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Chapter 1

Potential Production of Biofuel from Microalgae Biomass Produced in Wastewater

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1. Introduction

Microalgae are the principal primary producers of oxygen in the world and exhibit enormous potential for biotechnological industries. Microalgae cultivation is an efficient option for wastewater bioremediation, and these microorganisms are particularly efficient at recovering high levels of nitrogen, inorganic phosphorus, and heavy metals from effluent. Furthermore, microalgae are responsible for the reduction of CO_2 from gaseous effluent and from the atmosphere. In general, the microalgae biomass can be used for the production of pigments, lipids, foods, and renewable energy [1].

Much of the biotechnological potential of microalgae is derived from the production of important compounds from their biomass. The biodiversity of the compounds derived from these microorganisms permits the development of new research and future technological advances that will produce as yet unknown benefits [2].

Microalgae grow in open systems (turf scrubber system, raceways, and tanks) and in closed systems (vertical (bubble column) or horizontal tubular photobioreactors, flat panels, biocoils, and bags). The closed systems favor the efficient control of the growth of these microorganisms because they allow for improved monitoring of the growth parameters [3-4].

Because microalgae contain a large amount of lipids, another important application of microalgae is biodiesel production [5]. In addition, after hydrolysis, the residual biomass can potentially be used for bioethanol production [6]. These options for microalgae uses are promising for reducing the environmental impact of a number of industries; however, there

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is a need for optimizing a number of parameters, such as increasing the lipid fraction and the availability of nutrients [7].

Notably, the microalgae biomass can produce biodiesel [5], bioethanol [6], biogas, biohydrogen [8-9] and bio-oils [10], as shown in Figure 1.

The productivity per unit area of microalgae is high compared to conventional processes for the production of raw materials for biofuels, and microalgae represent an important reserve of oil, carbohydrates, proteins, and other cellular substances that can be technologically exploited [2,11]. According to Brown *et al*. [12], 90-95% of the microalgae dry biomass is composed of proteins, carbohydrates, lipids, and minerals.

An advantage of culturing algae is that the application of pesticides is not required. Furthermore, after the extraction of the oil, by-products, such as proteins and the residual biomass, can be used as fertilizer [13]. Alternatively, the residual biomass can be fermented to produce bioethanol and biomethane [14]. Other applications include burning the biomass to produce energy [15].

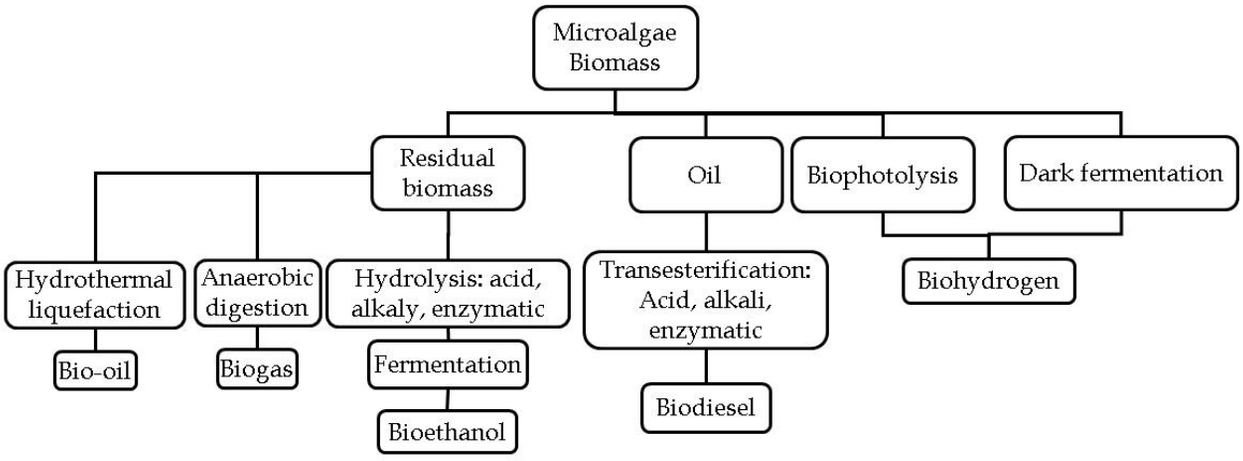


Figure 1. Diagram of the principal microalgae biomass transformation processes for biofuel production.

