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# A Holistic-Hierarchical Approach for Production of Energy Blocks from Microalgae

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**Abstract :** Microalgae biomass can potentially be exploited for the production of biofuels because their photosynthetic mechanism allowscollecting energy in a more efficient way than terrestrial plants. However, biotechnological use of microalgae biomass for biofuel production presents various bottlenecks at different stages to prevent the sustainable use of this energy crop.In this research paper, the joint use of experimental techniques, characterization, process modeling, sensitivity analysis and process simulation is shown in a holistic-hierarchical approach to improve the supply chain, emphasizing the production of fermentable sugars and oil as energy blocks or precursors of third generation biofuels is presented. The hierarchical part of the approach sets experimentation as a tool for selection of raw materials and improving efficiency, and uses modeling and simulation to minimize the use of natural resources allowing selecting technologies and processing routes. The results of implementation show in the experimental part an increase in the production of total sugars and cell wall disruption of 87 % of extraction efficiency for 120 minutes using acid hydrolysis, and 94% using a polyfunctional process. While the modeling and simulation results show that the technology of simultaneous saccharification and co-fermentation allows higher efficiency of ethanol production.
**Keywords :** Holistic approach, Microalgae, Modeling, Simulation.

## Introduction

The use of microalgae as an energy source has great potential due to its high efficiency in capturing and collecting solar energy and its easy adaptability to growth fast in different environmental conditions, such as fresh water or sea water[1]. It has been found that microalgae have several useful components, mainly lipids (7-23%), carbohydrates (5-23%)and proteins (6-52 %) which are extracted to be transformed into biodiesel[2,3], bioethanol and fertilizers or feed supplement for animals, respectively[4,5].Among the alternatives currently used for the metabolites extraction are found unitary operations carried out separately to break the cell wall, extract and purify. However, an alternative in development is the use of multifunctional processes where these operations be carried out simultaneously. On the other hand, the application of computer-aided process engineering in conjunction with the application of holistic[6,7]and hierarchical[8,9]approacheshave become indispensable to model a set of processes, due to they can be used for the evaluation and analysis of several levels thereof.This paper presents a holistic-hierarchical approach for the improvement of the microalgae biomass utilization chain based on three levels, where experimentation is used in the first one as a tool to select raw materials and increase efficiencies; in the second is found the modeling of processes for the decision

making at levels that allow to minimize the exhaustion of resources in order to select technologies and processing routes that can be finally compared in a third levels, the simulation. Different experimental techniques such as the study of fermentable sugars and lipid extraction from microalgae *Closterium* sp. and*Nannochloropsis* sp. as well as the characterization of the oil extracted from the two strainswere used as a decision-making tool in the selection of the species to be used; followed by process modeling and sensitivity analysis of fermentable sugars production under different scenarios of operating conditions and the process simulation for the comparison of three emerging alternatives of ethanol production from microalgal sugars: Simultaneous saccharification and co-fermentation, simultaneous saccharification and fermentation and hydrolysis and fermentation separated.

## Material and Methods

### Approach used

The holistic-hierarchical approach for the improvement of a productive chain is supported by three pillars: experimentation, as the primary source of technical information on usable raw materials, operating conditions, lipid profiles and efficiencies; process modeling and sensitivity analysis as a tool for analyzing pilot-scale operational scenarios to differentiate small-scale problems that lose relevance or can be solved on a larger scale; and computer-aided engineering as a tool to support decision-making for the evaluation of technologies and as a point of convergence of the synthesis of the process.

