**Environmental analysis of *Spirulina* cultivation and biogas production using experimental and simulation approach.**

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**Highlights**

* *Spirulina* growth is experimentally studied in both batch and continuous systems.
* Monod kinetic model describes well *Spirulina* growth.
* First order kinetic model is used to describe anaerobic digestion of *Spirulina*.
* Scaling up of biogas production from *Spirulina* is carried out by simulation.
* Nutrient production processes present the highest environmental impacts.

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**Abstract**

Microalgae is constituted by different compounds, interesting for the production of a wide range of end-products by using different technologies. Many potential possibilities have been developed under the context of a biorefinery. The aim of this work is to evaluate the environmental performance of biogas production from *Spirulina* (*Arthrospira maxima*) through LCA using experimental and simulation results. For this purpose, kinetic models for batch cultivation and anaerobic digestion (AD) were determined from experimental data. Thus, Monod kinetic model and a first order model describe well microalgal biomass growth and AD, respectively. This model was used to simulate growth of *Spirulina* in a continuous system by using SuperPro Designer 9.5. Calculated results were compared to continuous experimental ones, obtaining good agreement in all cases. On the other hand, the whole process (cultivation, dewatering and AD of *Spirulina* biomass) was also simulated and the obtained results (material and energy balances) were used to construct LCA inventory data. Thereafter, environmental impacts were quantified through CML-2001 methodology using software Gabi 6.0. LCA results show that abiotic depletion of fossil resources (ADFR) category presents the highest impact, being biomass cultivation the most important contributor (about 56%). This result is directly related to the high energy consumption required for nutrient production, which also leads to increase remarkably the global warming potential (GWP) category. Main conclusion of the work is that the total/partial substitution of mineral fertilizers as nutrient source is the key to improve the environmental performance of the studied process. In this sense, a potential alternative could be the use of nutrients from wastewater or other wastes.

**Keywords**

Microalgae; *Spirulina*, biorefinery; LCA; biogas.

1. **INTRODUCTION**

Biofuels from clean and renewable bioresources have been widely studied as an alternative to fossil fuels in the last decade [1,2]. In this context, microalgae appears as an interesting option because it is an autotroph microorganism that can accumulate lipids, can be grown in non-arable land and their cultivation does not compete with food production [2–4]. Furthermore, this microorganism can fix CO2 from air by the photosynthetic mechanism similar to the behavior of higher plants, thus reducing carbon dioxide emissions [3,5]. On the other hand, compounds present in microalgae composition, such as carbohydrates, protein, vitamins, minerals, carotenoids, long-chain omega-3 fatty acids, phytonutrients, etc, can be also interesting from an industrial point of view [6]. Thus, as recently reported, the use of these compounds for the production of a wide range of end-products (valuable chemicals, human and animal food, etc.) is potentially possible, although a number of drawbacks should be overcome [7,8]. Therefore, regarding to the desired product, different schemes based on bio-refinery approach can be used to optimize the use of microalgal sources. These processes consist of a first biomass fractionation step and a further stage in which separated compounds are transformed into a variety of products including biofuels, biomolecules, biomaterials and food [9,10]. Although there are many technologies to convert microalgal biomass, they can be classified in two groups: thermochemical processes (direct combustion for energy production, gasification for syngas production, liquefaction for biofuel production, pyrolysis for bio-oil production, etc.) and biochemical processes (anaerobic digestion for biogas production, alcoholic fermentation for bioethanol production, transesterification for biodiesel production, etc.) [11].

By taking into account energy related products, biofuels obtained from microalgae has been widely studied [5,12,13]. These works have concluded that current processes must be improved to become competitive because of their large energy requirements. Biogas production, combined biodiesel and biogas production; or extraction of valuable products including biogas production from resulting residues have been reported to improve energy performance of microalgae processing. [14–16]. Therefore, biogas production appears as an ideal option to be included in microalgae transformation and use processes.

Biogas production is carried out by anaerobic digestion of algal biomass, which consists of the conversion of organic matter in the presence of methanogenic bacteria, obtaining biogas (mainly composed of methane and carbon dioxide) and a solid waste (digestate). The obtained methane can be used for different purposes, such as electricity production, fuel for internal combustion engines, etc. [11,17,18].