The cultivation of microalgae does not compete with other cropsfor space in agricultural areas, which immediately excludes them from the "biofuels versus food" controversy. Similar to other oil crops, microalgae exhibit a high oil productivity potential, which can reach up to 100,000 L he^{-1}. This productivity is excellent compared to more productive crops, such as palm, which yield 5,959 L he^{-1} and thus contribute to the alleviation of the environmental and economic problems associated with this industry[16].

Although the productivity of microalgae for biofuel production is lower than traditional methods, there is increasing interest and initiatives regarding the potential production of microalgae in conjunction with wastewater treatment, and a number of experts favor this option for microalgae production as the most plausible for commercial application in the short term [17].

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2. Wastewater microalgae production

Photosynthetic microorganisms use pollutants as nutritional resources and grow in accordance with environmental conditions, such as light, temperature, pH, salinity, and the presence of inhibitors [18]. The eutrophication process (increases in nitrogen and inorganic phosphorus) of water can be used as a biological treatment when the microalgae grow in a controlled system. Furthermore, these microorganisms facilitate the removal of heavy metals and other organic contaminants from water [19-22].

In general, the use of microalgae can be combined with other treatment processes or as an additional step in the process to increase efficiency. Therefore, microalgae are an option for wastewater treatments that use processes such as oxidation [23], coagulation and flocculation [24], filtration [25], ozonation [26], chlorination [27], and reverse osmosis [28], among others. Treatments using these methods separately often prove efficient for the removal of pollutants; however, methods that are more practical, environmentally friendly, and produce less waste are desirable. In this case, the combination of traditional methods with microalgae bioremediation is promising [29]. The bioremediation process promoted by open systems, such as high rate algal ponds, combines microalgae production with wastewater treatment. In addition, the control of microalgae species, parasites, and natural bioflocculation is important for cost reduction during the production of the microorganism [20, 30].

Many microalgae species grow under inhospitable conditions and present several possibilities for wastewater treatments. All microalgae production generates biomass, which must be used in a suitable manner [31-32].

Microalgae are typically cultivated in photobioreactors, such as open systems (turf scrubbers, open ponds, raceway ponds, and tanks) or closed system (tubular photobioreactors, flat panels, and coil systems). The closed systems allow for increased control of the environmental variables and are more effective at controlling the growth conditions. Therefore, the specific cultivation and input of CO_2 are more successful. However, open systems can be more efficient when using wastewater, and low energy costs are achieved for many microalgae species grown in effluents in open systems [33-35]. Because of the necessity for renewable energy and the constant search for efficient wastewater treatment systems at a low cost, the use of microalgae offers a system that combines wastewater bioremediation, CO_2 recovery, and biofuel production.

In turf scrubber systems, high rates of nutrient (phosphorus and nitrogen) removal are observed. This phenomenon was observed in the biomass retained in the prototype turf scrubber system used in three rivers in Chesapeake Bay, USA. The time of year was crucial for the bioremediation of excess nutrients in the river water, and the best results demonstrated the removal of 65% of the total nitrogen and up to 55% of the total phosphorus, both of which were fixed in the biomass [32].

Compared to other systems, such as tanks and photobioreactors (Fig. 2), the algae turf scrubber system is an alternative for the final treatment of wastewater. The turf scrubber system offers numerous advantageous characteristics, such as temperature control in regions with

high solar incidence and the development of a microorganism community using microalgae, other bacteria, and fungi that promote nutrient removal. Under these conditions, it is possible to obtain biomass with the potential for producing biofuels. However, sufficient levels of oil in the biomass are an important consideration for the production of other biofuels, such as bioethanol, bio-oil, and biogas, among others, which would achieve the complete exploitation of the biomass.

Considering the possibility of using all the biomass, photobioreactors can be used to produce feedstock for biofuel, such as biodiesel and bioethanol, because the oil level of the biomass produced in closed systems is greater than in open systems. Table 1 shows the results obtained using a mixed system and a similar tubular photobioreactor with microalgae *Desmodesmus subspicatus* in the same effluent [36-37].



Figure 2. A) Mixed system prototype for microalgae production using a (1) scrubber, (2) tank, and (3) photobioreactor. B) Microalgae biomass in a mixed system separated by electroflotation [36].

