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MODELING AND SIMULATION OF THE BIOSURFACTANT PRODUCTION BY ENZYMATIC ROUTE USING XYLOSE AND OLEIC ACID AS REAGENTS

Article Highlights

- Simulation of sugar ester production by immobilized lipase using oleic acid and xylose as reagents
- Product separation is performed using precipitations by adding ethanol, water, and methyl ethyl ketone
- Simulation performed using EMSO software (Environment for Modeling, Simulation, and Optimization)
- Development of mathematical models that successfully described the process
- Presentation of economic analysis for the biosurfactant production

Abstract

The biosynthesis of sugar esters, molecules with biosurfactant properties, can occur through the esterification of sugars with fatty acids by enzymatic catalysis. An alternative to reduce the impact of raw materials on the final biosurfactant production cost and the reuse of industrial waste is to use residues from vegetable oil industries as a source of free fatty acids, such as oleic acid, and lignocellulosic residues of 2G ethanol as a source of sugar (xylose). In this scenario, the present work aimed at modeling the biosurfactants production via heterogeneous biocatalysis using lipase, oleic acid, and xylose. Product separation and purification were performed using a sequence of precipitations (adding ethanol, water, and methyl ethyl ketone). The simulation was performed using the equation-oriented software EMSO (Environment for Modeling, Simulation, and Optimization), CAPE-OPEN compliant. The percentage of biosurfactants in the product was around 86%, with a recovery of 88% in the purification. Regarding the study of energy expenditure, a value of -604.1 kW of heat associated with cooling and a value of 137.6 kW associated with heating was observed. Developed mathematical models successfully described the process. The initial economic analysis of the process indicates a minimum biosurfactant selling price of US$ 72.37/kg.

Keywords: biosurfactants, esterification, modeling and simulation, purification, precipitation.

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Biosurfactants are amphipathic molecules obtained by enzymatic or microbiological routes. Like surfactants, they have amphiphilic structures, which means the molecules have hydrophobic and hydrophilic regions [1]. This duality generates interfaces with different degrees of polarity, bringing the substance adsorption characteristic [2]. Emulsification capability, reduction of viscosity and surface tension, stabilizing effect, and solubilizing ionic strength are the main characteristics of these molecules [3]. Based on these characteristics, biosurfactants can be applied in the pharmaceutical, oil, textile, food, and cosmetics industries, among others [4]. However, despite low toxicity, good degradability, thermal stability, specific bioactivity, and other advantages, biosurfactants still lose market share to synthetic surfactants due to their

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high production cost [5].

It is important to note that the cost-benefit ratio in applying biosurfactants can be valued for processes that require a lower degree of purity since the separation and purification steps represent about 60% of the final operational cost of production [6]. Another important point that impacts production costs is the choice of reagents, which can reach 50% [7].

The literature reports different studies on the production of sugar esters (biosurfactants) by microbiological route, involving steps of purification using solvent extraction, acid precipitation, centrifugation, filtration, gel filtration chromatography, and lyophilization [8–11]. For enzymatic biosurfactants production, several studies focus on the development or immobilization of catalysts. The synthesis process can occur through the esterification of sugars with fatty acids, with lipase as the main enzyme that catalyzes the reaction [12]. Lipases are part of a group of hydrolytic enzymes found in animals or produced from fermentation using some species of microorganisms. They can catalyze interesterification, transesterification, and esterification reactions [13]. In particular, the immobilization of enzymes appears as a solution for the structural conformation of lipases in environments not conducive to them. In this way, they become stable for catalysis with their active sites exposed [14]. Another advantage of immobilization is the insolubilization of the enzyme in the liquid phase. Depending on the system, it facilitates their separation and reuses for long periods, contributing to reducing process costs. The work [15] achieved a good conversion in the production of glucose esters under specific enzymatic reaction conditions. Some other studies also use enzymes to produce sugar esters, but none focuses on the separation/purification process of the product obtained [16–19].

Several possibilities of employing less costly alternatives to the process exist regarding the used reagents and the biosurfactants' integration within a biorefinery concept. More specifically, within the framework of a biodiesel-bioethanol biorefinery, using by-products such as SODD (Soybean Oil Deodorization Distillate), obtained in the refining stage of soybean oil, and biomass residues, obtained in the production of bioethanol [20–23].

Brazil has geographic and climatic factors that favor the cultivation of various oleaginous species that can be used to produce oils. These species include pine nuts, castor beans, palm kernels, babassu coconuts, sunflower seeds, cotton, peanuts, linseeds, canola seeds, soybeans, corn, etc. On the other hand, residual vegetable oils result from domestic or industrial processes, citing examples of frying oils, industrial wastewater, or by-products originating from refining stages, such as SODD [24, 25]. Vegetable oils and their residues are mainly composed of mono, di, triglycerides, and fatty acids, justifying their use as raw material in the production of esters [26].

Process residues involving biomass (such as sugarcane bagasse), in turn, have a high potential for conversion into renewable products with high commercial value [27]. In particular, it is possible to obtain xylose from these residues. Lignocellulosic biomass needs pretreatment to disorganize the lignocellulosic complex and increase its surface area [20]. Following the pretreatment, hydrolysis occurs. Cellulose and hemicellulose are hydrolyzed and generate several products. Cellulose produces glucose, while hemicellulose is broken down into hexoses, pentoses (like xylose), glucuronic acid, and acetic acid [28].

