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Technoeconomic Evaluation of Microalgae Oil Production: Effect of Cell Disruption Method

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Abstract: Microalgae have a high capacity to capture CO_2. Additionally, biomass contains lipids that can be used to produce biofuels, biolubricants, and other compounds of commercial interest. This study analyzed various scenarios for microalgae lipid production by simulation. These scenarios include cultivation in raceway ponds, primary harvest with three flocculants, secondary harvest with pressure filter (and drying if necessary), and three different technologies for the cell disruption step, which facilitates lipid extraction. The impact on energy consumption and production cost was analyzed. Both energy consumption and operating cost are higher in the scenarios that consider bead milling (8.79–8.88 kWh/kg and USD 41.06–41.41/kg), followed by those that consider high-pressure homogenization (HPH, 5.39–5.46 kWh/kg and USD 34.26–34.71/kg). For the scenarios that consider pressing, the energy consumption is 5.80–5.88 kWh/kg and the operating cost is USD 27.27–27.88/kg. The consumption of CO_2 in scenarios that consider pressing have a greater capture (11.23 kg of CO_2/kg of lipids). Meanwhile, scenarios that consider HPH are the lowest consumers of fresh water (5.3 m^3 of water/kg of lipids). This study allowed us to develop a base of multiple comparative scenarios, evaluate different aspects involved in *Chlorella vulgaris* lipid production, and determine the impact of various technologies in the cell disruption stage.

Keywords: microalgae oil; cell disruption method; bioprocess simulation; techno-economic evaluation; energy demand; production cost

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1. Introduction

Microalgae biomass has become a source of commercial interest for various industries because it accumulates significant amounts of lipids, carbohydrates, and proteins. It is also an important source of other compounds such as pigments and vitamins of great economic interest for the pharmaceutical and cosmetics industries. Various studies have found diverse applications as food additives or food supplements. In addition, microalgal biomass can be used in the production of bioenergy or biolubricants, as well as being useful in capturing CO_2 and treating wastewater [1–4]. Due to the problem of clean water disposal and the increase in energy demand, there is a great need to search for sustainable processes for wastewater treatment, carbon sequestration methods, and sustainable fuels. In this way, researchers have considered microalgae in an integrated system in which wastewater and gases emitted by the same industry could be treated simultaneously, from which value-added raw material is produced [5]. Compared with fossil fuels, microalgae-derived biofuels can effectively reduce CO_2 emissions and could help maintain the balance between CO_2 generation and consumption [6]. Industrial, municipal, and agricultural wastewater have a large amount of organic and inorganic pollution and heavy metals. The idea of cultivating microalgae using wastewater could meet the goal of reducing biological oxygen demand, total suspended solids, pathogenic organisms, and nitrogen and phosphorous concentration; in turn, the biomass produced can be raw material to produce lipids and biofuels [7].

Microalgae are unicellular organisms, with the presence of chlorophyll and other pigments that help them carry out photosynthesis. During the photosynthesis process, CO_2 is used as the carbon source to be converted into organic compounds through the use of solar energy. It has been reported that lignocellulosic biomass captures approximately 0.77 kg of CO_2 per 1 kg of biomass produced [8], with which it is estimated that terrestrial plants only contribute 3–6% in the reduction of global CO_2 emissions [9,10]. Microalgae are capable of fixing up to 1.8 kg of CO_2 per 1 kg of biomass produced, being more efficient than terrestrial plants, due to their high photosynthetic capacity and growth rate [1,11–14]. In a previous study, a capture of 102.13 ton/year of CO_2 in 1 ha was estimated as a surface area for the cultivation of microalgae *Chlorella vulgaris* in a raceway pond [15]. Similarly, Gonçalves et al. report a capture of 110 tons of CO_2/year in 1 ha of cultivation [16]. Premalatha et al. report that an approximately 40 ha algal pond is required for mitigating the CO_2 emitted from 1 MW coal based power unit, at 50% capture efficiency [17]. Behera et al. reported the requirements of 279 ha algal ponds to capture the flue gas from the 240 MW power plant in Odisa [18]. CO_2 capture depends on the selected microalgae species, biomass productivity, and capture efficiency. Additionally, one of the advantages of microalgae over other types of biomass is that they do not belong to edible crops and do not compete for the use of arable or forest land, since they do not require fertile land for their cultivation, guaranteeing food security and better land use [1,19]. This has motivated the interest in using microalgae for various purposes, as well as being used as a system to reduce CO_2 emissions into the atmosphere.

Microalgae biomass is typically rich in lipids and can be produced throughout the year [19]. It has been reported that these can produce up to 70% of oils with respect to their dry weight in biomass, compared to other oil crops such as palm (36%), sunflower (40%), and jatropha (28%) [2,20], turning microalgae into a potential source for the production of lipids, and a source of biofuels, biolubricants, or other types of compounds of commercial interest.

Most of the research that analyzes the production or use of microalgae oils has focused on their use as a source for the production of biofuels (biodiesel, biogas, biohydrogen, bioethanol, biobutanol, among others [21]); however, it has been reported that these are not yet economically competitive with respect to conventional fuels [22,23]. Faried et al. report that the estimated minimum sales price for microalgal biocrude was USD 18.35/gal and microalgal biodiesel was USD 21.11/gal, whereas the price of similar fossil fuels in 2013 was USD 2.33/gal for fossil crude oil and USD 3.91/gal for fossil diesel [22]. Recently, techno-economic analyses and life cycle assessments of microalgae-based production systems have suggested that the only possible way to increase production is to fully utilize biomass in an integrated refinery configuration in which each component is extracted, processed, and valorized [21]. On the other hand, Hussaun et al. comment that the simultaneous use of microalgae for wastewater treatment and biofuel production has made these challenges feasible and economically viable [6]. The similarity in the chemical structure of the lipids of microalgae (which are mostly triglycerides, similar to vegetable oils) with the long-chain hydrocarbons contained in mineral lubricants, makes microalgae a potential source for the replacement for the widely used mineral-based lubricating oils that are commonly made from petroleum, coal, or natural gas [24,25].

Despite the great use of microalgae, there are many challenges in the development of technology for the use of this biomass. When bulk microalgae biomass is not the desired end product, it must undergo additional processing to obtain one or more cell fractions. The simplest option is to carry out cell lysis to release internal compounds, such as lipids and pigments contained in the cytoplasm and carbohydrates stored in the rigid cell wall; this leads to cell disruption being necessary for the extraction of oils. Some of the most common methods for extracting oil from microalgae are mechanical, chemical, and biological and they are used for cell lysis [26,27]. Oil extraction methods are discussed below.

Mechanical methods are widely used for the extraction of oil from microalgae, since the cell wall is broken through physical force, allowing the oil to escape. The efficiency of this technique depends on the microalgae species, for example, in *Spirulina* sp., cell rupture is easy since it does not have a rigid cell wall, while *Chlorella* sp. has a rigid cell wall [27]. Mechanical destruction can be carried out by various techniques, such as ultrasound, bead milling, microwaves, high pressure homogenization, pressing, among others [28,29]. The performance and energy consumption are different in all cell disruption treatment methods, and some of the techniques are only effective for certain species of microalgae.

Cell wall disruption can also be induced by chemical methods, commonly using chloroform, hexane, benzene, acids, and bases as solvents for extraction. Chemical extraction can be performed on dry or wet biomass, but there is greater efficiency in the extraction of dry biomass. However, drying and the high consumption of chemical products can modify the composition. For example, drying at 60 °C still retains a high concentration of triacylglycerides (TAG) in the lipids and only slightly decreases lipid yield, whereas higher temperatures decrease both TAG concentration and lipid yield [1]. On the other hand, solvent extraction may deem the residual biomass unfit for use as animal feed [30]. Another alternative is extraction with supercritical CO_2, which has several advantages: the extraction efficiency is high; it allows processing at low temperatures; it is non-toxic, non-flammable, and the supercritical solvent can be easily removed [23]. However, supercritical extraction equipment is expensive and operating costs are also high, compared to extraction with organic solvents [28].

The use of a single extraction method may not be enough to obtain the maximum yield of the extracted oil. Therefore, the use of pretreatments, such as ultrasound or microwave, is recommended to improve the extraction of microalgal lipids [31]. It has been reported that the combination of pressing and chemical extraction can increase lipid yield up to 75% by weight [32].