Parameters	Mixed system		Photobioreactor	
	without CO_2	with CO_2	without CO_2	with CO_2
Cultivation Days	20	15	7	7
Maximum Cell Division (x10^6 cell mL^{-1})	25.48 ± 0.02	26.97 ± 0.21	8.49 ± 1.02	25.98± 1.57
Average Cell Division (K)	0.29 ± 0.48	0.16 ± 0.33	-0.12 ± 0.60	0.34 ± 0.40
Biomass (g L^{-1})	0.62 ± 0.11	0.72 ± 0.15	0.18 ± 5.65	1.41 ± 1.40
Lipids (%)	1.36 ± 0.29	6.07 ± 0.12	18.73 ± 0.25	12.00 ± 0.28

Table 1. Microalgae biomass growth and total lipids in a mixed system and a tubular photobioreactor [36-37].

The removal of nutrients from the effluent produced excellent results using the genus *Scenedesmus*, as shown in Table 2. Other studies have also produced promising results. According to Ai *et al*. [38], the cultivation of *Spirulina platensis* in photobioreactors was satisfactory because of the photosynthetic performance. The pH, temperature, and dissolved oxygen levels

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were controlled effectively; however, continuous operation was required to ensure the reliability of photosynthetic performance in the photobioreactor.

The cultivation of the diatom *Chaetoceros calcitrans* in photobioreactors exhibited high growth rates; the maximum specific growth rate (μ) achievable was 9.65×10^{-2} h^{-1} and 8.88×10^{6} cells mL^{-1} in semicontinuous and batch systems, respectively. Even with a lower incidence of light, the results for the production of biomass were good [39].

The cultivation of microalgae *Chlorella* sp. in a semicontinuous photobioreactor produced a satisfactory level of biomass production (1.445 ± 0.015 g L^{-1} of dry cells). The growth, productivity and the amount of CO_2 removed obtained under conditions of increased control of the culture and a high concentration of inoculum using cells already adapted to the system increased the CO_2 assimilation[33]. The growth rate is also influenced by the concentration of microalgae until reaching an optimum concentration under the operational conditions used [40].

Therefore, microalgae can produce 3-10 times more energy per hectare than other land cultures and are associated with CO_2 mitigation and wastewater depollution [41]. Microalgae production is a promising alternative to land plants for reducing environmental impacts; however, the optimization of a number of the production parameters that are important for the viability of the process must be considered, such as the increase in lipid production [7].

Microalgae	System	Removal (%)	
		Nitrogen	Phosphorus
Melosira sp.; *Lygnbya* sp.; *Spirogyra* sp.; *Ulothrix* sp.; *Microspora* sp.; *Claophora* sp.; (seasonal succession) [32]	Turf scrubber	65	45-55
Chlorella sp.; *Euglena* sp.; *Spirogyra* sp.; *Scenedesmus* sp.; *Desmodesmus* sp.; *Pseudokirchneriella* sp.; *Phormidium* sp.; *Nitzschia* sp.[36]	Mix	99	65
Scenedesmus sp. [42]	Photobioreactor	98	98
Scenedesmus sp. [43]	Immobilized cell	70	94
Chlamydomonas sp. [44]	Photobioreactor	100	33
Scenedesmus obliquus [45]	Immobilized cell	100	-
Scenedesmus obliquus [46]	Photobioreactor	100	98

Table 2. Use of microalgae grown in different systems for the removal of nitrogen and phosphorus from wastewater.

The bioremediation of wastewater using microalgae is a promising option because it reduces the application of the chemical compounds required in conventional mechanical methods, such as centrifugation, gravity settling, flotation, and tangential filtration [21].

The feasibility of using microalgae for bioremediation is directly related to the production of biofuels because of the high oil content. Without the high oil levels, using other bacteria for

this purpose would be more advantageous because there are limitations to the removal of organic matter by microalgae. In the literature, emphasis is placed on the ability of microalgae to remove heavy metals from industrial effluents [47].

3. Biofuels

The term biofuel refers to solid, liquid, or gaseous fuels derived from renewable raw materials. The use of microalgal biomass for the production of energy involves the same procedures used for terrestrial biomass. Among the factors that influence the choice of the conversion process are the type and amount of raw material biomass, the type of energy desired, and the desired economic return from the product [30].