Thus, to contribute to the study of the production of biosurfactants in the context of a biodiesel-bioethanol biorefinery, the present paper aimed at modeling and simulating biosurfactants production via heterogeneous biocatalysis with immobilized lipases using oleic acid and xylose as reagents. The work was modeled and simulated in an EMSO environment (Environment for Modeling, Simulation, and Optimization). The EMSO simulator is an equation-oriented process simulator for modeling steady-state and dynamic processes. It is the CAPE-OPEN standard compliant. Pre-built models are available in the EMSO Modeling Library (EML). Besides, new models can be written in the EMSO modeling language. The behavior of the variables for the route under development was verified, and the energy costs were assessed throughout the process. Furthermore, the work aimed to simulate a separation/purification process as an alternative to the more complex and costly steps mentioned in the literature. Such an alternative for the separation/purification of the product is a sequence of precipitation processes.

MATERIAL AND METHODS

The main equipment used for computational simulations was a desktop with an Intel 7700K core I7 4.20 GHZ processor, 32 GB of RAM. In addition, a notebook with an Intel 3210M core I5 2.5 GHZ, 4 GB of RAM was also employed in the project. The software used was EMSO academic beta version 0.10.9.

The route evaluated in this work encompasses the process of producing biosurfactants via heterogeneous biocatalysis using immobilized lipases. Oleic acid and xylose were used directly as reagents. The process fol-

lows the lipase recovery and reuse and the product separation and purification by a sequence of precipitations. This production route is presented in detail in Figure 1.

Modeling

For the simulation of the process to be possible, it is necessary to model each equipment unit and specify the operating conditions and considerations used in the models. The main equations on which the models are based will be presented. Table 1 shows the components used throughout the modeling and simulation processes.

MIXER: The mixers used in this work were assumed

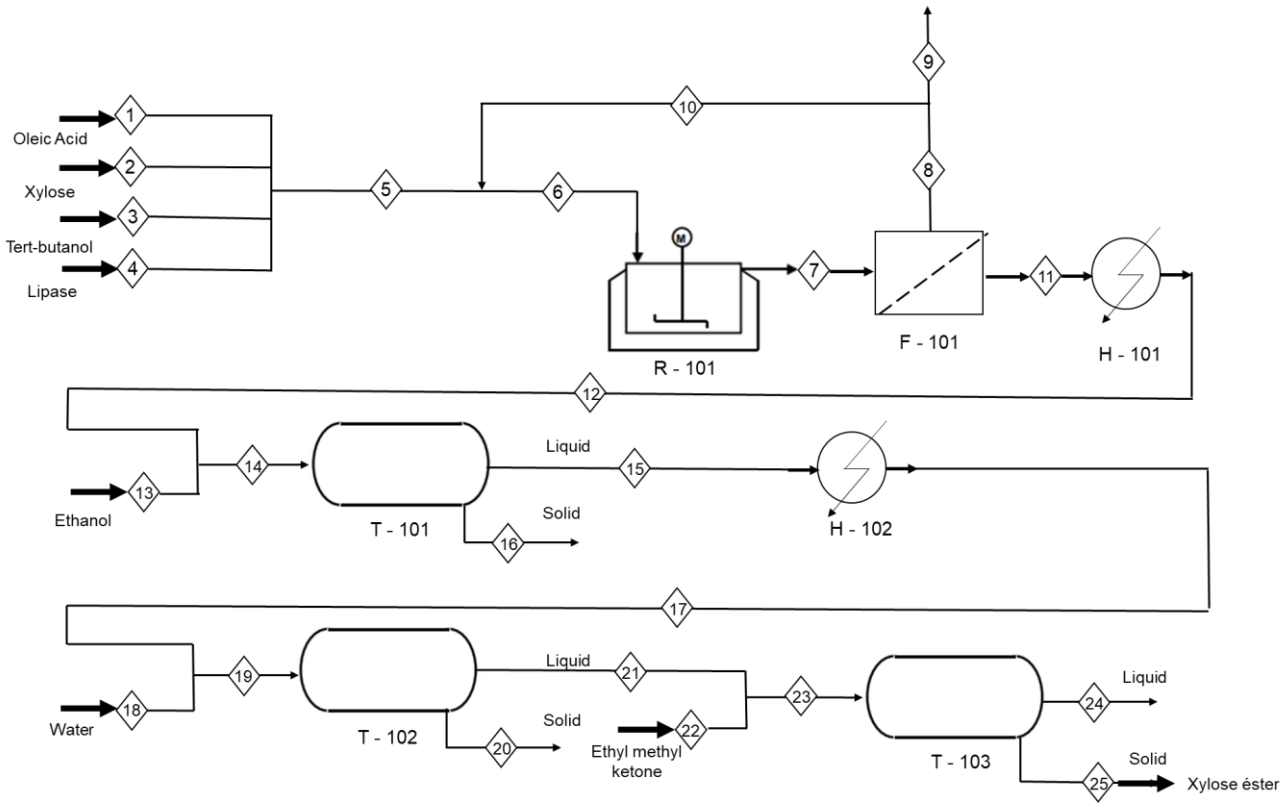

Figure 1. Representation of the production process of biosurfactants by the enzymatic route.

Table 1. Components phase used in the simulation

Components in the liquid phase	Components in the solid phase
Xylose	Immobilized Lipase
Oleic Acid	Xylose
Xylose Ester	Oleic Acid
Tert-butanol	Xylose Ester
Ethanol	
Water	
Ethyl Methyl Ketone	

to be static and adiabatic. This equipment has two inputs and one output, and the solid and liquid phases are considered. Thus, each balance is carried out for both phases.

"Global molar balance"

$$Inlet1.F + Inlet2.F = Outlet.F \quad (1)$$

where, *Inlet1.F* - the molar flow of the input 1 of the equipment (kmol/h); *Inlet2.F* - the molar flow of the input 2 of the equipment (kmol/h); *Outlet.F* - the molar flow of the equipment outlet (kmol/h).