The choice of cell disruption method depends on the cell wall structure of the microalgae species, the location of the product of interest, its size, and the energy applied [29]. For a given intracellular product, an ideal cell disruption method is one that selectively releases the target product while using as little energy as possible. On the other hand, the method used for extraction must be fast, easily scalable, efficient, and must not damage the extracted lipids. Biomass drying consumes a lot of energy, therefore, applying a methodology to extract lipids from microalgae by wet means can reduce this energy consumption [28].

The main objective in this work was to evaluate technically and economically three pretreatments to cause cell disruption: bead milling, high-pressure homogenization (HPH), and pressing for the extraction of oils. These techniques were considered because the literature review indicates that the most frequently used techniques are mechanical methods. Subsequently, extraction techniques can be applied with organic solvents and the extraction efficiency can be increased. Some of the pretreatment techniques do not require dry biomass, as is the case with milling and HPH. Therefore, the wet biomass is fed, and it is believed that this could be convenient to avoid the cost of drying operation in the scenarios that consider these techniques. However, those scenarios that consider the case of pressing as a pretreatment of cell disruption require dry biomass. Additionally, the consumption of CO_2 and fresh water per kg of lipids produced in the different production scenarios was analyzed.

2. Materials and Methods

2.1. Production Scenarios

For this study, three dry biomass production scenarios of the *Chlorella vulgaris* microalgae that have been evaluated in a previous study were considered as the basis [15], being those with the lowest unit cost of dry biomass production and the lowest energy consumption, Table 1. These base scenarios include open system cultivation (raceway pond, A), biomass harvesting by flocculation (three types of flocculants, B1, B2, B3), pressure filter (C), and the drying of the biomass in a drum dryer (D) [15]. These three scenarios were combined with technologies for cell disruption, bead milling (E1), high-pressure homogenization, (HPH, E2), and pressing (E3), resulting in nine scenarios for the production of microalgae oils (Figure 1, Table 2).

Table 1. Technical information of the base scenarios for the production of biomass of microalgae [15].

Scenario	Energy Consumption (kWh/kg [1])	Unit Production Cost (USD/kg [1])
A-B1-C	0.94	4.48
A-B2-C	0.93	4.37
A-B3-C	0.93	4.44
A-B1-C-D	0.97	4.75
A-B2-C-D	0.96	4.81
A-B3-C-D	0.96	4.87

[1] kg refer to the kg of DW of biomass produced up to the point indicated by the production scenario.

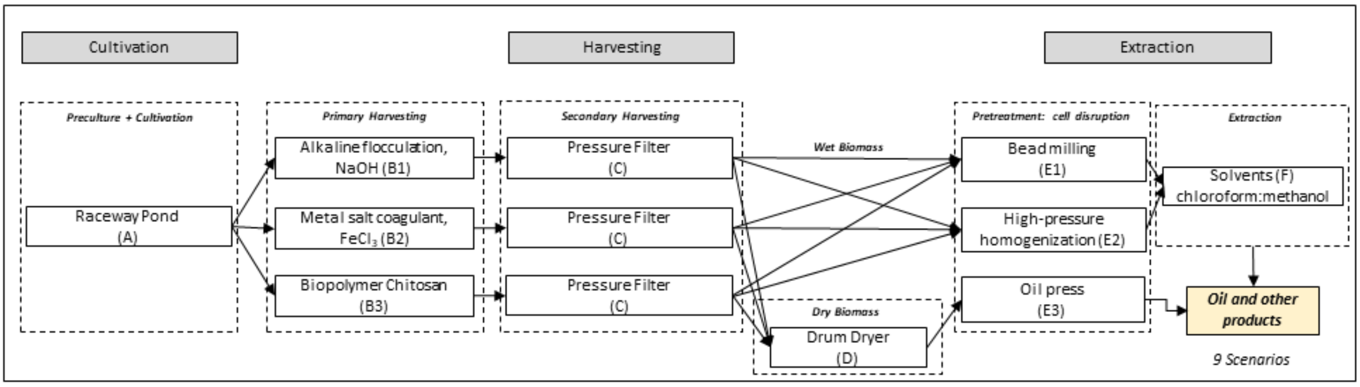

Figure 1. Microalgae oil production scenarios analyzed.

Table 2. Technological scenarios for the production of microalgal oil.

No.	Scenario	No.	Scenario	No.	Scenario
1	A-B1-C-E1-F	4	A-B2-C-E1-F	7	A-B3-C-E1-F
2	A-B1-C-E2-F	5	A-B2-C-E2-F	8	A-B3-C-E2-F
3	A-B1-C-D-E3	6	A-B2-C-D-E3	9	A-B3-C-D-E3

In the use of milling (Figure 2) and HPH (Figure 3), cell disruption is carried out with wet biomass, so drying technology is not considered in these production scenarios. However, in the use of these two technologies, a mixture of lipids, proteins, cell debris, and water can be generated as a product. To separate the oils from this mixture, the output current of this stage is sent to a unit where the lipids are extracted using chemical solvents (F), which are subsequently separated by evaporation (Figure 2). After extraction with chemical solvents, it is possible to have proteins and cellular remains that can be used if extra units are considered for their separation and purification, however, this is outside the scope of this study. The extraction is carried out with a mixture of chloroform:methanol as solvents in a 2:1 ratio. The proportions of the mixture of solvents with biomass is used in a

10:1 ratio. The biomass is put in contact with the mixture of solvents for 8 h, half of this time a mechanical mixing with the solvent will be carried out and the last 4 h will be subjected to decantation. Subsequently, the separation of the organic phase (lipids and solvents) from the aqueous phase and the biomass (cellular remains) will be carried out [33]. Finally, the organic phase passes to an evaporator where the solvents are separated from the lipids at a temperature of 60°, Figure 2. This temperature was set since at higher temperatures the oxidative degradation of lipids is promoted. This extraction method has the advantage of allowing the extraction of lipids from solutions with high moisture content.

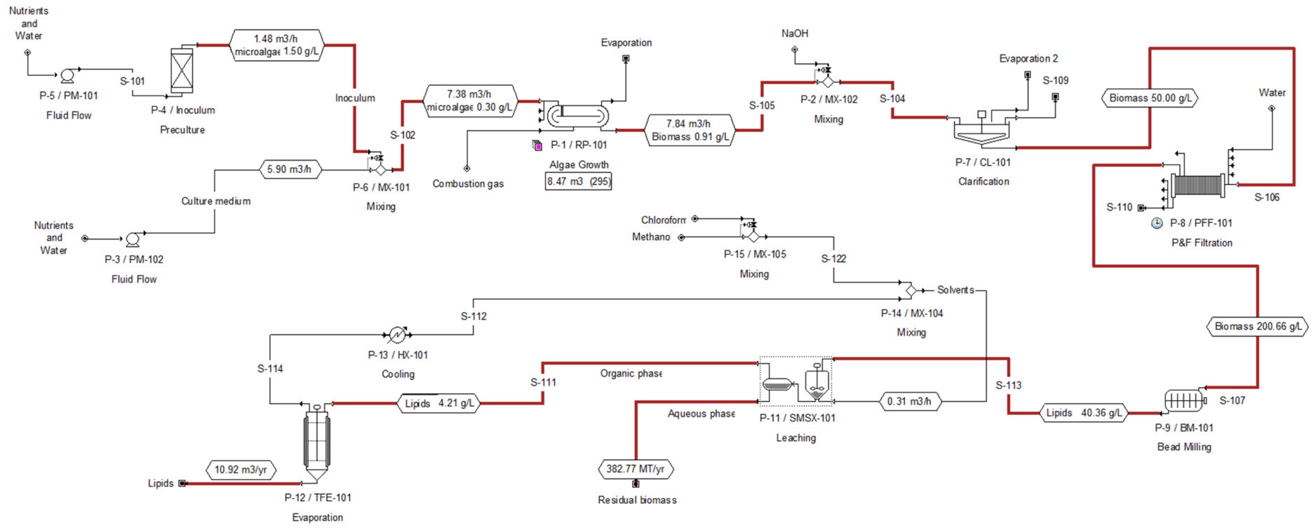

Figure 2. Flowsheet in SuperPro Designer for lipid production from wet biomass, scenario 1.