Microalgae have been investigated for the production of numerous biofuels including biodiesel, which is obtained by the extraction and transformation of the lipid material, bioethanol, which is produced from the sugars, starch, and carbohydrate residues in general, biogas, and bio-hydrogen, among others (Fig. 3) [8].

Between 1978 and 1996, the Office of Fuels Development at the U.S. Department of Energy developed extensive research programs to produce renewable fuels from algae. The main objective of the program, known as The Aquatic Species Program (ASP), was to produce biodiesel from algae with a high lipid content grown in tanks that utilize CO_2 waste from coal-based power plants. After nearly two decades, many advances have been made in manipulating the metabolism of algae and the engineering of microalgae production systems. The study included consideration of the production of fuels, such as methane gas, ethanol and biodiesel, and the direct burning of the algal biomass to produce steam or electricity [48].

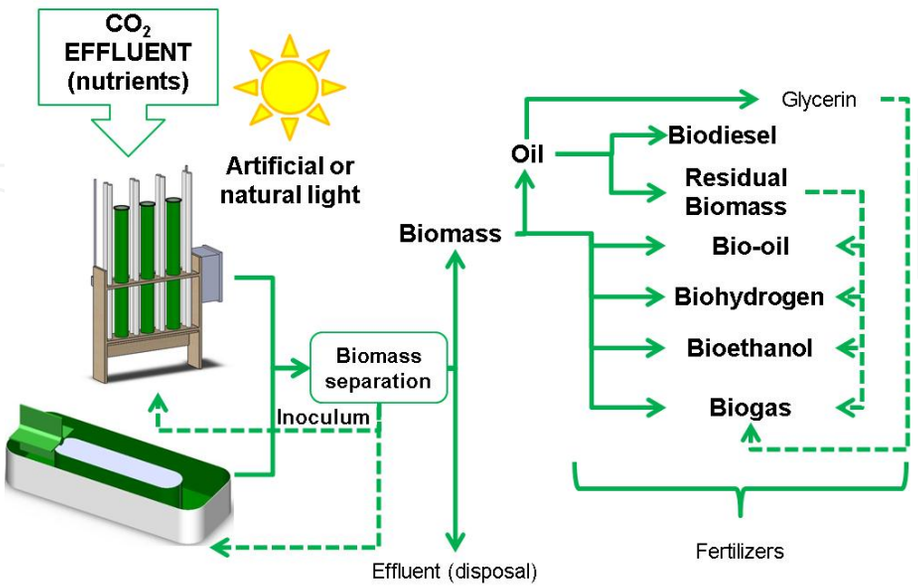


Figure 3. Utilization scheme for the microalgae biomass produced in wastewater.

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3.1. Biodiesel

The choice of raw material is a critical factor contributing to the final cost of biodiesel andaccounts for 50-85% of the total cost of the fuel. Therefore, to minimize the cost of this biofuel, it is important to assess the raw material in terms of yield, quality, and the utilization of the by-products [49-50].

A positive aspect of the production of biodiesel from microalgae is the area of land needed for production. For example, to supply 50% of the fuel used by the transportation sector in the U.S. using palm oil, which is derived from a plant with a high oil yield per hectare, would require 24% of the total agricultural area available in the country. In contrast, if the oil from microalgae grown in photobioreactors was used, it would require only 1-3% of the total cultivation area [49].

The biochemical composition of the algal biomass can be manipulated through variations in the growth conditions, which can significantly alter the oil content and composition of the microorganism [51]. Biodiesel produced from microalgae has a fatty acid composition (14 to 22 carbon atoms) that is similar to the vegetable oils used for biodiesel production [51-52].

The biodiesel produced from microalgae contains unsaturated fatty acids [53], and when the biomass is obtained from wastewater and is composed of a mixture of microalgae genera, it can exhibit various fatty acids profiles. Bjerk [36] produced biodiesel using a mixed system containing the microalgae genera *Chlorella* sp., *Euglena* sp., *Spirogyra* sp., *Scenedesmus* sp., *Desmodesmus* sp., *Pseudokirchneriella* sp., *Phormidium* sp. (cyanobacteria), and *Nitzschia* sp., identified by microscopy in accordance with Bicudo and Menezes [54]. The CO_2 input, the stress exerted by the nutrient composition, and the existence of a screen to fix the filamentous algae contributed to differential growth and differences in the fatty acid profiles (Table 3). Consequently, the biodiesel produced was relatively stable in the presence of oxygen.