The use of different microalgae for biogas production has been widely reported in the literature. Thus, Jankowska et al., [19] studied different algal biomass (*Scenedesmus*, *Chlorella*, *Nannochloropsis, Spirulina*, etc.). Capson-Tojo et al., [20] evaluated *Nannochloropsis gaditana* biomass after the lipid extraction to obtain biogas using a bio-refinery scheme. Ramos–Suárez et al [21] analyzed *Scenedesmus* extracted residues as substrates for methane production. Microalgae *Spirulina* has been describedin literature as an attractive substrate for anaerobic digestion process because of its high growth rate, and fermentability, superior to other microscopic algae [22,23].

One of the key points of these processes is the evaluation of their environmental and energy performance. In this sense, Life Cycle Assessment (LCA) methodology is a suitable tool to quantify positive or negative impacts of processes and products according to different environmental categories. This methodology allows a systematic estimation of the environmental changes related to the examined process, quantification of consumptions and emissions and their effects on human health, eco-systems and resources depletion. There are some LCA studies focused on biogas production from microalgae [17,24]. However, they are mostly based on literature data.

The aim of this work is to evaluate the environmental performance of biogas production from *Arthrospira maxima* through LCA using experimental and simulation results. For that purpose, kinetic models for cultivation and anaerobic digestion of *Arthrospira maxima* were determined from experimental data. Monod kinetic model was found to describe well microalgal biomass growth. On the other hand, experimental data on biogas production were fitted to a first order kinetic model. Thereafter, the whole process, including cultivation, dewatering and anaerobic digestion of *Spirulina* biomass, was simulated by using SuperPro Designer 9.5. This allows the scaling-up of the process. Besides, material and energy results obtained by simulation were used to construct LCA inventory data. Finally, environmental impacts were quantified through CML-2001 methodology using software Gabi 6.0.

1. **METHODS**
   1. **Microalgae cultivation**
      1. *Batch experiments*

Batch cultivation experiments were carried out in 50 ml photo-bioreactors (schematically represented in Figure 1a). F/2 Guillard medium (provided by Algaenergy) was used to supply the required nutrients. CO2 was fed into the culture solution at a flow rate of 3.5 l/min. These conditions ensure an excess of CO2 in the cultivation medium during the whole experiment according to theoretical CO2 fixation calculated in literature [25–27]. Light requirements were provided by white LED lamps (12 V/24 W) under an irradiance of 108µmol·photons·m-2s-1.

Different inoculum:culture medium ratios and light:darkness photoperiods were experimentally tested. In this work, inoculum:culture medium ratio of 5:45 (vol./vol.) and light:darkness photoperiod of 12:12 h. were selected as the optimal values of both parameters. The distance between the LED light panel and culture medium was 2 cm. These conditions were selected as the optimal ones according to our experimental previous work [28].

*Spirulina* algal cultivation was carried out at 20 ºC and pH=9.4. The biomass growth was monitored by taking culture samples each 24 h. Absorbance of these samples were measured by using a JASCO V-630 spectrophotometer at λ = 540 nm. A calibration between absorbance and biomass concentration was used.

* + 1. *Continuous experiments*

Continuous culture experiments were performed in a 12 L stirred reactor (Bioflo 110, New Brunswick Scientific Co., Inc.) by using experimental conditions previously optimized in batch experiments (as is schematically represented in Figure 1b). A reactor volume of 10 L was filled with a solution containing inoculum:culture medium ratio and using the light:darkness photoperiod above mentioned. CO2 was supplied at flow rate of 3.5 L/min only during light periods. In all the experiments, the system was stirred at 150 rpm.