## Experimental

Microalgae biomass of *Closterium* sp. and *Nannochloropsis* sp. was harvested by flocculation and dried in an oven at 105°C for 8 hours. The monosaccharaides production was carried outusingacid hydrolysis and Organosolv pretreatment. For the first method, solutions with 10 g of dry biomass of the microalga *Closterium*sp. and 150 mL of 0.5 mol $L^{-1}$ hydrochloric acid were prepared and subjected to a stirring speed of 500 rpm for 30, 60 and 120 minutes at room temperature. Subsequently, vacuum filtration was performed and the pH was adjusted near to 7 with the addition of distilled water to obtain two products: hydrolyzed biomass used for lipid extraction and water-soluble bioproducts in acid solution thatwere neutralized with NaOH to obtain reducing sugars. On the other hand, for Organosolv pretreatment, an experimental design $2^2$ was made to evaluate the effect of acid concentration and time on the reducing sugars yield.After this procedure, two phases were obtained and separated by vacuum filtration, a solid phase with pretreated biomass and a liquid phase that contains monosaccharaides desired. After that, pretreated biomass was used on the same way described above andneutralization of pretreatment liquor was made by adding $CaCO_3$ to a pH of about 5 or 6, finally, total reducing sugars were measured. Measurement of total reducing sugars for both monosaccharide production alternatives evaluated was performed using the method of dinitrosalicylic acid (DNS) proposed by Miller (1959)[10].

After monosaccharaides production, biomass of both microalgae was subjected to lipid extraction by CSE method using hexane as solvent and taking repeated wash times of 16 hours. Extract was filtrate to obtain the solvent with lipids anda portion of the solvent was removed by simple distillation and the other was allowed to volatilize. To calculate lipid extraction effectiveness, the term Relative Extraction Ratio is introduced; which is defined as the lipid yield reached using any extraction method evaluated related to lipid yield reached performing SHE method. Equations (1) and (2) were used for calculation of lipid yield and Relative Extraction Ratio respectively.

$$\text{Lipid yield (\%)} = \frac{\text{oil weight}}{\text{biomass weight}} \text{x100 \%} \quad (1)$$

$$\text{Relative Extraction Ratio (\%)} = \frac{\text{lipid yield}}{\text{total lipid determined}} \text{x100 \%} \quad (2)$$

Finally,multifunctional processwere conducted by joining cellular disruption, oil extraction and in situ transesterification[11]based on evaluation of ethanol and methanol as solvents/reagents. A ratio of biomass-alcohol 1:6 and sulfuric acid as catalyst were used for transesterification in an oil-acid ratio of 1:1. Reaction systems were continuously stirred at 500 rpm for 10 hours at 60 ºC, and 1 mL samples were taken at different time intervals to centrifugation for 10 min to separate hydrolyzed and water-soluble biomass. Liquor was neutralized by the addition of 50 mL of NaOH 1N, adjusting a pH near to 7. Then, 1.5 mL of hexane and 0.5 mL of distilled water were added in order to obtain a three-phase system consisting of hexane phase, residual biomass and hydro-alcoholic layer. Hexane phase was analyzed using Infrared Spectroscopy in order to detect products obtained using a FTIR-Fourier Transform Infrared Spectrophotometer in the wavelength range of 400-4000 $cm^{-1}$; hydro-alcoholic phase was treated using the DNS method andfor remaining biomass in each system, lipid extraction was carried out in order to quantify non-extracted and/or transesterified lipids.

**Modeling and sensitivity analysis**

Kinetic parameters for transformation of cellulosic components of *Nannochloropsis* sp. in reducing sugars and degradation of these sugars were found based on the model presented byTéllez-Luis et al.(2002)[11]for polysaccharides hydrolysis, which describes a first-order consecutive reaction with two irreversible steps, where PM (polysaccharides of microalgae) are transformed into RS (reducing sugars), and RS into DP(degradation products).Differential equations are also proposed for describing the changes in the concentration of polysaccharides, monosaccharaides and degradation products, Equation (3) expresses the reaction rate of monomerization of polysaccharides, Equation (4) describes the rate of production of reducing sugars, where C is the concentration of polysaccharides from microalgae and A is the concentration of total reducing sugars, and Equation (5) represents the Arrhenius equation which relates the rate constant $K_i$as a function of temperature. Kinetic parameters of reaction systems were determined using MATLAB software v.7.10 and the Solver tool in Microsoft Excel and with calculated kinetic data were modeled responses related to total reducing sugar concentration by modifying acid sulfuric concentration and system temperature.