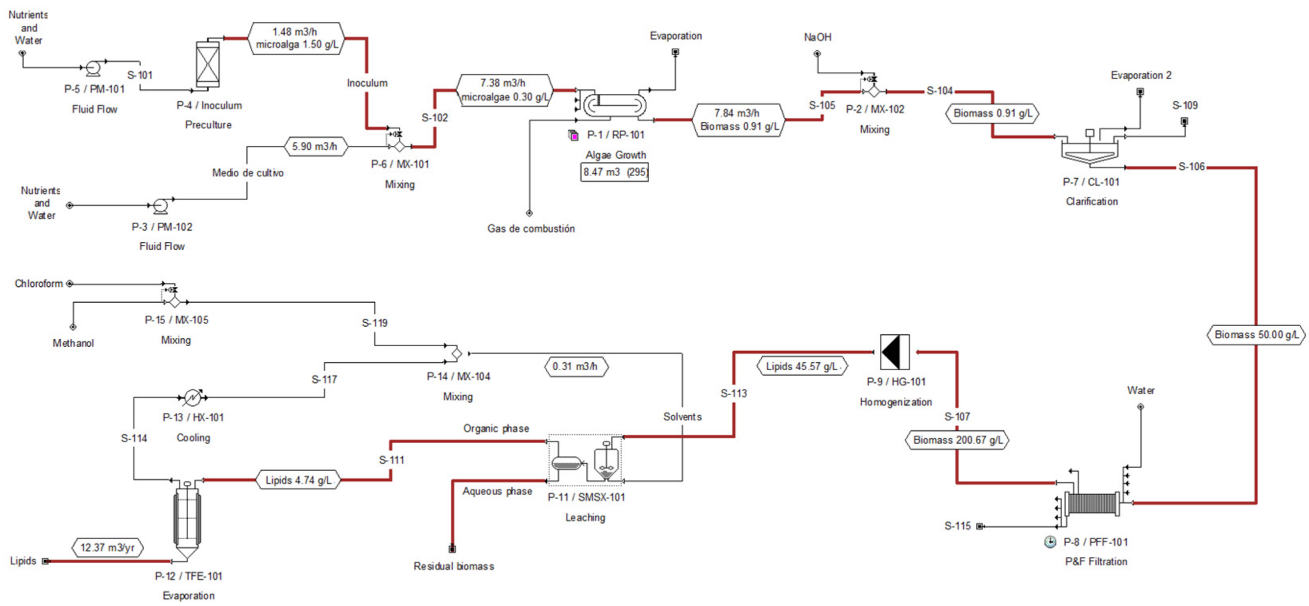

Figure 3. Flowsheet in SuperPro Designer for lipid production from wet biomass, scenario 2.

In the case of scenarios that consider pressing as a cell disruption pretreatment dry biomass is required, but lipid extraction with solvents is not required (see Figure 4). The products obtained in these scenarios are lipids and a biomass paste that can still be used for the extraction of valuable compounds, or as feed for livestock.

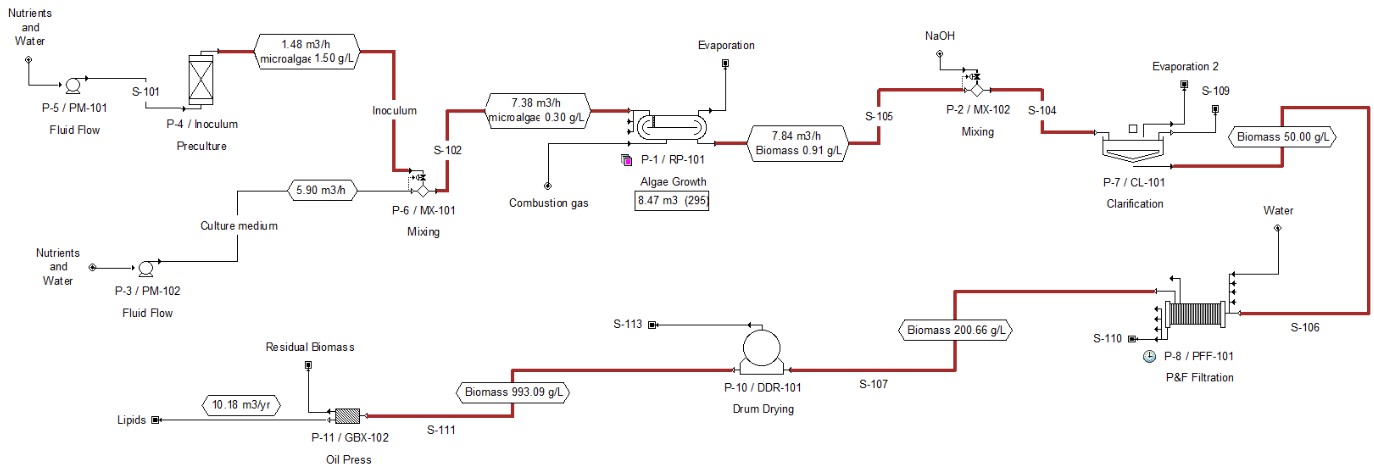

Figure 4. Flowsheet in SuperPro Designer for the production of lipids from dry biomass, scenario 3.

All the scenarios considered were evaluated through simulation in the SuperPro Designer v10® software (Intelligen, Inc., Scotch Plains, NJ, USA), considering energy consumption and production costs. Table 3 shows the operating conditions considered for each technology in the pretreatment stage. In addition to these operating conditions, each of the technologies has very particular conditions to meet the established objectives. These conditions were taken from the literature and some others were suggested by default in the simulator for the models of the technologies analyzed. For each technique or technology involved in the process, the mass and energy balances were made, and the necessary size of the equipment and production costs were estimated.

Table 3. Operating conditions for the technologies involved in the pretreatment stage (cell disruption).

Variable	Case E1	Case E2	Case E3
Initial concentration [1] (% DW)	20	20	95
Lipid recovery (%)	75 [34]	85 [35]	70 [36]
Energy consumption (kWh/kg DW) [2]	0.72 [37]	0.4 [29]	0.1375 [38]

[1] Biomass concentration in the feed stream to the pretreatment technology. [2] Consumption in kWh/kg of DW of fed biomass.

2.2. Economic Analysis

For the evaluation, 330 working days were considered in continuous operation mode. The cost of production is estimated with the help of the simulator, and this includes the sum of the costs related to raw materials, labor, services, and additional operating costs related to the use of the facility. The raw materials for the preculture and cultivation were those reported by Valdovinos et al. [15], and the costs reported for the year 2018 were considered. The costs associated with the services for the process are reported in Table 4; these were taken directly from the simulator. For the estimation of the rest of the costs, the simulator determines them by means of percentages related to the cost of purchasing equipment. Additionally, it has an extensive database that allows adjusting the costs over the years (Intelligen, Inc., Scotch Plains, NJ, USA).

Table 4. Service and raw material costs.

Type of Service	Cost	Unity
Electrical power	0.1	USD/kWh
Steam (heat)	12	USD/ton
Cooling water	0.05	USD/ton
NaOH	350	USD/ton
$FeCl_3$	650	USD/ton
Chitosan	1680	USD/ton
Chloroform	360	USD/ton
Methanol	350	USD/ton
Cost for labor (operator)	0.50	Labor-h/h

3. Results and Discussion

Once the production scenarios have been evaluated, we analyze the results in two sections; first, the technical and economic analysis of the technologies evaluated individually. Later, the analysis and evaluation of the scenarios, considering energy consumption, operating costs, CO_2 capture capacity, and fresh water consumption. To facilitate reference to the analyzed scenarios, they were numbered and, in this way, they will be named in the rest of the manuscript (Table 2).

3.1. Results of the Technical and Economic Analysis of the Individual Technologies

The cell wall of the microalga *Chlorella* is rigid, making it difficult to extract the compounds of interest [39]. There are several techniques and technologies to cause cell rupture, among the most common are bead milling, HPH, microwaves, and pressing However, not all of these techniques are candidates to be applied on an industrial scale. The results obtained in the individual evaluation of cell disruption technologies are shown in Table 5.

Table 5. Individual evaluation of cell disruption technologies.

Technology	Lipid Production (kg/Year)	Unit Production Cost (USD/kg)	Operation Cost (USD/Year)	Energy Consumption (kWh/Year)
Bead milling (E1)	9750	2.43	24,000	34,650
HPH (E2)	11,050	0.81	9000	8331
Oil press (E3)	9089	2.11	19,000	6633

We can observe in the results that HPH produces a greater amount of lipids. It is reported in the literature that 85% of cell rupture is reached. Therefore, this percentage was considered as the extracted amount of total lipids contained in the microalgae. In the case of press extraction, recoveries of 70% and 75% of the total lipids are reported. The composition considered for the biomass of *Chlorella* microalgae was reported by Alavijeh et al. [34]: lipids 27%, proteins 21%, carbohydrates 35%, and the remainder is considered to be made up of pigments and other compounds; all percentages are w/w.