"Molar balance per component"

$$Inlet1.F \times Inlet1.z + Inlet2.F \times Inlet2.z = Outlet.F \times Outlet.z \quad (2)$$

where, *Inlet1.z* - molar composition of the input 1 of the equipment (kmol of component/kmol total); *Inlet2.z* - molar composition of the input 2 of the equipment (kmol of component/kmol total); *Outlet.z* - molar composition of the output of the equipment (kmol of component/kmol total).

“Consideration for pressure”

$$Outlet.P = \min[(Inlet1.P, Inlet2.P)] \quad (3)$$

where, *Outlet.P* - pressure of the outlet stream of the equipment (kPa); *Inlet1.P* - pressure of the input stream 1 of the equipment (kPa); *Inlet2.P* - pressure of the input stream 2 of the equipment (kPa).

"Energy balance"

$$Outlet.F \times Outlet.h = Inlet1.F \times Inlet1.h + Inlet2.F \times Inlet2.h \quad (4)$$

where, *Outlet.h* - enthalpy of the output stream of the equipment (kJ/kmol); *Inlet1.h* - enthalpy of the input stream 1 of the equipment (kJ/kmol); *Inlet2.h* - enthalpy of the input stream 2 of the equipment (kJ/kmol).

ESTERIFICATION REACTOR: The reactor was assumed to be stoichiometric and steady-state, with the conversion specified based on the reaction limiting reagent. This equipment has a single input and one output. The solid and liquid phases are considered, so each balance is carried out for both phases.

“Reaction rate”

$$r = stoic \times conv \times z(\text{limit}) \quad (5)$$

where, *r* - reaction rate (dimensionless); *stoic* - matrix of stoichiometric coefficients (dimensionless); *conv* - reaction conversion (dimensionless); *z(limit)* - molar fraction of the limiting reagent for each reaction (dimensionless).

“Molar balance per component”

$$Outlet.F \times Outlet.z = Inlet.F \times Inlet.z + F \times r \quad (6)$$

where, *F* - total molar flow of the equipment inlet (kmol/h).

"Energy balance"

$$Outlet.F \times Outlet.h = Inlet.F \times Inlet.h + Q - F \times sum(h_R \times conv \times z(\text{limit})) \quad (7)$$

where, *Q* - heat removed from the reactor so that the temperature is maintained (kW or kJ/h); h_R - enthalpy of reaction (kJ/kmol).

“Reactor pressure”

$$Inlet.P = Outlet.P \quad (8)$$

FILTER: The filtration employed separates the solids present from the liquid phase in steady-state and adiabatic conditions. This equipment has a single inlet and two outlets, one with a higher concentration of solids and the other of liquids. The solid and liquid phases are considered, so each balance is carried out for both phases.

“Global molar balance”

$$Inlet.F = OutletS.F + OutletL.F \quad (9)$$

where, *OutletS.F* - the molar flow of solids output from the equipment (kmol/h); *OutletL.F* - the molar flow of liquids output from the equipment (kmol/h).

"Efficiency of liquid separation"

$$OutletL.Fluid.Fw = Inlet.Fluid.Fw \times frac\_liq \quad (10)$$

where, *OutletL.Fluid.Fw* - mass flow of the fluid in the liquid outlet stream (kg/h); *Inlet.Fluid.Fw* - mass flow of the fluid in the equipment inlet stream (kg/h); *frac_liq* - fraction of liquids from the inlet leaves the equipment in the liquid stream (kg of liquids/kg total).

“Efficiency of solid separation”

$$OutletS.Solid.Fw = Inlet.Solid.Fw \times frac\_sol \quad (11)$$

where, *OutletS.Solid.Fw* - mass flow of solids in the equipment solids outlet (kg/h); *Inlet.Solid.Fw* - mass flow of solids in the equipment entrance (kg/h); *frac_sol* - fraction of solids from the inlet leaves the equipment in the solid stream (kg of solids/ kg total).

“Humidity in the solid stream”

$$humidity = \frac{OutletS.Fluid.Fw}{OutletS.Total.Fw} \quad (12)$$

where, *humidity* - fraction of liquids in the equipment solids outlet (kg of liquids/kg total).

“Impurities in the liquid stream”

$$OutletL.Solid.Fw = impurity \times (OutletL.Total.Fw) \quad (13)$$

where, *OutletL.Solid.Fw* - mass flow of solids in the liquid outlet of the equipment (kg/h); *Impurity* - fraction of solids in the liquid outlet of the equipment (kg of solids/kg total); *OutletL.Total.Fw* - total mass flow of the equipment liquid outlet (kg/h).

"Thermal balance"

$$OutletS.T = Inlet.T \quad (14)$$

$$OutletL.T = Inlet.T \quad (15)$$

"Mechanical balance"

$$OutletS.P = Inlet.P \quad (16)$$

$$OutletL.P = Inlet.P \quad (17)$$

“Enthalpy of the streams”

$$OutletS.h = Inlet.h \quad (18)$$

$$OutletL.h = Inlet.h \quad (19)$$

COOLER: The cooler was used to cool the process stream in a steady-state and with no heat loss to the environment. This device has a single input and one output without changing the stream mass. The solid and liquid phases are considered, so each balance is carried out for both phases.

“Molar balance”

$$Inlet.F = Outlet.F \tag{20}$$

"Composition"

$$Outlet.z = Inlet.z \tag{21}$$

“Pressure Delta”

$$Outlet.P = Inlet.P - Pdrop \tag{22}$$

where, *Pdrop* - head loss in the heat exchanger (kPa).