In this mixed system, a difference between the fatty acid profiles of the biomass obtained in the photobioreactor compared to the biomass obtained on the screen was observed. The biomass from the screen contained the filamentous algae genera, and the oil did not contain linoleic acid.

This observation is important for biodiesel production because the oil produced was less unsaturated. The iodine index reflects this trend; oils from species such as *Spirulina maxima and Nanochloropsis* sp. have iodine indices between 50 and 70 mg I_2 g^{-1} of oil, whereas in species such as *Dunaliella tertiolecta* and *Neochloris oleobundans*, the iodine index is greater than 100 mg I_2 g^{-1} of oil [56].

The composition and proportion of fatty acids in the microalgae oil depends on the species used, the nutritional composition of the medium, and other cultivation conditions [57].

Table 4 shows the microalgae commonly used for oil production. The literature lacks information regarding the iodine index or the composition of saturated and unsaturated fatty acids, which could help identify the appropriate microalgae species for biodiesel production. Information on numerous parameters is important, such as the oil unsaturation levels, the productivity of the microalgae in the respective effluents, the growth rate, and the total

biomass composition. Using this information, a decision can be made regarding the economic and environmental feasibility of producing biodiesel and adequately allocating the waste.

Fatty acids*	without CO2 (%)	with CO2 (%)	with CO_2 (screen) (%)
Caprylic (C8:0)	0.05	0.08	-
Myristic (C14:0)	1.93	1.60	1.85
Pentadecanoic (C15:0)	0.50	0.44	0.52
Palmitoleic (C16:1)	1.28	2.02	4.20
Palmitic (C16:0)	29.58	24.68	32.50
Margaric (C17:0)	0.89	0.62	1.02
Linoleic (C 18:2)	15.12	9.51	-
Oleic (C 18:1n-9)	26.60	39.94	20.19
Estearic (C 18:0)	9.75	9.69	12.16
Araquidic (C 20:0)	0.70	1.43	1.72
Saturated and unsaturated not identified**	13.6	9.97	25.84

*The oil extraction method was adapted from the Bligh and Dyer (1959) method described by Gressler [37] using *Desmodesmussubspicatus* and the transesterification method described by Porte *et al.* [55] on a laboratorial scale.

Table 3. Relative proportion (%) of fatty acid methyl esters found in microalgae biomass cultivated in wastewater with and without CO_2 in a mixed system.

Among the microalgae shown in Table 4 that have an oil content that makes them competitive with land crops, twelve species (*Achnanthes sp., Chlorella sorokiniana, Chlorella* sp., *Chlorella vulgaris, Ellipsoidion sp., Neochloris oleoabundans, Nitzschia sp., Scenedemus quadricauda, Scenedemus sp., Schizochytrium sp., Skeletonoma costatum, and Skeletonoma sp.*) are from fresh water and can be investigated for the bioremediation of common urban and industrial effluents that do not have high salinity and contain pollutants that can be used as nutrients for the microorganisms. Because of their potential for oil production, a number of these microalgae species have been used for the production of biodiesel on a laboratory scale, although their potential industrial use associated with the bioremediation of industrial effluents is unknown. Studies using *Chlamydomonas* sp. [47] cultured in wastewater produced a rate of 18.4% oil and a fatty acid profile suitable for biodiesel production in addition to an excellent rate of nutrient removal (nitrogen and phosphorus).

Microalgae	Oil (%)	Microalgae	Oil (%)
Achnanthes sp.	44.5	*Nannochloris* sp.	20.0–35.0
Ankistrodesmus sp.	24.0–31.0	*Nannochloropsis oculata*	22.7–29.7
Botryococcus braunii	25–75	*Nannochloropsis* sp.	12.0-68.0
Chaetoceros calcitrans	39.8	*Neochloris oleoabundans*	35.0–54.0
Chaetoceros muelleri	33.6	*Nitzschia* sp.	45.0–47.0
Chlorella sorokiniana	19.3	*Phaeodactylum tricornutum*	18.7
Chlorella sp.	18.7–32	*Pavlova lutheri*	35.5 40.2
Chlorella vulgaris	19.2	*Pavlova salina*	30.9- 49.4
Chlorococcum sp.	19.3	*Phaeodactylum tricornutum*	18.0–57.0
Chlamydomonas sp.	18.4	*Synechocystis aquatilis*	18.5
Crypthecodinium cohnii	20.0	*Scenedemus quadricauda*	18.4
Cylindrotheca sp.	16–37	*Scenedemus* sp.	21.1
Dunaliella primolecta	23.0	*Schizochytrium* sp.	50.0–77.0
Ellipsoidion sp.	27.4	*Skeletonoma costatum*	21.0
Heterosigma sp.	39.9	*Skeletonoma* sp.	31.8
*Isochrysis*sp.	22.4-33	*Tetraselmis sueica*	15.0–23.0
Isochrysis galbana	7.0-40.0	*Thalassioria pseudonana*	20.6
Monallanthus salina	>20.0	*Thalassiosira* sp.	17.8