In continuous experiments, the biomass was firstly grown until finishing the exponential period according to batch results. Thereafter, the continuous experiment was started by feeding the culture medium solution (Guillard's F/2) at different flow rates (0.05, 0.1, and 0.2 L/h). Biomass concentration in the reactor output stream was measured each 24 h until obtaining similar results between consecutive measurements, thus ensuring steady state conditions.

a) b)

*Figure 1. Scheme of batch (a) and continuous (b) experimental systems.*

* + 1. *Elementary analysis.*

Elemental composition of cultivated biomass in batch reactors was determined by using an Elemental analyser (Vario EL III CHNS, Elementar Analysensysteme GmbH, Germany). The method used was that based on the sulphanilic acid standard.

* + 1. *Nitrate content determination.*

SAN++Skalar nutrient autoanalyzer was used to determine the nitrate concentration into cultivation medium. Samples were pre-filtered, using a 0,45 µm nylon filter, before being analyzed by means of a colorimetric method.

* 1. **Anaerobic digestion**

Data of biogas production used to determine the kinetic model for Spirulina anaerobic digestion (AD) were taken from a previous work [29]. In this work, author used the primary sludge of a municipal wastewater treatment plant as inoculum and the digestions are performed at mesophilic conditions (35ºC) in reactors of 1.2 L of capacity containing 0.7 L of liquid phase.

* 1. **LCA methodology**
     1. *Goal and scope definition*

This study is focused on evaluating the main environmental impacts related to biogas production by anaerobic digestion of *Arthrospira maxima*. The selected functional unit for this analysis has been 1 kg of dry biomass generated in a scaled plant with a production capacity of 1 kg of dry biomass per hour.

* + 1. *System boundaries*

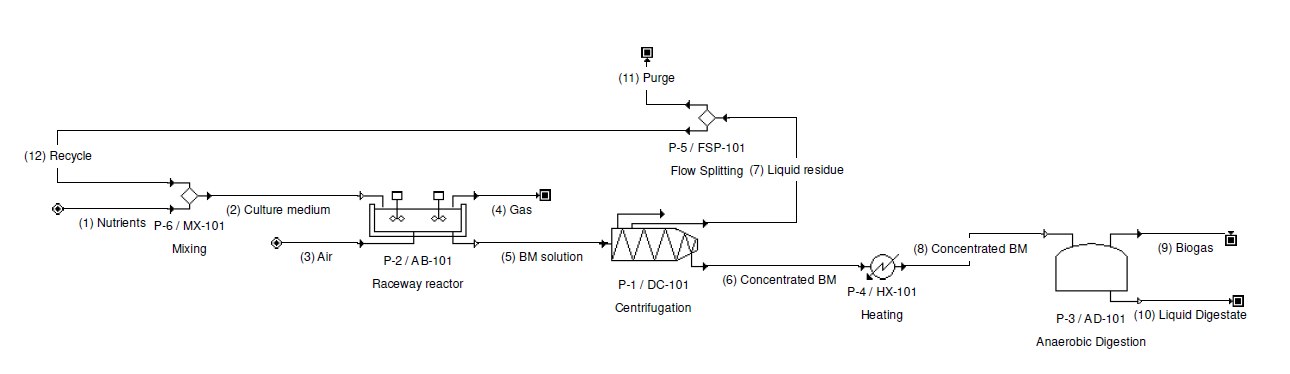
Figure 2 shows an overview of the system considered, from the cultivation of the microalgae to biogas production through anaerobic digestion. All the inputs and outputs of the processes were considered within the system boundaries. Capital goods are excluded of the study.



*Figure 2. System boundaries of the evaluated process*

* + 1. *Life Cycle Inventory*

The process shown in Figure 2 was simulated by using SuperPro Designer 9.5. For that purpose, experimental results, obtained from batch experiments for both biomass cultivation and biogas production, were used to determine kinetic models. Thus, the process was simulated (see scheme in Figure 3) and scaled up to plant conditions. Material and energy results obtained by simulation were used to construct inventory data of the system.



*Figure 3. Flow diagram used for the simulation of the evaluated process.*

Microalgae cultivation. *Arthrospira maxima* is cultivated in open raceways by using a F/2 culture medium, as explained before. Although the growth rate of microalgae in these systems is lower than in photobioreactors, the economic cost of raceway ones is remarkably lower [17]. They are modelled using the Monod kinetic parameters calculated from experimental batch results. According to these results, residence time used for cultivation stage was 200 h (to ensure that exponential growth step is finished). With this residence time and taking into account mass flows required for the production of 1 kg of dry biomass per hour, the volume of this open raceways reactor is 1129 m3. As a result of the cultivation process, biomass and gases are obtained as displayed in Figure 3.