$$\frac{dC}{dt} = -K_1[C] \quad (3)$$

$$\frac{dA}{dt} = K_1[C] - K_2[A] \quad (4)$$

$$K_i = C_{acid}^{n} A_i e^{-\frac{Ei}{RT}} \quad (5)$$

**Simulation of emergent chains**

The simulation of the bioethanol obtaining process was carried out using a commercial process simulation software, where bioethanol yield was evaluated from three simultaneous production routes: saccharification and co-fermentation (SSCF), saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF). The common chemical compounds were selected from the simulator library and the level 1 information, and those that were not found were modeled taking into account their physicochemical and thermodynamic properties making use of the molecular structures of the components in question. The thermodynamic model used to simulate the oil extraction routes was NRTL (Non-Random two liquids) due to it represents very well the mixtures of polar and non-polar components, as well as a strong non-ideality in the simulation; and the binary interaction coefficients not available in the software library were estimated using the UNIFAC (Universal Functional Group Activity Coefficient) method.

## Results and Discussion

**Experimental**

**Microalgae characterization**.

According to Table 1, *Nannochloropsis* sp., presents higher percentages of proteins than *Closterium* sp., ash percentage for both strains were normalized to 10%. Values reported does not presents significant differences, and both microalgae strains belong to the same order, for these reasons, microalgae biomass used can be considered comparable for the evaluation of routes.

**Table 1. Metabolites characterization of *Closterium* sp. and *Nannochloropsis* sp. microalgae**

| Microalgae Strain | Proteins (%) | Starch (%) | Cellulosic Material (%) | Lipids (%) | Ash (%) |
|---|---|---|---|---|---|
| ***Closterium*sp.** | 38.0 | 15.0 | 18.0 | 19.0 | 10.0 |
| ***Nannochloropsis*sp.** | 32.0 | 13.0 | 19.0 | 26.0 | 10.0 |

**Evaluation of hydrolysis-solvent extraction (HSE) route.**

The increase in contact time of hydrochloric acid to the microalga biomass of *Closterium sp*. influenced the release of lipids after solvent extraction. When the cellular lysis time was 120 minutes, a Relative Extraction Ratio of 87 % was reached, followed by 70 % and 42 % when the pretreatment time was 60 and 30 minutes, respectively. For this reason, 120 minutes of hydrolysis reaction was chosen for subsequent treatments.

**Evaluation of Organosolv pretreatment-solvent extraction (OSE) route.**

Organosolv pretreatment applied to the microalga *Closterium* sp. increased the lipid extraction efficiency compared with acid hydrolysis, reaching a Relative Extraction Ratio of 94 %.

**Evaluation of MSE and MSM routes: Alkyl esters production and fatty acids composition.**

By measurements of mid-infrared transmittance, the presence of alkyl esters or biodiesel in the hexane phase of the samples was evaluated. Table 2 shows that the spectra obtained by spectroscopic Fourier transform infrared (FTIR) indicated an increase in the band area corresponding to the carbonyl bond (C=O) around of 1750 $cm^{-1}$ and the strip forming of the aliphatic chains between 2800 and 3000 $cm^{-1}$, after 2 hours of reaction for the system with ethanol and 1 hour for the process with methanol. By comparing theFTIR spectra peaks for lipids, alkyl esters and petrodiesel, it can be said thatthese are very similar. In case of petrodiesel, vibration was found only for aliphatic chains between 2800 and 3000 $cm^{-1}$ due to the absorption of infrared bond olefin (CH).

**Table 2. Characteristic spectrum regions of lipids, biodiesel and petrodiesel using different feedstocks.**

| Sample | Vibration | Microalgae | Palm[12] | Petrodiesel[13] |
|---|---|---|---|---|
| | | Region ($cm^{-1}$) | | |
| **Lipids** | **Aliphatic chains ($CH_3$ y $CH_2$)** | 2954-3025<br>2918* | 2800-3000 | Absent |
| | **Carbonyl bond (C=O)** | 1654-1746<br>1704* | 1750 | |
| **Biodiesel/fossil diesel** | **Aliphatic chains ($CH_3$ y $CH_2$)** | 2970***** | 2800-3000 | 2800-3000 |
| | **Carbonyl bond (C=O)** | 1750* | 1750 | |
| | **Glycerol (bond OH)** | 3400 | | |

* Regions identified in this study using FTIR.