Regarding energy consumption, this is higher in bead milling (case E1) since this technique requires constant agitation to cause the breaking of the biomass due to collisions with the pearls. In addition, this technology generates a lot of heat and it is necessary to add a good cooling system [40]. Even so, this technology is widely used on an industrial scale for the pretreatment of microalgal biomass. HPH technology has a high energy requirement of 0.25 to 147 kWh/kg of treated biomass [41], which can be greater than the requirements of bead milling technology. This will depend on the required pressure and the time the sample passes through the equipment. In this case, a single pass and a pressure of 100 MPa were considered.

Regarding the cost of operation, this is higher in case E1 (bead milling), mainly due to the high cost of the equipment and its maintenance. In addition, the high energy consumption must be considered. Although the cost of operation is higher in bead milling, it has the advantage of not requiring the biomass drying stage before being processed by this technology, since wet biomass can be processed. Meanwhile, in the case that considers pressing (E3), biomass with a very low moisture content is required. This operation could hinder the use of other fractions of interest in the biomass. The use of pressing (case E3) has as its final product an oil stream mixed with other compounds such as dyes or pigments. Only if it is necessary to remove these limiting compounds from the microalgal oil stream is an extra purification step included. In the cases that include bead milling (E1) and HPH (E2), the product is a mixture of intracellular molecules, lipids, proteins, and the residual biomass. In such cases, it is necessary to carry out the separation of the lipids from this mixture.

For the extraction of lipids, the pretreatment output stream is mixed with solvents, which are subsequently separated by evaporation. In this extraction stage, it was observed that the amount of solvents is high; therefore, their recirculation to the process was considered. With 95% recovery of the solvents, the cost was reduced from USD 129/kg of lipids to USD 16.3/kg of lipids. On the other hand, the cost of equipment at this stage is very high, which contributes to high unit production costs.

3.2. Results of the Technical and Economic Analysis of the Production Scenarios

The combination of the pretreatment technologies with the biomass production base cases generated nine lipid production scenarios (Table 2). One of the main parameters to consider in process design is energy consumption. In the analyzed scenarios, this is mainly associated with the energy requirement in the cultivation of microalgae, harvesting, drying (whether considered or not), cell disruption, and solvent extraction. When evaluating and comparing the routes from an energy perspective (Figure 5a), we can observe that the scenarios that consider pressing (E3) are the ones with the lowest energy consumption per year, even including the biomass drying stage (scenarios 3, 6, and 9). In the group of scenarios that include the cases of bead milling (1, 4, and 7), the biomass enters the process with 80% moisture (just like the scenarios that consider HPH). However, although the biomass drying stage is avoided, this group of scenarios consumes approximately 63% more energy than those that consider pressing. This is due to the high energy consumption of bead milling. One of the stages with the greatest contribution to energy consumption is the extraction with solvents and their recovery, due to the time required to mix the solvents with the biomass and their evaporation. On the other hand, the scenarios that consider HPH (2, 4, and 6) show approximately 13% more energy consumption per year compared with the group of scenarios that considers the use of the press unit. However, when both groups are compared regarding energy consumption per kg of lipids produced (Figure 5b), the consumption is lower in those groups that consider HPH as pretreatment. Approximately 5.46, 5.39, and 5.40 kWh/kg in scenarios with HPH vs. 5.88, 5.80, and 5.80 kWh/kg in press scenarios. Meanwhile, for the scenarios that include milling, the energy consumptions are 8.89, 8.80, and 8.79 kWh/kg. This is due to the fact that in the scenarios that consider HPH there is a greater recovery of lipids than in those that consider pressing.

Although the groups of scenarios that consider the same technologies for cell disruption and lipid extraction do not indicate a variation in energy consumption, it is important to evaluate scenarios that use different types of flocculants. Flocculants are chemical compounds that could be toxic to microalgal biomass and limit their use [42]. Even if it is desired to evaluate the recovery of the culture medium that is extracted in the first and second harvest stages, the type of flocculant used must be taken into account, since it could contaminate the culture and reduce the potential growth of the microalgae. For example, it has been reported that the use of iron salts leads to a yellowish-brown coloration of microalgae, which limits the biomass for pigment extraction. On the other hand, the use of this type of flocculant can cause the sediment of unwanted metals that could contaminate the biomass, which would make it difficult to apply it as a raw material for biofuel or as animal feed [16]. In a previous publication [43], a discussion of the subject has been made, including some aspects to consider regarding the use of biomass when the three types of flocculants analyzed in this study are used. This information is important if you want to analyze or implement any of these scenarios for a specific purpose, and efficiently take advantage of microalgae biomass.

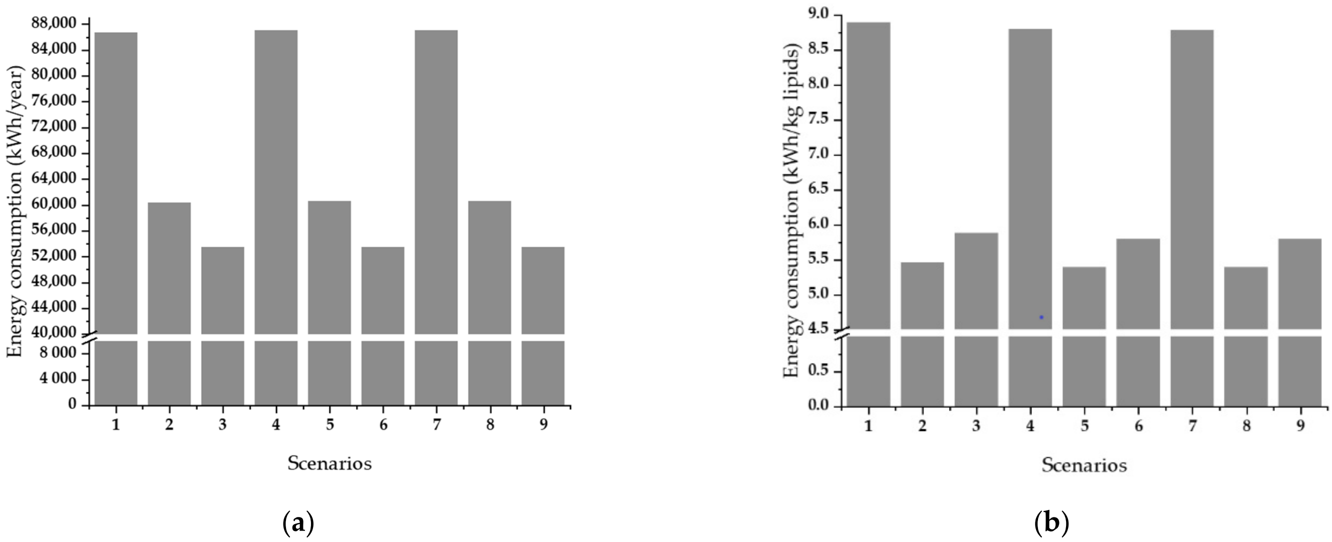

Figure 5. Energy consumption in the scenarios analyzed for lipid production: (**a**) Total energy consumption per year; (**b**) total energy consumption per kg of lipids produced.

At this point it is important to comment that there are several products obtained in the scenarios that consider bead milling and HPH. The use of bead milling and HPH require that the biomass be fed wet, producing complex mixtures that are very different from the initial biomass. After cell disruption, cell wall fragments and non-soluble compounds (such as lipids) remain suspended in the aqueous phase containing soluble compounds (proteins, carbohydrates, etc.). These cell disruption techniques allow the release of various compounds of commercial interest which provides an advantage if the aim is to fully exploit the microalgae biomass like in a biorefinery scheme. The separation of these compounds may involve centrifugation and molecular separation in membrane processes or by solvent extraction, which will increase energy consumption and production costs in the process. On the contrary, in the biomass pressing technique, the oil is filtered through small openings that do not allow the other components to filter through. The pressed microalgae biomass forms a cake and is removed from the machine. The pressures involved in the pressing of the expeller create heat in the range of 60–100 °C [44]. This would cause an increase in energy consumption since an additional mechanism is required to maintain the cooling of the biomass and prevent the deterioration of other intracellular compounds of interest. Most of the time, the extracted oil is accompanied by dyes extracted from biomass [45], which decreases the quality of the product or would cause an increase in the process operations to obtain a single product.