Biomass dewatering*.* In this step the water content of the cultivated biomass solution is reduced until the limit required by the further anaerobic digestion. There is a wide variety of technologies to carry out algal biomass concentration [30]. In this work, dewatering process is simulated by using a centrifugation step following similar schemes of biogas production available in the literature [17]. According to data reported elsewhere, the anaerobic digestion process does not require severe drying, since it reduces the methane yield. For that reason, solid concentration of microalgal solution must be within 4-6% (wt.) [30]. In this work, solid concentration of 5% (wt.) is fixed as target value in the simulation of the centrifugation step. As can be seen in Figure 3, the aqueous solution removed in the dewatering process is partially recirculated to the raceway reactor in order to recover nutrients.

Anaerobic digestion*.* Dewatered biomass is anaerobically digested in a digester working in the mesophilic temperature range (35 °C). The temperature conditions are the most adequate for algal biomass, as reported in the literature [30]. Residence time of 30 days is selected according to our experimental data on methane production. In these conditions, volume for the anaerobic digestion of 1 kg of dry biomass per hour is 1220 m3, yielding biogas (with methane content of 55 vol. %) and liquid digestate as products.

*2.3.4. Life Cycle Impact Assessment (LCIA).*

The evaluation of environmental impacts related to the process was carried out by using a mid-point methodology. This allows obtaining an environmental profile of a process/product through the quantification of environmental effects on different impact categories (global warming potential, abiotic depletion of resources, etc.). In this case, CML 2001 (nov. 2010) methodology was used since it considers most usual impact categories, and it has been previously applied to similar processes [17]. Abiotic depletion of fossil resources (ADFR), global warming potential (100 years) (GWP), freshwater aquatic ecotoxicity potential (FAEP) and human toxicity potential (HTP) were analyzed in this work. Besides, the total energy requirements of the process was quantified by means of cumulative energy demand (CED). This parameter represents the direct and indirect energy use throughout the life cycle (including the energy consumed during the extraction, manufacturing and disposal of the raw and auxiliary materials).

In this work, LCIA was carried out by means of Gabi 6.0 software using the inventory data above commented.

1. **RESULTS**

In this section, experimental results for microalgae cultivation and biogas production are presented and used for kinetic parameters calculation. These parameters were implemented into a bio-refinery scheme and the environmental and energy performance of the process was evaluated by LCA methodology.

* 1. **Kinetic parameters for *Spirulina* growth**

In order to determine the kinetic parameters of *Spirulina* growth, experimental data of biomass (X) production and substrate (S) consumption coming from batch experiments were adjusted by using the Monod equation. According to this model, the biomass production rate during the exponential period can be calculated as follows:

(Equation 1)

Whereas the consumption rate for the limiting substrate can be obtained by the Equation 2:

(Equation 2)

Where Y represents the yield of the substrate to biomass:

(Equation 3)

For applying Monod model, it is mandatory to define the limiting substrate, which depends on the elemental biomass composition and the nutrients amount used during growth experiments. Table 1 shows elemental analysis results corresponding to dry biomass obtained after 7 days of cultivation (once exponential stage has concluded). According to these values, the proposed molecular formula for *Spirulina* dry biomass was C13H26NO9 (molecular weight = 340 g/mol).