On the other hand, Table 3 show the fatty acids profile for both strains. Taking into account a desirable fatty acid for suitable biodiesel properties, which includes a high percentage of monounsaturated fatty acids and low percentages of saturated fatty acids, trienoic fatty acids and very long chain fatty acids[14], oil from *Closterium* sp. microalgae is more suitable for biodiesel production than *Nannochloropsis* sp. microalgae oil. However, taking into account selection criteria recommended by Moser and Vaughn (2012)[15], neither *Closterium* sp. nor *Nannochloropsis* sp. are suitable for a good quality biodiesel without adding an additive to the fuel produced.

**Table 3. Characterization of oil extracted from *Closterium* sp. and *Nannochloropsis* sp. microalgae**

| Fatty acid | % Fatty acids | |
|---|---|---|
| | ***Closterium*sp.** | ***Nannochloropsis*sp.** |
| **Saturated fatty acids (%)** | 14.3 | 38.5 |
| **Monounsaturated fattyacids (%)** | 7.0 | 3.2 |
| **Polyunsaturated fatty acids (%)** | 55.4 | 34.7 |

**Modeling reducing sugars yield and sensitive analysis**

Triglyceride molecules released from the cellular disruption step reacts with ethanol or methanol under the catalytic action, yielding reducing sugars, fatty acids esters and glycerin. In this multifunctional process, sulfuric acid acts as a catalyst for hydrolysis and in situ transesterification reactions. Equation (6) was obtained by mathematical development of Equations (3) to (5) and relates the concentration of total reducing sugars with constant speed and time. Numerical value in the equation is based on microalgae polysaccharide material reported by Ververis et al. (2007)[16].

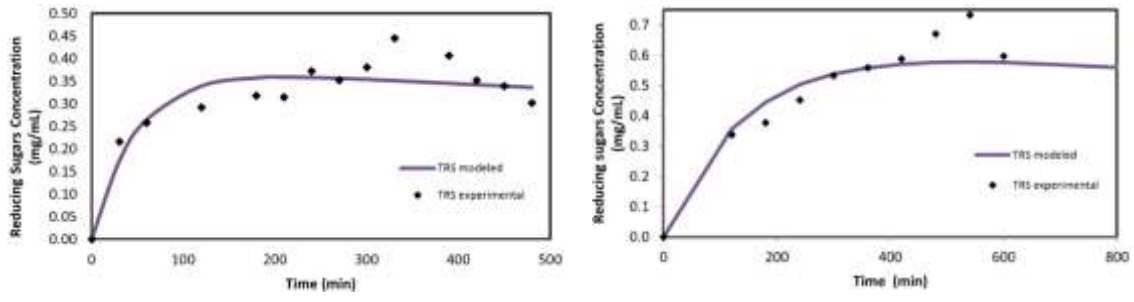
$$C_{RS} = \frac{25.32\ K_1}{K_1 - K_2}\left[(e)^{-K_1 t} - e^{K_2 t}\right) \qquad (6)$$

Table 4 shows the kinetic parameters obtained from multifunctional process route using ethanol (MSE) and methanol (MSM), where*n* is an exponential factor obtained experimentally that power the acid concentration, *A* is a pre-exponential factor, *E* is the activation energy of the reaction, *K* represents the rate constant, $X_{E/M}$ the relationship between the experimental concentration of reducing sugars obtained respect to reducing sugars concentration modeled in time and$Y_{E/M}$ is the ratio of the logarithm of the experimental concentration of RS compared to the logarithm of the concentration of reducing sugars modeled in time.