Since some scenarios, i.e., those that include the same cell disruption technologies, do not differ greatly in energy consumption, the distribution of energy consumption is analyzed only for scenarios 1, 2, and 3. The distribution of energy consumption in scenarios 1, 2, and 3 is presented in Figure 6. In scenario 1, 44% of energy consumption is associated with biomass cultivation, 8% with biomass harvesting, 40% with bead milling pretreatment, and the rest with lipid extraction (mixed with solvents and evaporation of the solvent). The same behavior is observed in the scenarios of the same group (1, 4, and 7). Regarding scenario 2, 64% of energy consumption is associated with cultivation, 11% with harvest, 14% with pretreatment, and the remaining 11% with lipid extraction. Scenario 3 differs a little from the other two scenarios: 72% is associated with cultivation, 13% with harvest, only 3% with biomass drying, and 12% with lipid extraction with a press. The distribution of energy consumption could change slightly if the energy consumption considered in each of the technologies involved in each stage is modified. However, the impact would be minimal, since biomass cultivation is the stage with the highest energy consumption in all scenarios. That is why the scientific community interested in the use of microalgae biomass has focused on finding alternatives to reduce energy consumption at this stage.

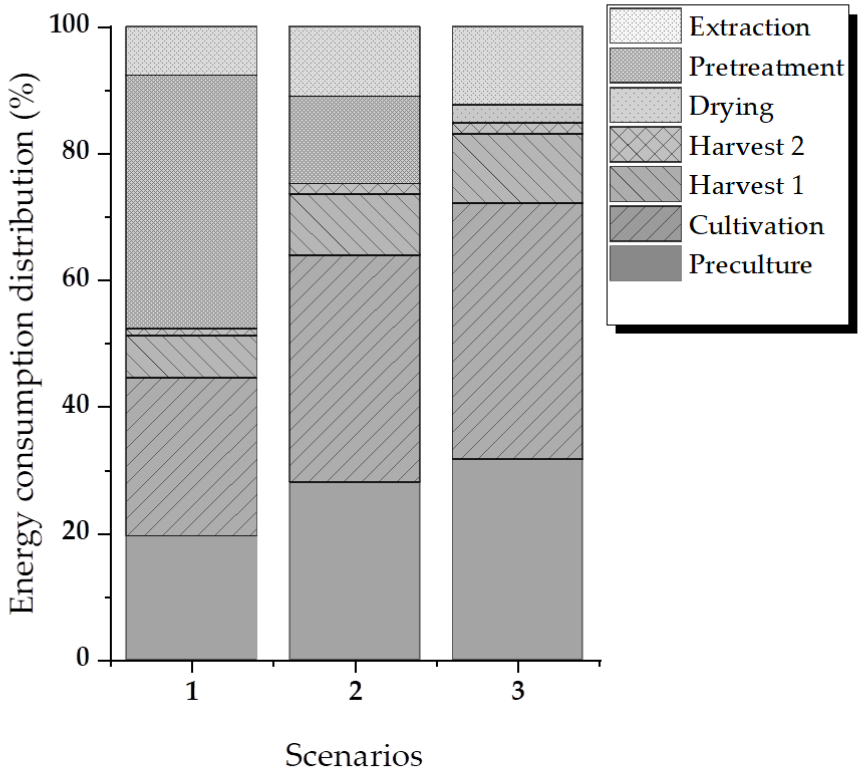

Figure 6. Distribution of energy consumption for scenarios 1, 2, and 3.

Regarding production costs, in Figure 7a we can see that the scenarios that consider pressing (3, 6, and 9) have the lowest total annual operating cost. The group of scenarios that include HPH (2, 5, and 8) have a slightly lower operating cost than the scenarios that consider pearl grinding (1, 4, and 7). A disadvantage of pressing as a cell disruption technique is that it can be a very slow process [46] and increase production costs, mainly due to the costs associated with labor. Although the application of this method is not widely discussed in the literature for the extraction of lipids from microalgae, it should be considered as an option with the possibility of being viable for industrial applications. On the contrary, bead milling is a technique widely used in industry for the extraction of DNA in biological samples [46].

When analyzing the operating costs per year in the scenarios (Figure 7a), we observe a behavior like energy consumption. The scenarios that consider bead milling (1, 4, and 7) are approximately 65% more expensive than the scenarios that consider pressing (3, 6, and 9), and 8% more than those that consider HFH (2, 5, and 8). With the operating costs per kg of lipids produced (Figure 7b) we observe that the group of scenarios that include pressing are the ones with the lowest cost. These costs are approximately USD 27.3 to 27.9/kg of lipids vs. USD 41.1 to 41.4/kg for scenarios that consider bead milling. The costs for the scenarios that consider HPH are USD 34.3 to 34.7/kg. The big difference in operating costs is because, in the scenarios that include bead milling and HPH, an extraction stage with solvents and their separation by evaporation was added, causing these scenarios a higher energy consumption and higher operating cost due to solvents and additional equipment. The cost of solvents had the greatest impact on the cost of operation followed by the cost associated with the equipment and its maintenance, and finally the high energy consumption.

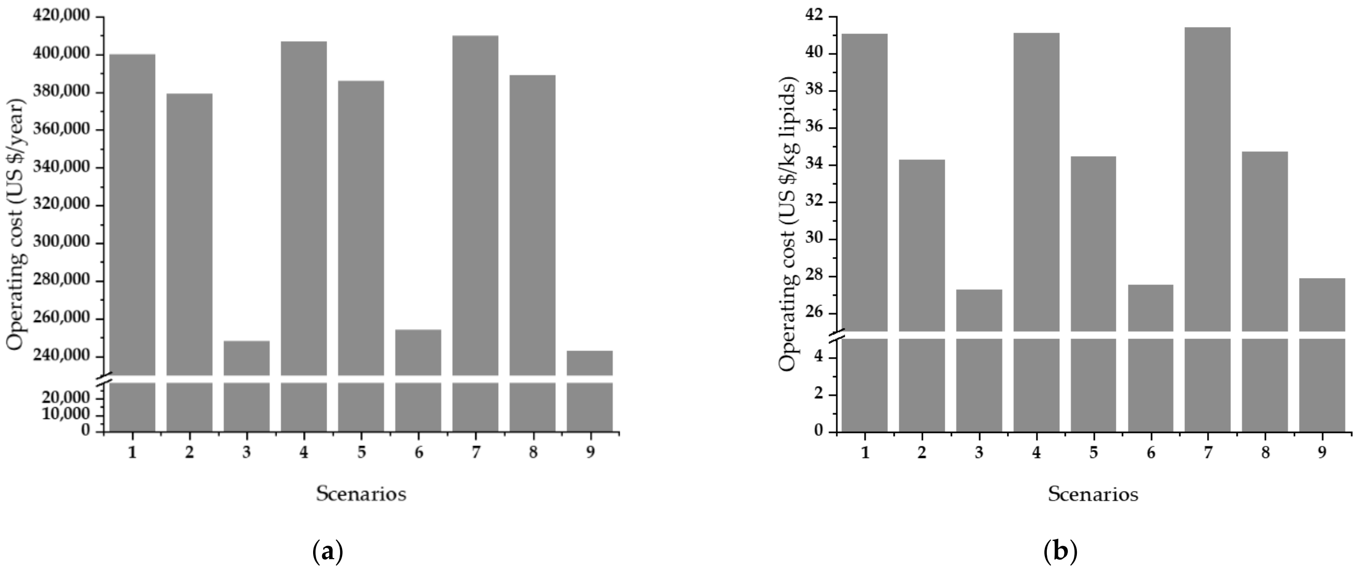

Figure 7. Operating cost in the scenarios analyzed for lipid production: (**a**) Total operating cost per year; (**b**) total operating cost per kg of lipids produced.