*Table 1. Elemental analysis results of dry biomass (after 7 days of cultivation)*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Composition | H | C | N | S | O(\*) |
| Weight (%) | 7.56 | 47.30 | 4.17 | 0.70 | 40.27 |
| Mol (%) | 7.56 | 3.94 | 0.30 | 0.02 | 2.52 |

(\*) Calculated by difference: 100-H-C-N-S

As explained in experimental section, an excess of carbon dioxide was used for all the cultivation experiments (in both, discontinuous and continuous reactors) leading to nitrogen as the limiting element for *Spirulina* growth. Since nitrogen is provided as nitrate (coming from NaNO3 of the F/2 medium) and one mol of biomass requires one mol of nitrate, the parameter Y calculated by applying the Equation 3 for the limiting substrate is:

Figure 4 (a) shows the evolution of *Spirulina* biomass concentration with the cultivation time starting from an inoculum of 0.19 g/L. As observed, production rate drastically decreases above 6 days, indicating that the exponential period has finalized. Therefore, from 1 to 5 days *Spirulina* biomass growth can be described according to the exponential equation obtained by integration of the Equation 1. Besides, if we assume that substrate limitations are negligible during this period the integrated expression will be:

Thus, data of biomass concentration and cultivation time can be adjusted for max calculation. Figure 4 (b) displays this linear adjustment. As observed, a reasonable correlation is obtained (R2=0.945), indicating that experimental results can be adequately described by Monod model. The line slope leads to a value of max=0.011 h-1. Despite specific growth rate of algae is strongly dependent on cultivation conditions, works previously published about *Spirulina* cultivation showed max similar results [31–33].



*Figure 4. (a) Evolution of Spirulina biomass concentration with cultivation time and (b) linear adjustment for max calculation.*

On the other hand, semi-saturation constant (KS) of Monod model could be estimated by using the substrate consumption rate (Equation 2):

So, by depicting (-t/S)·X versus 1/S it is possible to calculate Ks from the slope of the obtained line. Figure 5 shows the evolution of limiting substrate concentration (NaNO3) by increasing the cultivation time (a) and linear adjustment for KS calculation (b). NaNO3 profile indicates that nitrate availability is high up to the sixth cultivation day, which confirms that between 1 and 5 days there are not substrate limitations that can affect to the specific growth rate. From the line slope of Figure 5 (b) (0.0473 h·g·L-1) and taking into account Y and max values (above calculated) the obtained semi-saturation constant is KS=3.1 mg/L. By using the y-intercept value of this graph for max calculation, we obtain a very similar value as that yielded by the integration and adjustment of Equation 1 (max=0.011 h-1 from Equation 1 and 0.0115 h-1 from Equation 2). These results indicate that Monod model can be properly used for kinetic description of our *Spirulina* cultivation results obtained in batch experiments.



*Figure 5. (a) Evolution of substrate (NaNO3) concentration with cultivation time and (b) linear adjustment for semi-saturation constant calculation.*

* 1. **Validation of *Spirulina* kinetic model: continuous cultivation experiments.**

According to the experimental section, *Spirulina* was cultivated not only in batch reactors but also in a continuous one using different flow rates (0.05, 0.1 and 0.2 L/h) and, thus, different residence time. The objective of these tests is to compare experimental values of biomass production and substrate consumption obtained in the continuous reactor with those calculated by simulating the same cultivation system with SuperPro Designer 9.5 software. For these simulations, we have used the Monod kinetic parameters previously calculated (from batch tests). If experimental and simulated results compare well, Monod kinetic parameters will be properly validated.

Figure 6 shows the comparison between experimental and simulated biomass concentration reached in the continuous reactor working with three different flow rates. It can be observed that higher flow rates leads to slightly lower biomass concentration due to the decrease of the residence time. Likewise, it must be remarked that experimental and simulated results are very similar in all the cases (differences are lower than 10 %) indicating an adequate validation of Monod kinetic parameters. Finally, it can be also noted that experimental biomass concentration is slightly lower than the results obtained by simulation in all cases. These results could are related to small irradiation differences between batch and continuous tests since the light way is not the same in both installations (2 cm for batch reactor and 5 cm for continuous one). This light effect has been previously observed for other authors by comparing different algae cultivation set-ups [34,35].