“Heat exchanged”

$$Q = U \times A \times lmtd \tag{23}$$

$$lmtd = \frac{\Delta T_1 - \Delta T_2}{\ln\left(\frac{\Delta T_1}{\Delta T_2}\right)} \tag{24}$$

where, *Q* - heat exchanged in the equipment (kW); *U* - global coefficient of thermal exchange (kW/m²/K); *A* - heat exchange area (m²); *lmtd* - logarithmic mean temperature difference (K).

SPLITTER: The separators used in this work were assumed to be static and adiabatic. This equipment has a single input and two outputs. The solid and liquid phases are considered, so each balance is carried out for both phases.

“Global molar balance”

$$Inlet.F = Outlet1.F + Outlet2.F \tag{25}$$

“Molar balance per component”

$$Inlet.F \times Inlet.z = Outlet1.F \times Outlet1.z + Outlet2.F \times Outlet2.z \tag{26}$$

“Consideration for pressure”

$$Outlet1.P = Inlet.P \tag{27}$$

$$Outlet2.P = Inlet.P \tag{28}$$

"Energy balance"

$$Outlet1.F \times Outlet1.h + Outlet2.F \times Outlet2.h = Inlet.F \times Inlet.h \tag{29}$$

PUMP: The pump operates in a steady-state to correct a particular pressure drop. The equipment has a single entrance and a single exit. Therefore, there is no heat exchange with the environment, and, again, all balances occur for liquid and solid phases.

“Molar balance for the liquid phase”

$$Inlet.Fluid.F = Outlet.Fluid.F \tag{30}$$

“Molar balance for the solid phase”

$$Inlet.Solid.F = Outlet.Solid.F \tag{31}$$

“Molar fraction for the liquid phase”

$$Outlet.Fluid.z = Inlet.Fluid.z \tag{32}$$

“Molar fraction for the solid phase”

$$Outlet.Solid.z = Inlet.Solid.z \tag{33}$$

"Head loss"

$$Outlet.P = Inlet.P + Pln \tag{34}$$

where, *Pin* - pressure delta.

"Energy balance"

$$Inlet\_p.W = Inlet.Fluid.F \times (Outlet.Fluid.h - Inlet.Fluid.h) + Inlet.Solid.F \times (Outlet.Solid.h - Inlet.Solid.h) \tag{35}$$

“Work”

$$Inlet\_p.W = (Inlet.Fluid.Fw + Inlet.Solid.Fw) \times \frac{Pln}{n \times density} \tag{36}$$

where, *n* - pump efficiency (dimensionless).

Simulation

According to Figure 1, the process starts by mixing the reactants (oleic acid and xylose), the solvent (tert-butanol), and the enzyme (lipase) to form stream 5, which is mixed to the system recycle and enters the bioreactor. Next, the reactor outlet, stream 7, goes downstream to filtration, where part of the immobilized enzyme is recycled (a fraction is removed from the process (stream 9) while another is reinserted (stream 10)). Afterward, the enzyme-free stream goes to the purification process, where stream 11 is cooled and mixed with ethanol in a separation tank. As a result, a solid-liquid equilibrium is formed, and the solid phase is removed from the process. In the sequence, stream 15 is cooled, and water is added to the stream to create a new solid-liquid equilibrium in a tank where the solid formed is removed. Soon after, ethyl methyl ketone is inserted into the process in a way that the formed precipitated contains the product of interest, xylose ester (stream 25).

The process simulation occurs to approximate the operating conditions of a given operational scheme and verify the behavior of some variables. Thus, the EMSO software was used to solve the equations describing the process based on the previously presented modeling. Tables 2 and 3 show the main specifications for the process simulation. As the simulated process has 2554 variables and 2419 equations, 135 specifica-

tions are needed. Thus, the remaining specifications are found in the supplementary material (Table A1).

It is worth mentioning that the value of 1 10^{-6} present throughout the tables refers to the value zero and is used to avoid convergence problems. The information present in [29] was used for the reaction conditions, with the xylose to free fatty acids (FFA; here represented by the Oleic Acid) molar ratio of 1:5, so for every 2.16 mmoles of xylose-FFA, there is 6 mL of tert-butanol (as organic solvent) and 0.6 g of lipase, the temperature of 60 °C and conversion of 70%. As detailed elsewhere [29], a suspension containing octyl-silica and enzyme solution was used for the immobilization. The reaction of esterification of xylose with FFA follows the stoichiometry of Equation 37.

In the second stage of the process, there are precipitation tanks to purify the biosurfactant (Figure 1). Wagner *et al.* [30] presented a method for separating and purifying sugar esters based on the precipitation of compounds. That method consisted of adding ethanol, water, and ethyl methyl ketone to purify the ester (a sucrose ester, in the specific case addressed by the authors [30]). The first tank aims to precipitate the xylose (by adding ethanol), removed from the process, following Eq. (38). The next two tanks (the second and the third) are intended to precipitate/purify the xylose ester. In the second tank, the ester precipitation occurs with the presence of water, but part of the FFA is precipitated together with the ester. The last precipitation tank uses ethyl methyl ketone, which solubilizes part of the FFA, leaving the xylose ester more concentrated in the solid phase to achieve a higher level of purification. The second process separation tank has three equations to represent what happens in precipitations and solubilizations. Equation 39 is the representation of the xylose ester precipitation. Equation 40 represents the precipitation of the FFA, and Equation 41 the solubilization of xylose in water. The third and last separation tank aims to solubilize the FFA and thus increase the concentration of ester in the product. Equation 42 represents the solubilization of FFA in ethyl methyl ketone.