Figure 8 shows the distribution of operating costs for scenarios 1, 2, and 3. Regarding scenario 1, 46% of the operating cost is associated with raw materials, of which fresh water occupies 43.4% of the cost, 32.7% is due to solvents, and the rest (23.9%) is due to nutrients for the culture and the flocculant. Facility-dependent costs account for 38% of operating costs, of which 35% is associated with maintenance (estimated as 10% of equipment purchase) and the remaining 65% is for insurance payments, taxes, depreciation, etc. The cost of labor consumes 5% of the total cost of operation, for which only operators were considered for the process, so if other types of personnel are included, the cost could increase. The rest of the operating cost (12%) is associated with utilities, of which 18% of this cost corresponds to energy consumption and the rest (82%) to other services for the process, mainly cooling water. Scenario 2 has a similar distribution of operating costs: 48% is associated with raw materials, 35% is facility-dependent, 11% is associated with utilities, and 5% with labor. In both scenarios, energy consumption has a low impact on operating costs (Figure 8), however, the cost associated with fresh water consumption (98% for cultivation) has the greatest impact (Figure 9a), prompting the need to look for alternatives to reduce the consumption of fresh water and nutrients in the culture, in addition to considering other solvents for extraction that minimize their consumption and increase extraction efficiency.

Regarding scenario 3 (Figure 8), 50% of the operating cost is associated with raw materials, of which fresh water occupies 64% of the cost, and the rest is due to the nutrients for the culture and the flocculant. Facility-dependent costs account for 40% of operating costs, the labor cost consumes 6% of the total operating cost, and the rest of the operating cost (3%) is associated with utilities, of which 64% of this cost corresponds to energy consumption, and the rest (35.62%) to other services for the process such as steam, cooling water, etc. In scenario 3, the biggest impact on the cost of operation is also the consumption of fresh water (Figure 9b).

The difference in energy consumption and operating costs in each group of scenarios that consider the same technology for cell disruption (for example 1, 4, and 7), is due to the amount of biomass that is fed to process. The amount of biomass fed to this stage depends on the type of flocculant that has been used in the harvest, since they have different collection efficiencies [15].

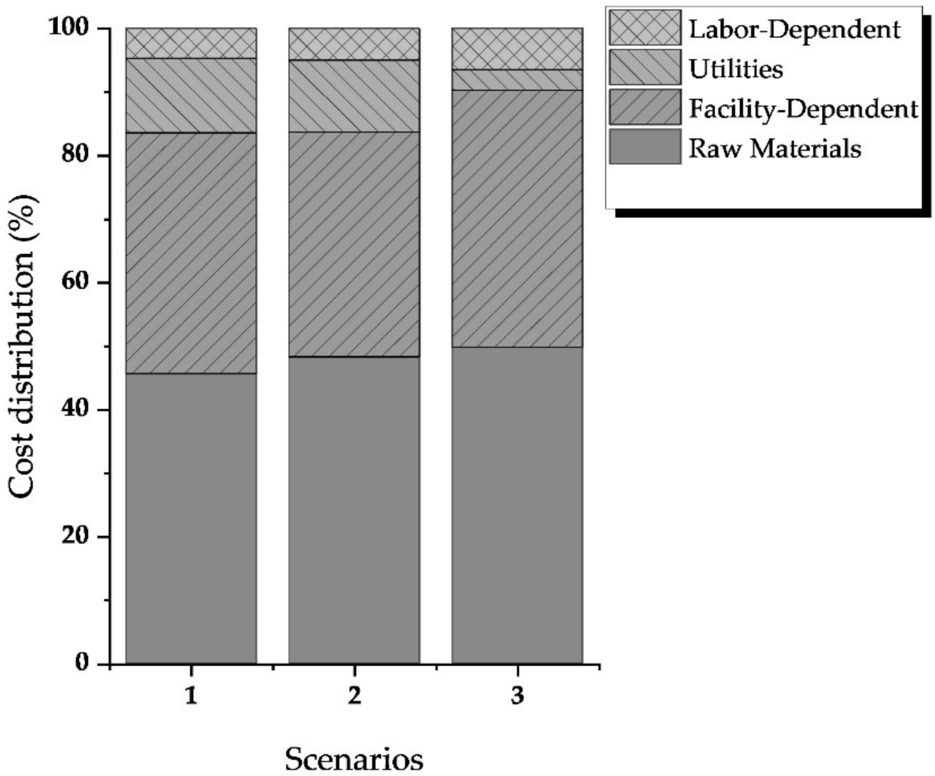

Figure 8. Distribution of the operating cost for scenarios 1, 2, and 3.

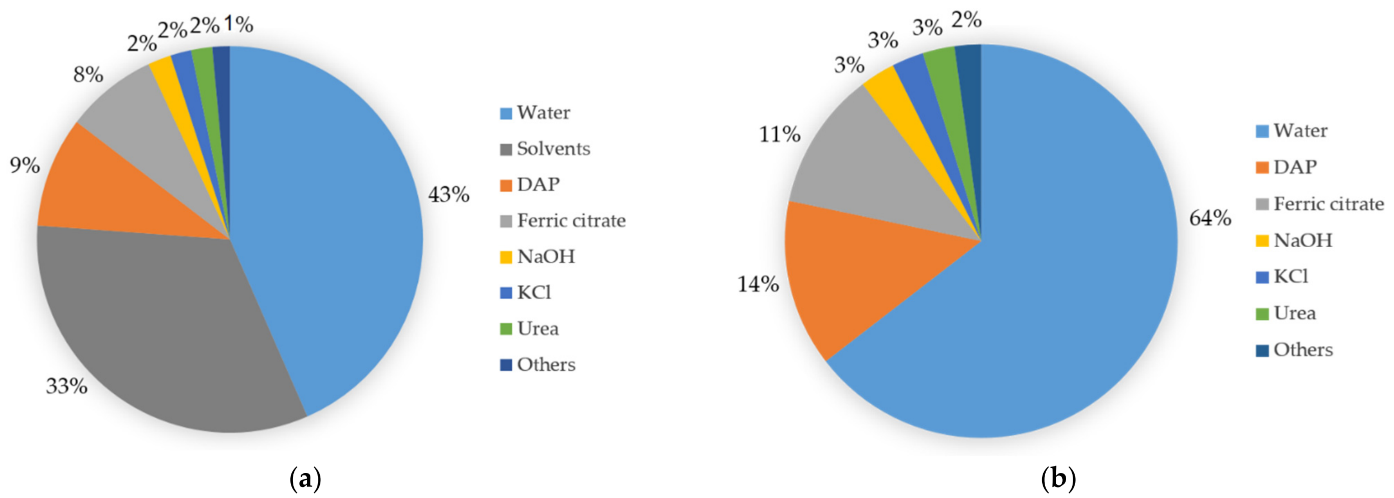

Figure 9. Distribution of raw material cost in the scenarios analyzed for lipid production: (**a**) scenario 1 and 2; (**b**) scenario 3.

In this study we analyze which scenarios have the lowest energy impact and the lowest production cost, considering the production of 1 kg of lipids (see Figure 10). Three groups are clearly observed, which are differentiated by the type of cell disruption method. In the upper right corner are the scenarios that consider cell disruption by bead milling: 1, 4, and 7. In the upper left corner are the scenarios that consider HPH: 2, 5, and 8. Finally, those of the lower left corner are the scenarios that consider pressing: 3, 6, and 9.

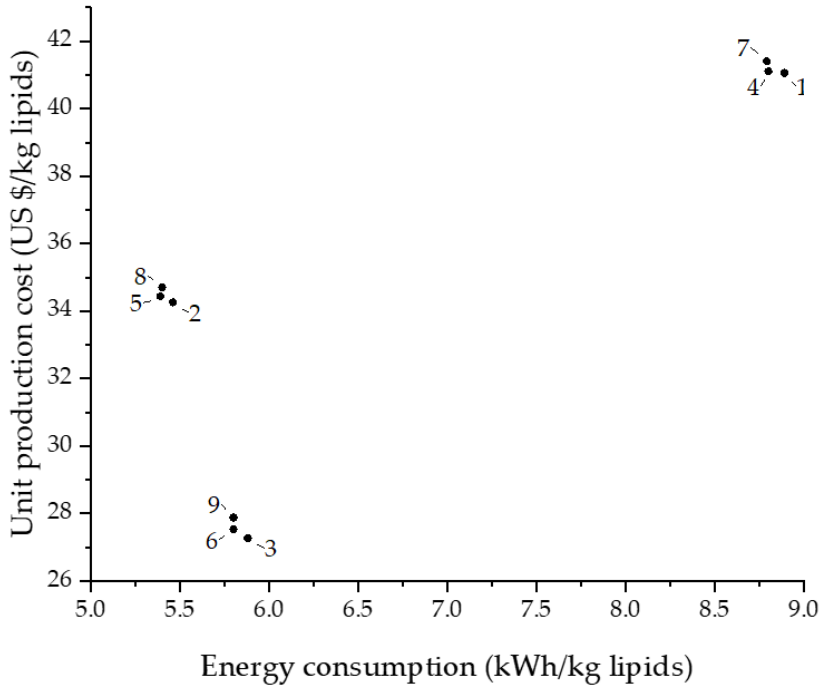

Figure 10. Total energy consumption vs. total production cost per kg lipids produced in each scenario.