Adapted from [5,16,44,52,58-60], considering the values found under the respective production condition.

Table 4. Oil-producing microalgae with potential for biodiesel production.

3.2. Bioethanol

Bioethanol production from microalgae has received remarkable attention because of the high photosynthetic rates, the large biodiversity and variability of their biochemical composition, and the rapid biomass production exhibited by these microorganisms [1].

Furthermore, bioethanol derived from microalgae biomass is an option that demonstrates the greatest potential. John *et al*. [61] assessed microalgae biomass as a raw material for bioethanol production and argued that it is a sustainable alternative for the production of renewable biofuels. Examples of the genera of microalgae that fit the parameters for bioethanol production include the following: *Chlorella, Dunaliella, Chlamydomonas, Scenedesmus, Arthrospira,* and *Spirulina.* These microorganisms are suitable because they contain large amounts of starch and glycogen, which are essential factors for the production of bioethanol. The carbohydrate composition of these genera can be 70% of the biomass [62].

Traditionally, bioethanol is produced through the fermentation of sugar and starch, which are produced from different sources, such as sugarcane, maize, or a number of other grains [62].

After the oil extraction, the residual biomass contains carbohydrates that can be used for bioethanol production. This process represents a second-generation bioethanol and may be an alternative to the sugar cane ethanol produced in Brazil and corn or beet ethanol produced in other countries. The process requires pretreatment with a hydrolysis step before fermentation [63-65].

In bioethanol production, the processes vary depending on the type of biomass and involve the pretreatment of the biomass, saccharification, fermentation, and recovery of the product. The pretreatment of the biomass is a critical process because it is essential for the formation of the sugars used in the fermentation process (Table 5). Before the traditional fermentation process, acid hydrolysis is widely used for the conversion of carbohydrates from the cell wall into simple sugars. The acid pretreatment is efficient and involves low energy consumption [63].

Other techniques, such as enzymatic digestion [74] or gamma radiation [75], are interesting alternatives for increasing the chemical hydrolysis to render it more sustainable. Through analysis of the process in terms of energy, mass, and residue generation, it is possible to determine the best route. With enzymatic hydrolysis, the process can be renewable. Another technique for pretreatment of the biomass is hydrolysis mediated by fungi. Bjerk [36] investigated the *Aspergillus* genera for this purpose, and the bioethanol produced was monitored by gas chromatography using a headspace autosampler. The study demonstrated that seven strains (four isolates from *A. niger*, one from *A. terreus*, one from *A. fumigatus*, and one from *Aspergillus* sp.) were more efficient at hydrolyzing the residual biomass.

However, it is worth noting the importance of developing a well-designed and efficient system for the cultivation of these microorganisms, which can remove compounds that cause impurities in the final product. In addition, more studies should be undertaken to select strains that are resistant to adverse conditions, especially studies related to genetic engineering.

According to Yoon *et al*. [75], the use of gamma radiation is of potential interest for the hydrolysis of the microalgae biomass because compared to chemical or enzymatic digestion, gamma radiation raised the concentration of sugar reducers, and the saccharification yield was 0.235 g L^{-1} when gamma radiation was combined with acid hydrolysis. Acid hydrolysis alone produced a saccharification yield of only 0.017 g L^{-1}.