Table 2. Variables specified in the process input streams

Stream	Phase	Variable	Name	Unit	Value
Streams 1, 2,3 and 4	All	T	Temperature	K	333.15
Streams 1, 2,3 and 4	All	P	Pressure	atm	1
Streams 1, 2 and 3	Solid	F	Molar flow	kmol/h	1 10^{-6}
Stream 1	Liquid	F	Molar flow	kmol/h	50
Stream 2	Liquid	F	Molar flow	kmol/h	10
Stream 3	Liquid	F	Molar flow	kmol/h	175
Stream 4	Liquid	F	Molar flow	kmol/h	1 10^{-6}
Source 4	Solid	F	Molar flow	kmol/h	79.6
Stream 1	Solid	z(1)*	Composition	dimensionless	1
Stream 1	Solid	z(2–4)*	Composition	dimensionless	0
Stream 1	Liquid	z(1)*	Composition	dimensionless	0
Stream 1	Liquid	z(2)*	Composition	dimensionless	1
Stream 1	Liquid	z(3–7)*	Composition	dimensionless	0
Streams 2, 3 and 4	Solid	z(1)*	Composition	dimensionless	1
Streams 2, 3 and 4	Solid	z(2–4)*	Composition	dimensionless	0
Stream 2 and 4	Liquid	z(1)*	Composition	dimensionless	1
Stream 2 and 4	Liquid	z(2–7)*	Composition	dimensionless	0
Stream 3	Liquid	z(1–2)*	Composition	dimensionless	0
Stream 3	Liquid	z(3)*	Composition	dimensionless	0
Stream 3	Liquid	z(4)*	Composition	dimensionless	1
Stream 3	Liquid	z(5–7)*	Composition	dimensionless	0

* Component number: Liquids: 1-Xylose; 2-Oleic Acid; 3-Xylose Ester; 4-Tert-butanol, 5-Ethanol; 6-Water; 7-Ethyl Methyl Ketone. Solid: 1-Immobilized Lipase; 2-Xylose; 3-Oleic Acid; 4-Xylose Ester.

Table 3. Variables specified for the equipment

Equipment	Variable	Name	Unit	Value
Reactor 101	conv	Conversion	dimensionless	0.7
Reactor 101	T	Temperature	K	333.15
Filter 101	frac_sol	Fraction of solids in the liquid stream	dimensionless	0.99
Filter 101	humidity	Fraction of liquid in the solid stream	dimensionless	0.10
Splitter between streams 8 and 9	frac	Fraction of the stream that will be removed from the process	dimensionless	0.10
Cooler 101	Pdrop	Pressure loss	atm	0
Cooler 101	Outlet.T	Output temperature	K	323.15
Cooler 101	U	Global heat exchange coefficient	kW/m²/K	0.6945

$$C_5H_{10}O_5(l) + C_{18}H_{34}O_2(l) \Leftrightarrow C_{23}H_{42}O_6(l) + H_2O(l) \quad (37)$$
$$(Xylose)\ (Oleic\ Acid)\ (Xylose\ ester)\ (Water)$$

$$C_5H_{10}O_5(l) \Leftrightarrow C_5H_{10}O_5(s) \quad (38)$$
$$(Liquid\ xylose)\ (Solid\ xylose)$$

$$C_{23}H_{42}O_6(l) \Leftrightarrow C_{23}H_{42}O_6(s) \quad (39)$$
$$(Liquid\ xylose\ ester)\ (Solid\ xylose\ ester)$$

$$C_{18}H_{34}O_2(l) \Leftrightarrow C_{18}H_{34}O_2(s) \quad (40)$$
$$(Liquid\ FFA)\ (Solid\ FFA)$$

$$C_5H_{10}O_5(s) \Leftrightarrow C_5H_{10}O_5(l) \quad (41)$$
$$(Solid\ xylose)\ (Liquid\ xylose)$$

$$C_{18}H_{34}O_2(s) \Leftrightarrow C_{18}H_{34}O_2(l) \quad (42)$$
$$(Solid\ FFA)\ (Liquid\ FFA)$$

RESULTS AND DISCUSSION

Simulation

Table A2 presented in the supplementary material presents the data for stream 7, found immediately after the reactor output. It is noticed that there is recovery and recycling of enzymes in the process. The flow rate of the lipase source (line 8 of Table 2) was adjusted so that the flow of the enzyme into the esterification reactor (line 15 of Table A2) was in accordance with the literature since 10% of this current is removed from the process. It is important to note that the lipase loses activity during the reactions. Therefore, the splitter is present in the simulation, representing a fraction of the enzyme stream removed from the process. In the study by Vescovi *et al.* [29], the immobilized enzyme loses its total activity in about 100 h of reactions. The results for the recycle stream (Stream 10) and the stream that removes lipase from the process (Stream 9) are shown in Table A2. In the simulation, it was necessary to add a pump to correct the numerical errors inserted in the pressures. As in some cases of mechanical equilibrium, the pressure at the output of the equipment was given by the minimum between the pressures of the inlet streams, and the solution of the set of equations subtly modified these pressures and, consequently, the minimum pressure. This phenomenon led to the numerical non-convergence of the simulation. After countless tests and verifications to identify the problem, a loss of pressure was noticed throughout the process, resulting from the numerical solution. A pump was used in the recycle to circumvent this problem. The pump is located before the reactor, just after the separator (Stream 10).

After the esterification reaction and the filtration for reuse of the enzymes, the stream has the composition shown in Table 4. Notice that FFA is in greater quantity since it was placed in excess to get the degree of conversion of 70%.

