols. Applying a recirculation in the purification zone allowed us to decrease the product loss percentage from 19 % to 5.8 %, with 94.2 % of process efficiency. Implementing a gasification as a strategy to supply the energy needs of the bioprocess, is a viable option for the alternative treatment of residual biomass and a solution to the serious environmental problems they cause, in the simulation only used the 1.14 % sugarcane bagasse, industry generated in milling. This residue can used of a higher percentage to cover over 37 % of the required energy needs.

One of the most important results in the simulations, strategy II and III to VP 24000-25000 L, this is the production cost of a tablet of 100 mg of polyphenols with a composition of 50 % polyphenols and 50 % of excipients, with values of

0.234 to 0.232 USD / tablet, which are below 1-2 USD / tablet price corresponding to a polyphenol tablet in the market, value dependent on the amount of active ingredient presents in the tablet. Therefore, the simulation of strategy II and III in SuperPro Designer V 8.5 meets the objective of decreasing the production cost of a polyphenols tablet and being below the cost of a tablet on the market.

Nomenclatures

GAE	Gallic acid, unit of measurement of total phenols.
Unit	Unit or tablet produced.

Greek Symbols

µM	Micro molar, molar concentration of a solute in a solution.

Abbreviations

BG	Bagasse
BM	Bead Milling
C	Chromatography
CR	Crystallization
DE	Dead-End Filtration
DR	Rotary Drying
DS	Centrifugation
E	Equilibrate
EL	Elute
EZ	Enzyme
EER	Economic evaluation reports
ENR	Energy
FR	Stoich Fermentation
GC	Gas Compression
GR	Grinding
GSF	Gasification
LV	Wash
MJ	Methyl Jasmonate
MS	Murshige and Skoog, culture medium.
PLPH	Polyphenol
RT	Stoich Reaction
ST	Sterilization
SFR	Seed Fermentation
TP	Tablet Press
VP	Volume of Production
VR	Blending Storage
WSH	Washing
WST	Biochar and Ashes
Xo	Inoculum
Y p / s	Yield of polyphenols produced with respect to the glucose consumed.
Y x / s	Yield of biomass produced with respect to the glucose consumed.

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