*Figure 6. Comparison of experimental and simulated biomass concentration results obtained in a continuous reactor*

* 1. **Biogas production kinetic model**

Experimental data of biogas production through anaerobic digestion (AD) of *Spirulina* coming from a previous work [29] have been used in order to determine the kinetic model. The experimental variation of methane yield with the digestion time is depicted in Figure 7. As can be observed, maximum methane yield is achieved in 30-35 days, obtaining about 400 mL CH4/g volatile solid (VS). These results are in good agreement with other reported in the literature, like those by Samson and LeDuyt (1985) [22] and El-Mashad (2013) [36]. They reported reported 350 mL CH4/g VS and 355 mL CH4/g VS, respectively for anaerobic digestion of *Spirulina* algal biomass at same digestion time.



*Figure 7. Experimental values of methane yield of Spirulina as a function of digestion time.*

In order to obtain a kinetic model describing these experimental results, the anaerobic reaction of the biomass is adjusted according to the following scheme proposed by Sialve et al. (2009) [37]:

(Equation 4)

By considering this approach and the biomass formula above indicated, the anaerobic digestion reaction is:

(Equation 5)

Kinetic parameters of this reaction can be calculated by adjusting the experimental data of methane production shown in Figure 7. For that purpose, these data were expressed in terms of substrate consumption (*Spirulina* biomass (X), in this case) by considering the VS content of the *Spirulina* biomass (0.87 g VS/g dry biomass) and the amount of produced methane per gram of VS. Obtained variation of biomass concentration results are displayed in Figure 8 (a).

These data were fitted to a first order kinetic model, expressed as follows:

(Equation 6)

which is shown in Figure 8 (b), being the calculated kinetic constant k=0.004 h-1. It must be noticed that biomass concentration does not vary significantly after 17 days (see Figure 8 (a)) and consequently these data were not considered in the model fit.



*Figure 8. (a) Variation of biomass concentration as a function digestion time. (b) Kinetic model data fit*

* 1. **Environmental performance: LCA results**

Environmental impacts of biogas production from *Spirulina* were calculated by means of CML 2001 (Nov. 2010) using Gabi 6.0 software, which incorporates Ecoinvent Database 2.2. For that purpose, inventory data of the process (inputs and outputs) are required. In this work, the life cycle inventory data were obtained by simulation of all the stages with SuperPro Designer 9.5 and reported in Table 2. Likewise, calculated environmental impacts of biogas production are depicted in Figure 9. As can be observed, abiotic depletion of fossil resources (ADFR) category presents the highest value for the studied process, being the biomass cultivation the most important contributor (about 56%). This result is directly related to the energy required for nutrients production since these processes are highly energy demanding. On the other hand, electricity needed for biomass dewatering process contributes in a remarkable way to ADFR category (about 34%), since it is necessary to remove large amount of water to increase the solid content until 5%, as required in the anaerobic digestion step. Unlike biodiesel production, the impact related to the energy consumed in the dewatering step is lower since less amount of water must be removed.

Finally, the anaerobic digestion step contributes to ADFR impact about 10%, due to heating (supplied as natural gas) necessary to increase the temperature of the biomass entering to the digester, and the electricity related to the reactor stirring. It must be noticed that the electricity used in the studied process is assumed to be supplied by Spanish electricity mix, having about 40-50% contribution of non-renewable resources. That is the reason why electricity contributes remarkably to ADFR impact.

By analyzing global warming potential (GWP) category, nutrient production process shows the largest contribution (1.61 kg CO2-eq./kg dry biomass). This result is expected, since this process implies high energy requirements as above mentioned. It must be remarked that biomass cultivation present negative contribution to GWP because CO2 is fixed in this step (1.72 kg CO2-eq./kg dry biomass), which reduce the contribution of the process to GWP category. The overall GWP impact per unit of energy output for the studied process resulted 0.06 kg CO2-eq./MJ, which is lower than those reported for biodiesel production from different microalgae evaluated in similar cultivation systems (0.1-0.5 kg CO2-eq./MJ) [38–40].

Regarding the rest of impact categories, freshwater aquatic ecotoxicity potential (FAEP) and human toxicity potential (HTP), nutrients production process exhibits the largest contribution (82% and 86%, respectively) due to the release of heavy metals, mainly vanadium and chromium.