Mass balances are consistent with the results presented by Vescovi *et al.* [29], obtaining the production described by the authors. However, as the mass fraction of xylose ester in the stream is approximately 10.3%, some purification steps are necessary to achieve higher biosurfactant concentrations in the product. Therefore, the reaction followed by the enzyme filtration step alone is insufficient for this process.

The purification of the xylose ester consists of consecutive steps of the precipitant addition followed by precipitation. Figure 2 shows a graph of the composition of the streams after each purification step. The product enters this process at 10.3% (step 1: reactor exit after enzyme removal) in mass composition and reaches 10.4% in the first precipitation process (step 2: first precipitation process with ethanol). In the second precipitation, it reaches 37.6% (step 3: second

Table 4. Results for stream 12

Phase	Variable	Name	Unit	Value
Liquid	Fw	Mass flow	kg/h	28408.4
Solid	Fw	Mass flow	kg/h	166.793
All	T	Temperature	K	323.15
All	P	Pressure	atm	1
Liquid	zw(1)*	Composition	dimensionless	0.0154
Liquid	zw(2)*	Composition	dimensionless	0.4236
Liquid	zw(3)*	Composition	dimensionless	0.1030
Liquid	zw(4)*	Composition	dimensionless	0.4537
Liquid	zw(5)*	Composition	dimensionless	0
Liquid	zw(6)*	Composition	dimensionless	0.0045
Liquid	zw(7)*	Composition	dimensionless	0
Solid	zw(1)*	Composition	dimensionless	1
Solid	zw(2–4)*	Composition	dimensionless	0

* Component number: Liquids: 1-Xylose; 2-Oleic Acid; 3-Xylose Ester; 4-Tert-butanol, 5-Ethanol; 6-Water; 7-Ethyl Methyl Ketone**.** Solid: 1-Immobilized Lipase; 2-Xylose; 3-Oleic Acid; 4-Xylose Ester.

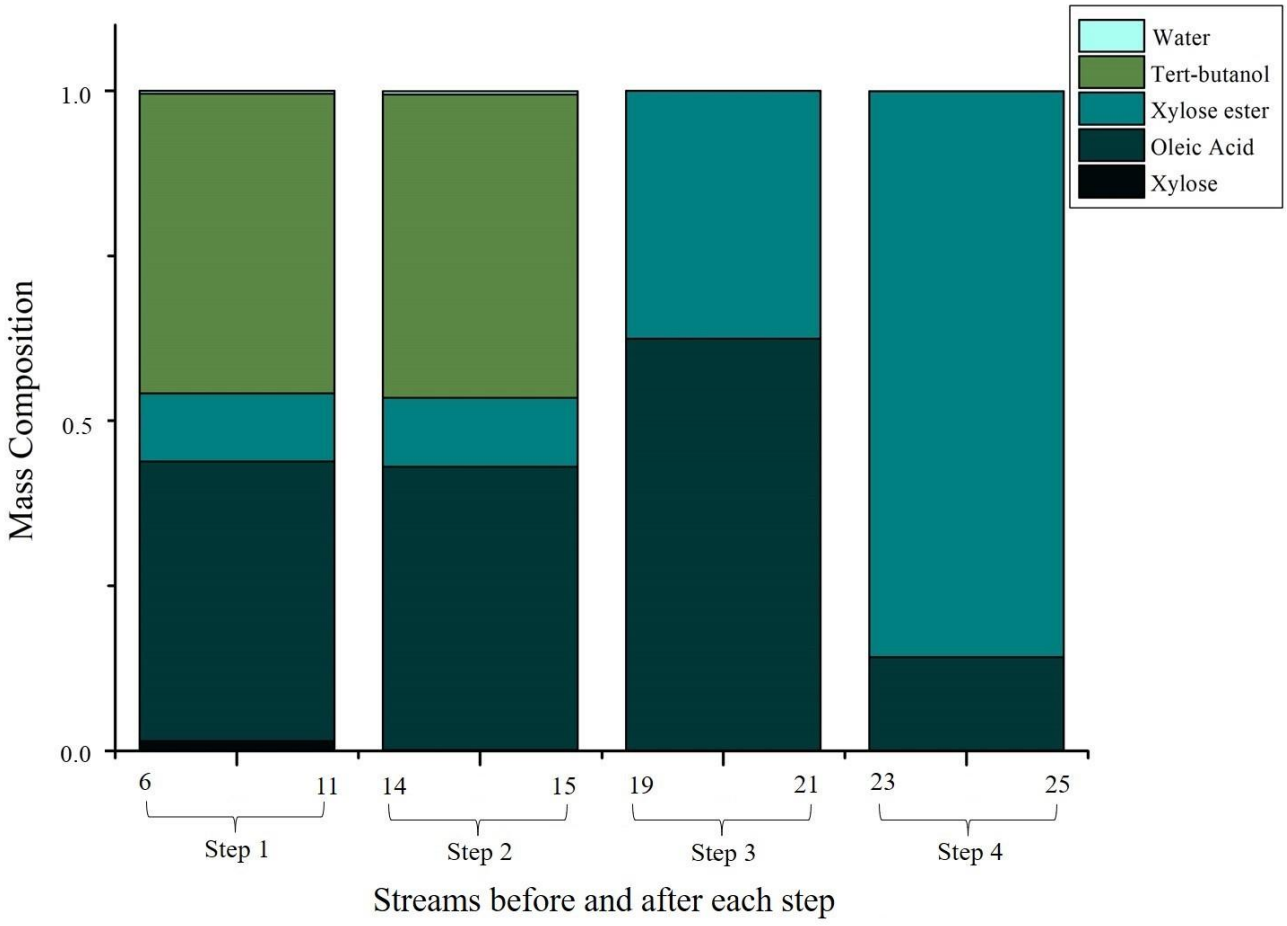

Figure 2. Mass composition of the streams after each stage (1, 2, 3, and 4) and streams identification (associated with each step, before and after them).

precipitation process with water), and in the third (last) precipitation process, it reaches 85.7% (step 4: third precipitation process with ethyl methyl ketone, Table 5).