Microalgae	Pre treatment	Reaction condition		Fermenter	Bioethanol yield (%)	Ref.
		Temp. (°C)	Time (min)			
*Chlamydomonas reinhardtii**	acid	110	30	*Saccharomyces cerevisiae*	29.2	[66]
Chlorococcum sp.	alkaline	120	30	*Saccharomyces cerevisiae*	26.1	[67]
	acid	140	30	*Saccharomyces cerevisiae*	10-35	[68]
Chlorococcum humicola	acid	160	15	*Saccharomyces cerevisiae*	52	[63]
*Nizimuddinia zanardini***	acid	120	45	-	-	[69]
Kappaphycus alvarezii	acid	100	60	*Saccharomyces cerevisiae*	2.46	[70]
*Scenedesmus obliquus****	acid	120	30	-	-	[71]
Spirogyra	alkaline	-	120	*Saccharomyces cerevisiae*	20	[72]
	enzymatic	-	-	*Saccharomyces cerevisiae*	4.42	[73]
	enzymatic	-	-	*Zymomonas mobilis*	9.7	

Glucose yield: * 58%; **70.2%; *** 14.7%

Table 5. Conditions of bioethanol production from microalgae.

3.3. Other biofuels

Several articles describe the thermochemical processing of algal biomass using gasification [63,76] liquefaction [77], pyrolysis [78], hydrogenation [79], and biochemical processing, such as fermentation [80-81]. However, engineering processes have not been investigated as a potential biotechnological method for the production of other biofuels from microalgae.

Currently, the energy derived from biomass is considered one of the best energy sources and can be converted into various forms depending on the need and the technology used, and biogas is chief among the forms of energy produced by biomass. [82].

Anaerobic digestion for biogas production is a promising energy route because it provides numerous environmental benefits. Biogas is produced through the anaerobic digestion of organic waste, drastically reducing the emission of greenhouse gases. As an added benefit, the

by-products of fermentation, which are rich in nutrients, can be recycled for agricultural purposes. Adding anaerobic digestion to the use of biomass waste from which the oil has been removed produces an environmental gain and results in the complete exhaustion of the possible uses for the biomass. This strategy enables biomass waste to be an end-of-pipe technology for industrial processes that generate high amounts of organic matter containing phosphorus and nitrogen. A proposed system for this purpose is shown in Figure 4, which represents a simplification of the work performed by Chen *et al*. [83] and Ehimen *et al.* [84].

Therefore, using the residual microalgae biomass as a source of biogas is similar to other agricultural residue uses [85] in which the organic substrate is converted into biogas through anaerobic digestion, producing a gas mixture containing a higher percentage of carbon dioxide and methane [86].

The use of microalgae for biomethane production is significant because fermentation exhibits high stability and high conversion rates, which makes the process of bioenergy production more economically viable. For example, Feinberg (1984) (cited in Harun*et al*. [87]) considered exploiting *Tetraselmissuecica* for biomethane production in conjunction with the possibilities of producing other biofuels. The production of the following biofuels were proposed: biomethane alone (using total protein, carbohydrate, and lipids); biomethane and bioethanol (using carbohydrate for bioethanol production and protein and lipids for biomethane production); biomethane and biodiesel (using carbohydrate and protein for biomethane production and lipids for biodiesel production); and biomethane, biodiesel, and biomethanol (using carbohydrate for bioethanol production; lipids for biodiesel production, and proteins for biomethane production).

Harun *et al*. [47] also reported that the main factors influencing the process are the amount of the organic load, the temperature of the medium, the pH, and the retention time in the bioreactors, with long retention periods combined with high organic loads exhibiting greater effectiveness for biomethane production.

Converti *et al*. [82] demonstrated this effect, reporting the increased production of total biogas at 0.39 ± 0.02 m^3 kg^{-1} of dissolved organic carbon after 50 days of maturation and 0.30 ± 0.02 m^3 of biomethane.

When considering total biomass use, in addition to biogas, it is possible to produce biohydrogen and bio-oils using enzymatic and chemical processes.

The chemical processes that can be used for hydrogen production include gasification, partial oxidation of oil, and water electrolysis. In the literature, cyanobacteria are primarily used for the production of biohydrogen through a biological method, and the reaction is catalyzed by nitrogenases and hydrogenases [88]. Studies with *Anabaena* sp. also demonstrate that this biomass is promising for the production of biohydrogen and that adequate levels of air, water, minerals, and light are necessary because the process can be photosynthetic [9,89].

Bio-oil can be produced from any biomass, and for microalgae, a number of investigations have been performed using *Chlamydomonas, Chlorella*, *Scenedesmus* [90], *Chlorella vulgaris* [91-92], *Scenedesmus dimorphus, Spirulina platensis, Chlorogloeopsis fritschiiwer* [91], *Nannochloropsis oculata* [93], *Chlorella minutissima* [94], and *Dunaliella tertiolecta* [10].