*Figure 9. Environmental impacts calculated by CML-2001 methodology.*

Energy performance of biogas production from *Spirulina* was evaluated by means of the Net Energy Ratio (NER), expressed as follows:

(Equation 7)

Input energy is calculated by means of Cumulative Energy Demand (CED), which represents the direct and indirect energy use throughout the life cycle (including the energy consumed during the extraction, manufacturing and disposal of the raw and auxiliary materials). CED is calculated by using Gabi 6.0 obtaining a value of 27.6 MJ/kg dry biomass. Energy output of the process is determined according to the amount of methane produced. In this work, 0.254 kg CH4/kg dry biomass are obtained from the anaerobic digestion process. By considering a calorific value of 42 MJ/kg for CH4, the energy produced in the studied process is 10.67 MJ/kg dry biomass. From calculated input and output energy values, the obtained NER is 0.4, which indicates the need to reduce the energy requirements, mainly related to nutrient production processes. Obtained NER value is higher than that reported in literature for biodiesel production from microalgae in similar cultivation conditions (0.1-0.2) [38,40].

As overall conclusion of the obtained results, it can be stated that the improvement of the environmental and energy performance of the studied process requires mandatorily the total/partial substitution of mineral fertilizers as nutrients source for *Spirulina* biomass cultivation. In this sense, a potential alternative could be the use of nutrients contained in wastewater.

*Table 2. Material and energy balance results obtained by simulation.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Variable*** | ***Operation*** | | | | | |
| *Algae cultivation* | | *Centrifugation* | | *Anaerobic digestion* | |
| *INPUTS* | | | | | | |
|  | Medium culture | Air | Biomass  solution | | Concentrated biomass | |
| T (°C) | 25 | 25 | 25 | | 35 | |
| P (bar) | 1 | 1 | 1 | | 1 | |
| Total mass flow  (kg/h) | 1706.23 | 5896.73 | 1706.39 | | 20.29 | |
| Composition  (wt.%) | NaH2PO4:0.021  NaNO3: 0.015  H2O: 99.964 | N2: 76.862  O2: 23.100  CO2: 0.038 | BM: 0.060  Na2HPO4: 0.025  NaH2PO4 < 0.001  H2O: 99.915 | | BM: 5.033  Na2HPO4: 0.012  NaH2PO4 < 0.001  H2O: 94.955 | |
| Energy (MJ) | 0.76 | | 3.31 | | 113.93 | |
| *OUTPUTS* | | | | | | |
|  | Biomass solution | Gas | Concentrated biomass | Liquid residue | Biogas | Liquid digestate |
| T (°C) | 25 | 25 | 25 | 25 | 35 | 35 |
| P (bar) | 1 | 1 | 1 | 10 | 1 | 1 |
| Total mass Flow  (kg/h) | 1706.39 | 5896.57 | 20.29 | 1686.10 | 0.83 | 1678.63 |
| Composition  (wt. %) | BM: 0.060  Na2HPO4: 0.025  NaH2PO4 < 0.001  H2O: 99.915 | N2: 76.864  O2: 23.127  CO2: 0.009 | BM: 5.033  Na2HPO4: 0.012  NaH2PO4 < 0.001  H2O: 94.955 | Na2HPO4: 0.025  NaH2PO4 < 0.001  H2O: 99.975 | CH4: 30.656  CO2:69.344 | BM: 1.350  Na2HPO4:0.012  NaH2PO4 < 0.001  NH3: 0.195  H2O: 98.440 |

1. **CONCLUSIONS**

Kinetic models for batch cultivation (Monod model) and anaerobic digestion (first order model) were determined from experimental data. Cultivation kinetic model was validated by simulating continuous cultivation experiments, obtaining differences lower than 10% respect to experimental results.

Environmental and energy performance of the studied process was carried out by LCA. Inventory data were obtained by simulation. Environmental impact results show that abiotic depletion of fossil resources is the most affected category due to the high energy required for nutrient production. This process also presents the largest contribution to global warming potential. Consequently, the total/partial substitution of mineral fertilizers as nutrient source is key to improve the environmental performance of the studied process.

Biogas production shows a better energy performance than biodiesel production, since lower GWP impact and higher NER value were obtained.

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