The scenarios that include bead milling are the most energy demanding and have the highest unit production cost, as expected. Meanwhile, the scenarios that consider pressing are those with lower energy consumption and lower production cost.

Seongwhan et al. [47] report a production cost of USD 6.5/kg of lipids obtained from the microalgae *Chlorella vulgaris*, much lower than in this study. It is also mentioned that the scenario analysis shows that economic results change significantly when processing technologies are changed. Sun et al. [48] published a study comparing the production costs of algae oil for biofuels reported in different studies. They comment that even though there is a variety of techno-economic studies, there is a significant difference in production costs, and this can be largely attributed to the assumptions and uncertainties in the economic evaluation of the process [48–50]. To address this disparity, they collaborated on a payback study to estimate algal oil production costs based on a common framework. They found that the updated cost comparison based on a normalized set of input assumptions greatly reduces economic variability, resulting in oil production costs ranging from USD 10.87 to 13.32/gallon. Batan et al. [49] carried out a techno-economic analysis for the production of microalgae in a photobioreactor with an annual production of 37.85 million liters of biofuel, obtaining a production cost of crude oil from algae of USD 3.46/L. Llamas et al. [50] developed a techno-economic analysis of different scenarios to evaluate the production of microalgae considering the fixation of CO_2. The cost of biomass production ranged between 0.41 and 13.06 €/kg. They also comment that the most relevant parameters that influenced the cost of biomass production were biomass productivity and the number of working days. All these reports were evaluated under different conditions and in different reference years.

This information allows us to observe that it is necessary to optimize the processing route individually for each species for a fair evaluation. The production costs of microalgal oil will depend on various technical factors, such as the lipid content in the microalgal species, the biomass production by volume, the technologies used, the recovery efficiency of the microalgal biomass, the energy consumption of each technology, the cost of nutrients for the crop, operating times, among others. This will cause studies related to this topic to generate a wide variety of results. That is why it is recommended that once the objective of microalgal biomass production is established, the most appropriate scenarios to achieve it are evaluated.

The objective of this research is to study the lipid production scenarios with the lowest energy consumption and the lowest unit cost of production. Considering this, an approximate production of 10.92 m^3/year of lipids would be obtained if milling is used for cell disruption and solvent extraction, 10.18 m^3/year if only pressing is used, and 12.37 m^3/year if it is biomass pretreated with HPH. All scenarios consider biomass cultivation in an open system with a productivity of 12.71 g/m^2/day in an area of 1 ha [15]. Quinn and Davis [51] report that various techno-economic studies estimate lipid productivities ranging from 2.3 m^3/year/ha to 136.9 m^3/year/ha. However, these values are estimated by linear extrapolation from laboratory-based growth and lipid production data, leading to a wide range of values. This causes some uncertainty in the results obtained, so it is recommended to use data obtained from a real production, or at least data obtained from a pilot plant. It is also important to indicate which species of microalgae will be used, their composition, and culture conditions considered, since the quality of the biomass depends on the latter.

Also, microalgae can be used as CO_2 capture systems due to their high capacity to do so. In a previous study, it was estimated that the cultivation of microalgae in an open system with a biomass productivity of 12.71 g/m^2/day could achieve a capture of 102.13 tons of CO_2/year in 1 ha of cultivation area [15]. Based on this data, the amount of CO_2 that the lipid production scenarios would be capable of capturing was estimated and compared with energy consumption and unit production costs (Figure 11).

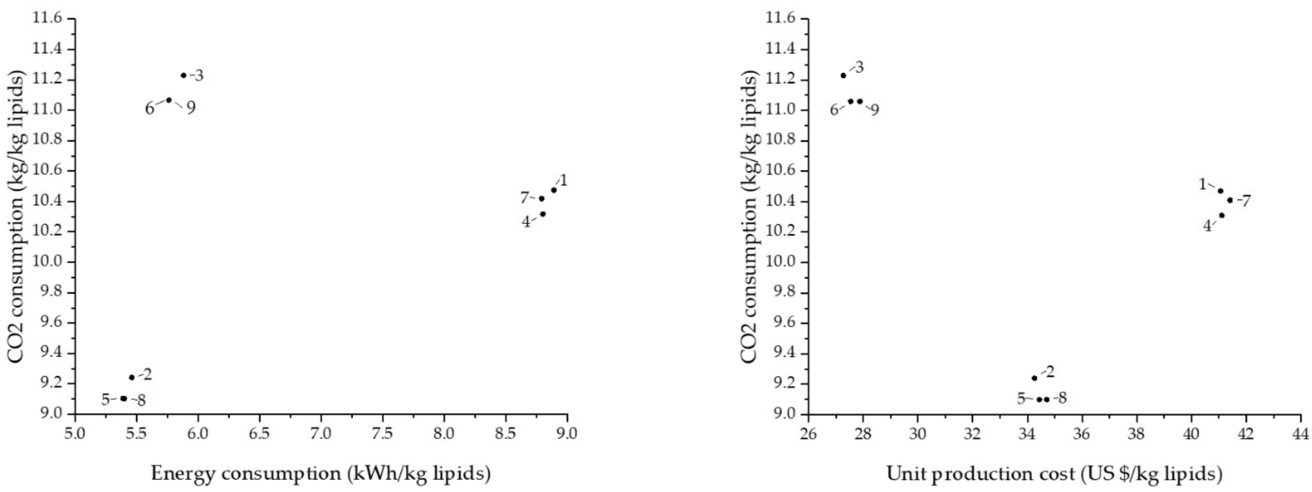

Figure 11. CO_2 consumption in the scenarios analyzed for lipid production: (**a**) Energy consumption vs. CO_2 consumption; (**b**) unit production cost vs. CO_2 consumption.

In Figure 11a we can see that the scenarios that consider HPH—2, 5, and 8—are the ones that consume the least amount of CO_2 per kg of lipids produced, although they are the ones that consume less energy. Meanwhile, the scenarios that consider pressing—3, 6, and 9—consume the most CO_2 for each kg of lipids produced. In Figure 11b we can observe the same behavior of the unit cost of production vs. CO_2 capture. If all the scenarios reached the same amount of lipids produced, there would be no difference between the amount of CO_2 consumed, but there would be in terms of the amount of energy required. In this study, the amount of CO_2 that can be generated by the use of energy for the production of lipids was not evaluated. Considering the CO_2 captured and the CO_2 emitted due to energy consumption is a very important parameter if you want to consider the use of microalgae cultivation as a system for capturing. We must remember that these results were obtained from considering 1 ha as a surface destined only for the microalgae cultivation stage. If a larger area of land is used for the cultivation of microalgae, an increase in biomass production yield will be observed. Furthermore, although energy consumption and operating costs are increased, there will be a positive impact on consumption and production cost per kg of lipids obtained, making the process more profitable. However, a sensitivity analysis is needed to validate this information.

Another important parameter to consider is the amount of fresh water used, mainly in the cultivation stage (98%). Figure 12 shows that the scenarios that consider HPH—2, 5, and 8—are those with the lowest consumption of fresh water per kg of lipids produced, either compared to energy consumption (Figure 12a) or to the unit production cost (Figure 12b). The scenarios with a high consumption of fresh water, and high energy consumption and unit production cost are those that consider bead milling—scenarios 1, 4, and 7. However, the scenarios that consider use of the press unit—3, 6, and 9—are the largest consumers of fresh water per kg of lipids produced. However, they are the least energy consumers and have the lowest unit production cost.