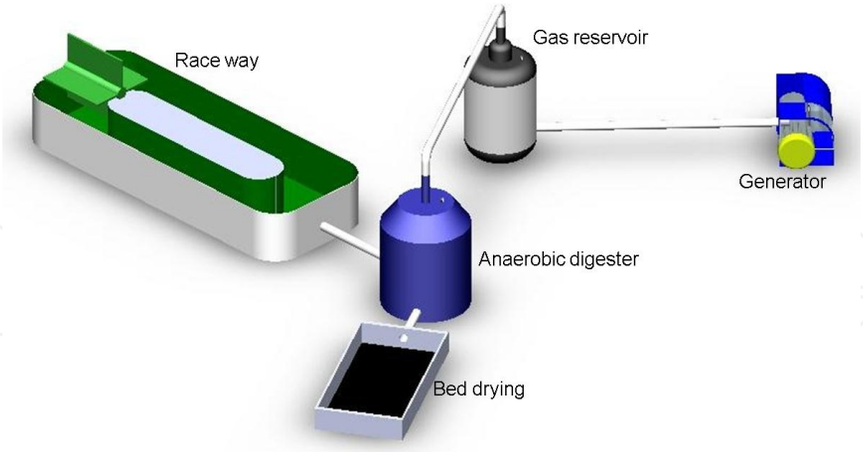


Figure 4. Anaerobic digestion of biomass waste in a unit of bioenergy production associated with an effluent treatment plant.

These initiatives highlight the potential use of hydrothermal liquefaction, which is a process that converts the biomass into bio-oil at a temperature range of 200-350°C and pressures of 15-20 MPa. According to Biller et al. [91], yields of 27-47% are possible, taking into account that microalgae can be produced using recycled nutrients, providing greater sustainability to the system.

A different bio-oil can be produced using pyrolysis in which the oil composition features compounds exhibiting boiling points lower than the hydrothermal liquefaction product [93]. In pyrolysis, the nitrogen content of the microalgae is converted into NOx during combustion. NOx is an undesirable emission that increases depending on the microalgae and their protein content; however, NOx emissions can be reduced by 42% using a hydrothermal pretreatment process.

In terms of waste recovery, the use of *Dunaliella tertiolecta* cake under various catalyst dosage conditions, temperatures, and times were used in hydrothermal liquefaction, and the yield was 25.8% using 5% sodium carbonate as catalyst at 360°C [10].

Therefore, in addition to producing microalgae in urban or industrial effluents, it is possible that after the extraction of the oil for biodiesel production and the production of bioethanol from carbohydrates, biogas or bio-oil can be produced from the waste material.

4. Conclusions

This chapter reviews the initiatives for biofuel production from microalgae cultivated in wastewaters. The exploitation of the total microalgae biomass was considered, and the potential for biodiesel and bioethanol production was explored.

The various systems for microalgae production using wastewater and the consequences for biodiesel and bioethanol production were discussed in detail.

Microalgae have been used to produce biodiesel and bioethanol with excellent results; however, the use of microalgae must be expanded to include bioremediation combined with biofuel production. The commercial initiatives for this purpose will depend on the composition and volume of the effluent, on the selected microalgae species, and on the temperature and light conditions of the region. The initiatives will also depend on the particular biofuel of interest to the region or that required for local consumption. Therefore, each situation must be analyzed on an individual basis, and there is no single model; however, because of the wide biodiversity of microalgae and the extensive ongoing research capacity of many countries, it is likely that a conditions for viable microalgae production can be achieved anywhere.

Finally, it should be noted that microalgae that are adapted to the environment could produce biomass that, depending on the composition of cells, can be used as the raw material for the production of one or more biofuels.

The research and development of microalgae production in urban or industrial effluents involve principles of sustainable development, clean technology, and the ecology of the productive sectors, prioritizing preventive and remediation steps with the decreased use of energy and inputs. Therefore, there is an emphasis on the methods of treatment, the transformation processes, and the biotechnological products (biofuels), prioritizing the use of wastewater for biomass and bioenergy production. These developments will decrease the impact on activities of anthropogenic origin from the industrial, commercial and service sectors, among others.

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