**Table 4. Modeled kinetic parameters for the microalga *Closterium* sp. using ethanol and methanol**

| Reaction System | Product | n | A ($min^{-1}$) | E (kJ/mol) | K ($min^{-1}$) | $X_{E/M}$ | $Y_{E/M}$ |
|---|---|---|---|---|---|---|---|
| **(MSE)** | **Reducing Sugars** | 0.16 | 0.05 | 15.52 | $3.02x10^{-4}$ | 1.01 | 0.99 |
| | **Degradation Products** | 0.35 | 1.13 | 14.08 | $2x10^{-2}$ | | |
| **(MSM)** | **Reducing Sugars** | 0.16 | 0.03 | 15.52 | $1.75x10^{-4}$ | 0.99 | 1.04 |
| | **Degradation Products** | 0.34 | 0.45 | 14.38 | $6.98x10^{-3}$ | | |

Through the calculated kinetic parameters, concentration of reducing sugars was modeled. As it is observed in Figure 1 and according to the model established for the MSE route, reducing sugars production reached a plateau value after 200 minutes of reaction, after this time there was a minimal degradation of sugars, which can be attributed to the role of sulfuric acid, due to it breaks the cellular walls releasing sugar molecules, but after some time of contact, these monomers are gradually degraded.

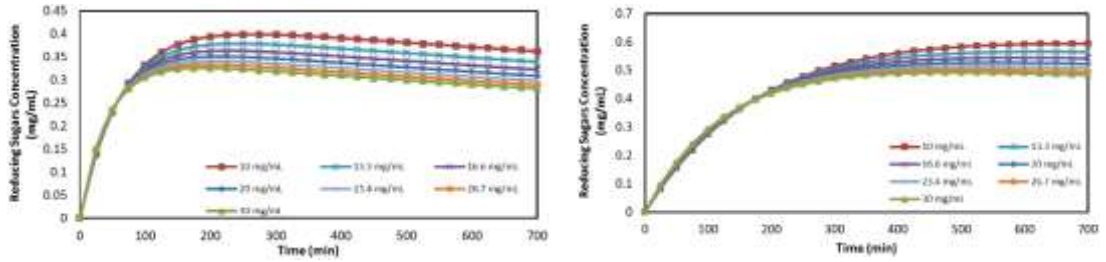



**(a)** **(b)**

**Figure 1. Modeling the production of total reducing sugars in time using a) MSE and b) MSM route**

This behavior also appears in the MSM route, the difference lay in the total reducing sugar concentration reached in the process. After 420 minutes of reaction the highest concentration of reducing sugars was reached, after this time, concentration of sugars remains relatively constant. Subsequently, free reducing sugars are degraded by action of acid.

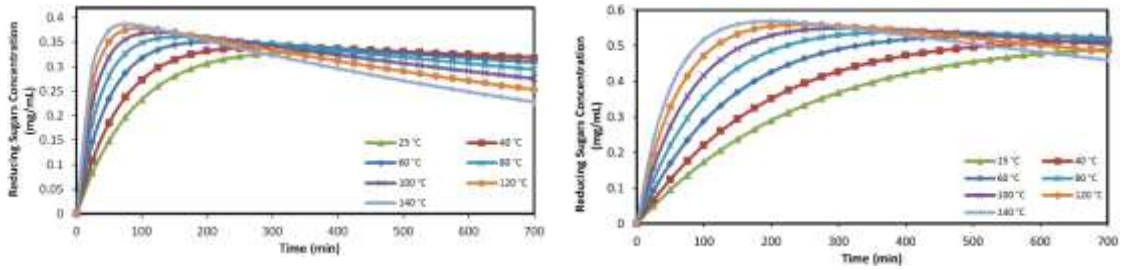
On the other hand, effect of sulfuric acid concentration on the total reducing sugars production and degradation for MSE and MSM were modeled through a sensibility analysis. According to Figure 2 (a), for MSE route, the different concentrations of acid conducted a proportional cellular disruption for 75 minutes, after this time are generated differences in the concentration of reducing sugars, so that the lowest sulfuric acid concentration(10 mg/mL) allows obtaining higher amounts of reducing sugars (0.40 mg/mL) for a time of 275 minutes of reaction. Regarding MSM system, at different concentrations of sulfuric acid, the concentration of reducing sugars during the first 175 minutes of process was similar (0.40 mg/mL, approximately). After 675 minutes, it was obtained the highest concentration of total reducing sugars (0.59 mg/mL) at 10 mg/mL of acid.Multifunctional system using methanol yielded higher reducing sugars concentrations for the same reaction time in comparison to the same system using ethanol.