Thus, the purification process indicates an overall efficiency of 88% ((0.857-0.103)/0.857). The literature does not report purification processes for obtaining biosurfactants by enzymatic route, using oleic acid and xylose as reagents. However, Mukherjee *et al.* [8] reported purification for biosurfactants obtained by the microbiological route. The separation proceeds from several complex steps (acidification, cooling, gel filtration chromatography, and lyophilization) to reach a lyophilized product with high purity. Regarding the degree of complexity of the proposed steps, the present work has reached a satisfactory purity using simpler equipment.

Energy costs throughout the process are shown in Table 6, separated by equipment.

Table 5. Results for stream 25

Phase	Variable	Name	Unit	Value
Liquid	Fw	Mass flow	kg/h	367.413
Solid	Fw	Mass flow	kg/h	3306.72
All	T	Temperature	K	298.15
All	P	Pressure	atm	1
Liquid	zw(1)*	Composition	dimensionless	0.0004
Liquid	zw(2)*	Composition	dimensionless	0.8860
Liquid	zw(3)*	Composition	dimensionless	0.0002
Liquid	zw(4)*	Composition	dimensionless	0.1037
Liquid	zw(5)*	Composition	dimensionless	0.0002
Liquid	zw(6)*	Composition	dimensionless	0.0011
Liquid	zw(7)*	Composition	dimensionless	0.0084
Solid	zw(1)*	Composition	dimensionless	0.0005
Solid	zw(2)*	Composition	dimensionless	0
Solid	zw(3)*	Composition	dimensionless	0.1424
Solid	zw(4)*	Composition	dimensionless	0.8571

* Component number: Liquids: 1-Xylose; 2-Oleic Acid; 3-Xylose Ester; 4-Tert-butanol, 5-Ethanol; 6-Water; 7-Ethyl Methyl Ketone. Solid: 1-Immobilized Lipase; 2-Xylose; 3-Oleic Acid; 4-Xylose Ester.

Table 6. Energy costs of the simulated process

Equipment	Heat (kW)
Esterification reactor	137.58
Cooler 101	- 178.74
Cooler 102	- 425.39

In general, greater heat is associated with cooling (coolers 101 and 102, -604.14 kW) than heating (esterification reactor, 137.58 kW), with total heat of - 466.56 kW. The energy expenditure figures exposed do not consider an energy integration of the process. If a possible energy integration is evaluated to feedback heat into the process, expenses can significantly reduce operating costs. However, when assessing global energy costs, the used equipment requires less heat than equipment used in producing biosurfactants by microbiological routes [8].

Finally, to demonstrate the potential of the developed tool, a simple sensitivity analysis was carried out, showing the effect of the conversion of the esterification reactor on the mass composition of the final product.

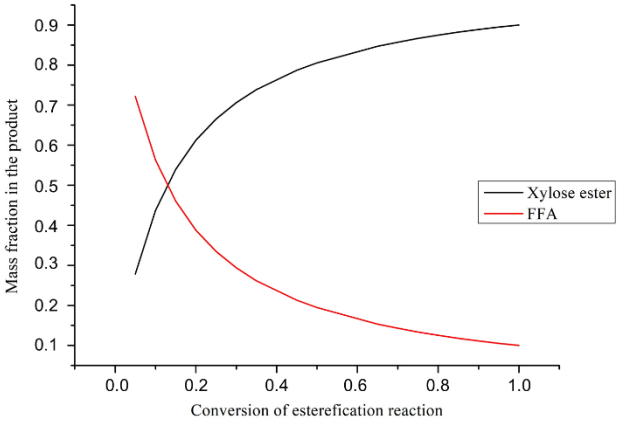

Figure 3. Ester/FFA fraction in the final stream of process versus conversion of the reactor.

From Figure 3, the gain of purity of xylose ester in the final product increases according to the conversion

of reagents to ester in the reactor. Note that the behavior of the final product concentration has an exponential correlation with the conversion of the esterification reaction. For values around 15% conversion, the concentration of esters is greater than FFA in the final stream. It is worth mentioning that the final product concentration increases subtly at conversion values close to 100%. Therefore, there is no need for further research to increase the conversion above 70% to impact the final purity of the product.

Economic analysis

The process simulated in this work was calculated and analyzed for capital and operating costs. All the details of the calculations for this analysis are in the supplementary material (Appendix B). For equipment costs, the methodology from [31] was used. Their parameters are shown in Table 7. The capacity of the equipment units and their calculated costs are shown in Table 8.

For the operating costs evaluated in the economic analysis of the process, the raw material, labor, utility, operating supervision, and maintenance costs were considered. These costs are presented in Table 9. Table 10, in turn, shows the cash flow for the process.

Table 7. Equipment cost parameters to be used in economic analysis

Equipment	K_1	K_2	K_3	FBM or (B1 and B2)	LF_B	A_{min}	A_{max}	Unit
Esterification reactor (Jacketed agited)	4.1052	-0.4680	-0.0005	4.00	1.14	0.1	35	m³
Filter (Gravity)	4.2756	-0.648	0.0714	1.65	1.14	0.5	80	m²
Heat exchanger (Fixed tube)	4.3247	-0.3030	0.1634	1.63 1.66	1.14	10	1000	m²
Process Vessel (Horizontal)	3.5565	0.3776	0.0905	1.49 1.52	1.14	0.1	628	m³

* Adapted from [31].