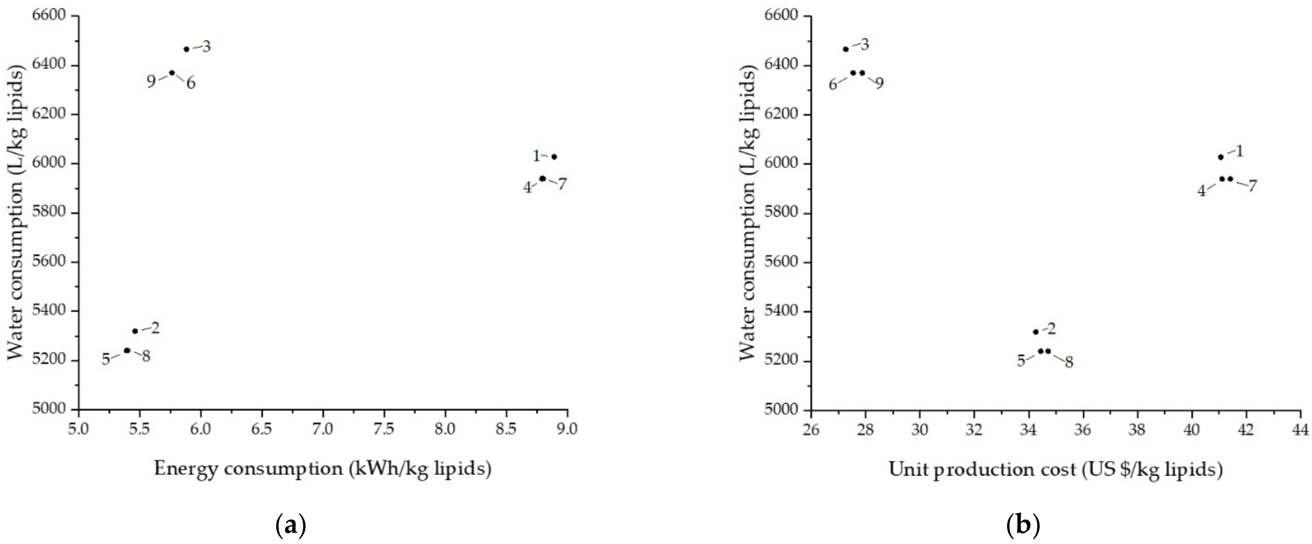

Figure 12. Water consumption in the scenarios analyzed for the production of lipids: (**a**) Energy consumption vs. water consumption; (**b**) unit production cost vs. water consumption.

The consumption of fresh water is not only in the cultivation stage. The remaining 2% is associated with the use of water for washing the biomass in the secondary harvest stage. Thereby, remains of compounds that interfere in the stage of cell disruption or direct use of dry biomass are eliminated. There are several alternatives to reduce the consumption of fresh water, such as the partial or total use of wastewater, the recirculation of effluents from the cultivation stage or from the harvest stages. Referring to scenario 5, most of the operating costs correspond to raw materials (49%), of which 46.1% is due to the consumption of fresh water, and the rest to synthetic nutrients for the crop (14.58%), flocculant for primary harvest (7.39%), and solvents for extraction (31.93%). Gouveia et al. mention that the use of wastewater contributes to cost reduction, since the demand for fresh water and synthetic nutrients is reduced due to the use of nitrogen, phosphorus, and other nutrients present in wastewater [52]. Rossi et al. report that they evaluated the efficiency of a pilot-scale open-air raceway pond treating wastewater generated by a large-scale pig farm in northern Italy and comment that biomass production without using wastewater nutrients resulted in much higher costs, due to the cost of synthetic nutrients. Rossi et al. also mention that it should be noted that an important limitation of the use of wastewater to grow algae is that it makes it difficult to exploit the biomass for food and feed applications, since bacterial contamination cannot be prevented [53]. The production of microalgae using wastewater allows the concentration of excess nutrients in the microalgae biomass, which reduces their release to the environment and allows the recovery of water for further use. In this case, microalgae are considered an organic fertilizer that has the potential to prevent nutrient loss through a gradual release of N, P, and K. Biomass production of microalgae from wastewater can valorize residual nutrients into sustainable and innovative biofertilizers with commercial opportunities in crop production [54]. On the other hand, the recirculation of the effluents from the harvest stages will be viable if the type of flocculant used is not an impediment. One drawback to the use of flocculants is the unavoidable release of chemicals in the processed water, which can limit the recycling of water as a growth medium and its direct discharge into the environment. Flocculants can contaminate harvested biomass, with the possible inhibition of photosynthesis and growth by residual chemicals, and contamination can prevent some recovery alternatives, e.g., animal feed, biofuels, and biofertilizers production [55]. Ketife et al. mention that water recirculation has the potential to reduce energy consumption, nutrient loss, and water demand. However, this also carries a risk of infection and growth inhibition from the accumulation of pathogenic microorganisms and refractory organic and inorganic chemicals, and residual metabolites from the destroyed algal cells [56]. This study does not consider any of these options. However, an interesting proposal could be to evaluate these alternatives and analyze the impact of integrating them into the process.

One of the advantages of using the simulator is that it allows you to carry out an analysis of the processes, evaluate the impact of the different variables of the process, execute the economic evaluation in less time, and estimate the various costs associated with the process. For this, more detailed information on the process is required, for example, service data, main equipment, dimensioning, and some other design specifications, which are challenging if not directly available. These estimates can be used to decide whether to continue with the project or not, and to create more detailed studies where spending more resources is justified.

4. Conclusions

This study analyzed various scenarios for the microalgae lipid production process. These scenarios were formed with the cultivation of microalgae in a raceway pond, primary harvest with three flocculants, secondary harvest with a filter press (and drying if necessary), and three different technologies for the cell disruption stage, facilitating the extraction of lipids. The impact on energy consumption and production costs were analyzed, first of the individually evaluated cell disruption technologies and later of the scenarios. This provided us with a clear view of the contribution of each technology in the lipid production process.

Regarding the individual evaluation, cell disruption by bead milling has higher energy consumption (3.55 kWh/kg), compared to extraction with pressing (0.73 kWh/kg) or HPH (0.75 kWh/kg). Although HPH technology consumes much more energy than the press, it achieves the highest lipid recovery, which makes consumption similar when comparing per kg of lipids produced. However, the energy consumption will depend on the pressure used and the passes made to the biomass in the equipment, which can considerably increase consumption. Lipid production costs are USD 2.11/kg for pressing, USD 0.81/kg for HPH, and USD 2.43/kg for bead milling. The high cost of production for the press is due to the cost of the equipment and the maintenance associated with it.

When analyzing energy consumption and operating costs in the production scenarios, both parameters are higher in the scenarios that consider bead milling (8.8–8.9 kWh/kg and USD 41.1–41.4/kg), followed by those that consider HPH (5.4–5.5 kWh/kg and USD 34.3–34.7/kg). The high energy consumption and operating costs are due to the fact that the scenarios that consider bead milling and HPH require an extra stage for the separation of lipids (extraction with solvents and evaporation). The use of bead milling and HPH require that the biomass be fed in a wet state, producing complex mixtures. These technologies allow the release of various compounds of commercial interest, which can generate the addition of supplementary separation stages, increasing production costs. On the other hand, extraction with a press presents a greater advantage if the main objective is to obtain microalgal oil and a biomass with uniform characteristics. For the scenarios that consider the production of lipids by press, the energy consumption ranges from 5.8–5.9 kWh/kg and the operating cost is USD 27.3–27.9/kg. Operating costs are high, so considering the use of lipids as feedstock for biofuel production may be economically unfeasible. However, considering various current research perspectives, lipids have the potential to be used as the basis to produce biolubricants which can be economically competitive. Although the groups of scenarios that consider the same technologies for cell disruption and lipid extraction do not indicate a significant variation in energy consumption or operating costs, it is important to evaluate scenarios that use different types of flocculants, since these could be toxic for microalgal biomass, limiting their use and the use of lipids.

Although the consumption parameters of CO_2 and fresh water are not directly linked to the cell disruption stage, they are linked to the final process, regardless of the stage where they are needed. Analyzing the consumption of CO_2 that could be achieved in the production of lipids, scenarios that consider pressing are the ones with the highest capture of CO_2 per kg of lipids produced (11.23 kg of CO_2/kg of lipids). Meanwhile, scenarios that consider HPH are the lowest consumers of fresh water per kg of lipids produced—approximately 5.3 m^3 of water/kg of lipids.

This study allowed us to develop a base of multiple comparative scenarios to evaluate different aspects involved in the production of lipids from microalgae of a specific species, in this case for *Chlorella vulgaris*, and to determine the impact of the various technologies in the cell disruption stage.

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