**(a)** **(b)**

**Figure 21. Modeling the concentration of total reducing sugars in time, using different concentrations of sulfuric acid in a) MSE and b) MSM routes**

Influence of temperature on the concentration of total reducing sugars was also evaluated due to changes on it, increases cellular lysis significantly. However, this variable also contributes to their faster degradation. For the MSE route, Figure 3 (a) shows that higher temperatures triggered the concentration of sugars, reaching 0.39 mg/mL in 75 minutes of reaction; while Figure 3 (b) shows that using a reaction temperature of 140 °C, concentration of reducing sugars reaches a value of 0.57 mg/mL for a reaction time of 200 minutes, after this point relevant, reducing sugars begins to degrade slowly. Behavior of reducing sugars production and degradation differs drastically if reaction systems are compared, showing that the type of alcohol used also affects the shape of the curves when effect of temperature is evaluated.




**(a)** **(b)**

**Figure 3. Effect of temperature on the total reducing sugar production in the a) MSE and b) MSM route**

Multifunctional system model results shows that temperature effect is more significant than acid concentration effect, allowing to obtain higher reducing sugars amounts in less time, however, degradation products concentration is also increased at long times when temperature is increased, for this reason is recommended to stop the reaction when plateau value is reached, for all cases studied, is clear that use of methanol as solvent is more convenient than the use of methanol for a multifunctional reaction system focused on reducing sugars production.

**Simulation of emergent chains**

In thefirst stage of microalgal bioethanol purification, conventional distillation was used in order to increase ethanol concentration to 50%, followed by a rectification column were ethanol purity of 91.4% was reached, finally, extractive distillation with glycerol was used to obtain a final bioethanol concentration of 99.5 %. Table 5 shows the specifications of output streams for fermentation steps in each route. Simulation results of each technological alternative for microalgal bioethanol production shows that for a lipid extraction flow of 34 t/h, SSCF, SSF and SHF routesallowed toobtain a bioethanol flow of 8,028 kg/h, 6,840 kg/h and 6,290 kg/h, respectively, which corresponds to bioethanol yields of 23.6%, 20.1% and 18.5%.

**Table 51.Composition of product streams for routes evaluated in microalgal bioethanol simulation**

| **Streams** | **Stages** | | |
|---|---|---|---|
| **SSCF Route** | | | |
| Mass flows (kg/s) | | Fermentation | Bioethanol separation |
| Total mass flow | | 56.66 | 2.26 |
| Bioethanol flow | | 2.74 | 2.23 |
| Water | | 47.52 | 0.03 |
| Xylose | | 0.01 | 0.00 |
| Hemicellulose | | 0.00 | 0.00 |
| Cellulose | | 0.00 | 0.00 |
| Glucose | | 0.01 | 0.00 |
| Oxygen | | 0.02 | 0.00 |
| Ammonia | | 3.60 | 0.00 |
| Carbon Dioxide | | 2.60 | 0.00 |
| *Z. mobilis* | | 0.66 | 0.00 |
| *S. cerevisiae* | | 0.45 | 0.00 |
| Temperature (K) | | 314.15 | 315.31 |
| **SSF Route** | | | |
| Mass flows (kg/s) | | Pentoses fermentation | Hexoses fermentation |
| Total mass flow | | 73.86 | 7.78 |
| Bioethanol flow | | 0.76 | 0.33 |
| Water | | 70.16 | 6.61 |
| Xylose | | 0.07 | 0.00 |
| Hemicellulose | | 0.00 | 0.00 |
| Cellulose | | 0.00 | 0.00 |
| Glucose | | 0.00 | 0.01 |
| Oxygen | | 0.01 | 0.00 |
| Ammonia | | 0.25 | 0.50 |
| Carbon Dioxide | | 0.72 | 0.31 |
| *Z. mobilis* | | 0.10 | 0.00 |
| *S. cerevisiae* | | 0.00 | 0.41 |
| Temperature (K) | | 308.15 | 308.15 |
| **SHF Route** | | | |
| Mass flows (kg/s) | Hydrolysis | Pentoses fermentation | Hexoses fermentation |
| Total mass flow | 92.69 | 62.69 | 31.56 |
| Bioethanol flow | 0.00 | 1.25 | 0.63 |
| Water | 83.49 | 58.63 | 25.13 |
| Xylose | 2.74 | 0.11 | 0.03 |
| Hemicellulose | 0.01 | 0.00 | 0.00 |
| Cellulose | 0.00 | 0.00 | 0.00 |
| Glucose | 1.34 | 0.00 | 0.02 |
| Oxygen | 0.00 | 0.01 | 0.00 |
| Ammonia | 0.00 | 1.35 | 1.35 |
| Carbon Dioxide | 0.00 | 1.18 | 0.60 |