Table 8. Equipment capacity according to process simulation and associated costs obtained

Equipaments	Capacity	Cost
Esterification reactor (Jacketed agited)	1451.87 m³ (Volume)	MUS$ 0.7931
Filter (Gravity)	39.75 m² (Area)	MUS$ 0.0086
Heat exchanger 1 (Fixed tube)	25.74 m² (Area)	MUS$ 0.1077
Heat exchanger 2 (Fixed tube)	61.26 m² (Area)	MUS$ 0.1303
Process Vessel 1 (Horizontal)	28.43 m³ (Volume)	MUS$ 0.1169
Process Vessel 2 (Horizontal)	27.99 m³ (Volume)	MUS$ 0.1158
Process Vessel 3 (Horizontal)	0.89 m³ (Volume)	MUS$ 0.0204
Total	-	MUS$ 1.2927
Total plant installation	-	MUS$ 1.5254

* MUS$ = Million US$.

Table 9. Operating costs

Raw material costs	MUS$ 1.0240 10³/year
Labor costs	MUS$ 0.5119/year
Utility costs	MUS$ 0.0197/year
Operating supervision costs	MUS$ 0.0256/year
Maintenance costs	MUS$ 0.0763/year
Total operating costs	MUS$ 1.0243 10³/year

The cash flow presented in Table 10 refers to the case of a null net present value, which represents the minimum biosurfactant selling price (MBSP). The value obtained for a minimum attractive rate of return of 11% per year and project life of 25 years was US$ 72.37/kg. This price is coherent with some references presented in the literature [32, 33].

Table 10. Process cash flow

Total operation cost	MUS$ 1.0243 10³/year
Total revenue	MUS$ 1.0338 10³/year
Cash flow	MUS$ 9.5703/year

A sensitivity analysis was also performed regarding the process scale (Figure 4).

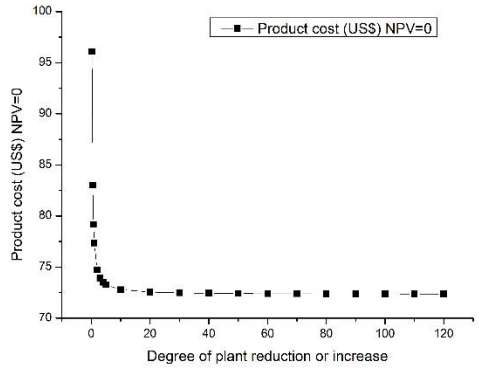

Figure 4. Product sales value depending on the degree of reduction or increase of the original plant (100%), with NPV = 0.

No gain in the MBSP is observed for scales between 20% and 120% of the base case. Also, a rapid increase in MBSP is seen for scales below 20% of the base case.

CONCLUSION

For the proposed esterification process, by the immobilized lipase of oleic acid with xylose, recovery and reuse of enzymes, and separation/purification of the product, it was possible to develop mathematical models that successfully described the process. The simulation of the purification steps indicated a product with 86% in biosurfactants, which increased their recovery by 88%. It was also possible to obtain estimates of energy costs for the process. Compared to the works reported in the literature, the proposal presents itself as a more energy-efficient and less complex alternative that uses cheaper equipment to purify biosurfactants. In addition, it is a suggested use for co-products from soy oil and 2G ethanol productions. It is worth mentioning that energy expenditure does not yet consider the integration of the process. Therefore, energy integration analysis can still significantly reduce these in future works. The initial economic analysis of the process indicates a minimum biosurfactant selling price of US$72.37/kg for a minimum attractive rate of return of 11%.

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NAUČNI RAD

MODELOVANJE I SIMULACIJA ENZIMSKE PROIZVODNJE BIOSURFAKTANTA KORIŠĆENJEM KSILOZE I OLEINSKE KISELINE

Biosinteza estara šećera, molekula sa svojstvima biosurfaktanata, može se izvesti enzimski katalazovanom esterifikacijom šećera sa masnim kiselinama. Alternativa za smanjenje uticaja sirovina na konačnu cenu proizvodnje biosurfaktanata i ponovnu upotrebu industrijskog otpada je korišćenje ostataka iz industrije biljnih ulja kao izvora slobodnih masnih kiselina, kao što je oleinska kiselina, i lignoceluloznih ostataka iz proizvodnje etanola kao izvor šećera (ksiloza). U ovom scenariju, ovaj rad je imao za cilj modelovanje proizvodnje biosurfaktanata putem heterogene biokatalize korišćenjem lipaze, oleinske kiseline i ksiloze. Razdvajanje i prečišćavanje proizvoda izvršeno je korišćenjem niza taloženja (dodavanje etanola, vode i metil etil ketona). Simulacija je izvedena korišćenjem softvera EMSO (Environment for Modeling, Simulation, and Optimization) orijentisanog na jednačine, usklađenog sa CAPE-OPEN-om. Procenat biosurfaktanata u proizvodu je bio oko 86%, sa prinosom od 88% posle prečišćavanja. Što se tiče studije utroška energije, uočene su vrednosti od -604,1 kW i 137,6 kW toplote povezane sa hlađenjem i grejanjem, redom. Razvijeni matematički modeli uspešno su opisali proces. Početna ekonomska analiza procesa ukazuje na minimalnu prodajnu cenu biosurfaktanta od 72,37 USD/kg.

Ključne reči: biosurfaktanti, esterifikacija, modelovanje i simulacija, prečišćavanje, precipitacija.