| | | | |
|---|---|---|---|
| *Z. mobilis* | 0.00 | 0.07 | 0.00 |
| *S. cerevisiae* | 0.00 | 0.00 | 1.25 |
| Temperature (K) | 394.15 | 303.15 | 308.15 |

According to simulation results, SSCF route shows the highest efficiency of microalgal ethanol production, in addition, acid hydrolysis shows lower efficiencies in terms of reducing sugars production in comparison to obtained data from enzymatic hydrolysis, this can be explained by the selectivity of enzymes in comparison to acid hydrolysis reaction which presents low efficiencies in cellulose hydrolysis.The studies previously carried out by the authors using the energy integration methodology showed that the SSCF technology presents the highest yield of bioethanol (24.1 %) and low energy requirements after energy integration[17].

## Conclusions

A holistic-hierarchical approach was used for decision-making in the improvement of the microalgae biomass utilization chain. In a first level, the experimentation was used as primary source of information to choose a promissory strain. Fatty acid composition of lipids extracted reveals that despite neither *Closterium* sp. nor *Nannochloropsis* sp. oil satisfies completely the parameters for a good quality biodiesel, oil of *Closterium*sp. is more suitable for biodiesel production taking into account percentage of monounsaturated fatty acids, saturated fatty acids, trienoic fatty acids and very long chain fatty acids.For acid hydrolysis-solvent extraction (HSE) route, the hydrolysis and extraction time had a positive effect on microalgae oil release, therefore, for 120 minutes of hydrolysis and a solvent extraction time of 16 hours. Implementation of Organosolv pretreatment-solvent extraction route (OSE) presented a lipid extraction efficiency of 94% and a reducing sugars yield of 1.47 mg/mL, so it can be concluded that the presence of an organic solvent and the increase of temperature in cell disruption stage produces higher process efficiencies. Alkyl esters production in MSE and MSM routes was confirmed by FTIR experiments by comparing microalgae crude oil, palm oil, petrodiesel and hydrophobic phase obtained after performing multifunctional system experiments ratifying the potential of this alternative for obtaining multiple products in the same volume unit, however, separation of extracted and/or transformed microalgae components must be studied and could decrease the viability of this route.In a second level, modeling of multifunctional-system routes using ethanol (MSE) and methanol (MSM) to obtainmonosaccharaidesshowed a similar behavior, however reducing sugars concentration peak is reached faster in MSE system. Sensitivity analysis of reactions modeled shows that lower concentration of sulfuric acid and higher temperatures increases the production of reducing sugars.Finally, at the third level, and to offer the holistic characteristic to the approach used, simulation was used to describe the industrial scale behavior of emerging technologies based on the fermentation of reducing sugars and the separation of the products obtained by three alternatives for the bioethanol production. Simultaneous saccharification and co-fermentation SSCF technology shows the highest performance, while the separate hydrolysis and fermentation SHF show the lowest yield of bioethanol.

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