We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Potential Production of Biofuel from Microalgae Biomass Produced in Wastewater

Rosana C. S. Schneider, Thiago R. Bjerk,
Pablo D. Gressler, Maiara P. Souza,
Valeriano A. Corbellini and Eduardo A. Lobo

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/52439>

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 CO₂ 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, bio-coils, 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

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 crops for 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].

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 biofloculation 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 CO₂ 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, CO₂ 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

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 $9.65 \times 10^{-2} \text{ h}^{-1}$ and $8.88 \times 10^6 \text{ cells mL}^{-1}$ 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 ($1.445 \pm 0.015 \text{ g L}^{-1}$ 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
<i>Melosira</i> sp.; <i>Lygnbya</i> sp.; <i>Spirogyra</i> sp.; <i>Ulothrix</i> sp.; <i>Microspora</i> sp.; <i>Claophora</i> sp.; (seasonal succession) [32]	Turf scrubber	65	45-55
<i>Chlorella</i> sp.; <i>Euglena</i> sp.; <i>Spirogyra</i> sp.; <i>Scenedesmus</i> sp.; <i>Desmodesmus</i> sp.; <i>Pseudokirchneriella</i> sp.; <i>Phormidium</i> sp.; <i>Nitzschia</i> sp.[36]	Mix	99	65
<i>Scenedesmus</i> sp. [42]	Photobioreactor	98	98
<i>Scenedesmus</i> sp. [43]	Immobilized cell	70	94
<i>Chlamydomonas</i> sp. [44]	Photobioreactor	100	33
<i>Scenedesmus obliquus</i> [45]	Immobilized cell	100	-
<i>Scenedesmus obliquus</i> [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.

3.1. Biodiesel

The choice of raw material is a critical factor contributing to the final cost of biodiesel and accounts 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 CO₂ 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 I₂ g⁻¹ of oil, whereas in species such as *Dunaliella tertiolecta* and *Neochloris oleabundans*, the iodine index is greater than 100 mg I₂ 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
Stearic (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 *Desmodesmus subspicatus* 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 CO₂ 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 (%)
<i>Achnanthes</i> sp.	44.5	<i>Nannochloris</i> sp.	20.0–35.0
<i>Ankistrodesmus</i> sp.	24.0–31.0	<i>Nannochloropsis oculata</i>	22.7–29.7
<i>Botryococcus braunii</i>	25–75	<i>Nannochloropsis</i> sp.	12.0-68.0
<i>Chaetoceros calcitrans</i>	39.8	<i>Neochloris oleoabundans</i>	35.0–54.0
<i>Chaetoceros muelleri</i>	33.6	<i>Nitzschia</i> sp.	45.0–47.0
<i>Chlorella sorokiniana</i>	19.3	<i>Phaeodactylum tricornutum</i>	18.7
<i>Chlorella</i> sp.	18.7–32	<i>Pavlova lutheri</i>	35.5 40.2
<i>Chlorella vulgaris</i>	19.2	<i>Pavlova salina</i>	30.9- 49.4
<i>Chlorococcum</i> sp.	19.3	<i>Phaeodactylum tricornutum</i>	18.0–57.0
<i>Chlamydomonas</i> sp.	18.4	<i>Synechocystis aquatilis</i>	18.5
<i>Cryptocodinium cohnii</i>	20.0	<i>Scenedemus quadricauda</i>	18.4
<i>Cylindrotheca</i> sp.	16–37	<i>Scenedemus</i> sp.	21.1
<i>Dunaliella primolecta</i>	23.0	<i>Schizochytrium</i> sp.	50.0–77.0
<i>Ellipsoidion</i> sp.	27.4	<i>Skeletonoma costatum</i>	21.0
<i>Heterosigma</i> sp.	39.9	<i>Skeletonoma</i> sp.	31.8
<i>Isochrysis</i> sp.	22.4-33	<i>Tetraselmis sueica</i>	15.0–23.0
<i>Isochrysis galbana</i>	7.0-40.0	<i>Thalassioria pseudonana</i>	20.6
<i>Monallanthus salina</i>	>20.0	<i>Thalassiosira</i> 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)			
<i>Chlamydomonas reinhardtii</i> *	acid	110	30	<i>Saccharomyces cerevisiae</i>	29.2	[66]
<i>Chlorococcum</i> sp.	alkaline	120	30	<i>Saccharomyces cerevisiae</i>	26.1	[67]
	acid	140	30	<i>Saccharomyces cerevisiae</i>	10-35	[68]
<i>Chlorococcum humicola</i>	acid	160	15	<i>Saccharomyces cerevisiae</i>	52	[63]
<i>Nizimuddinia zanardini</i> **	acid	120	45	-	-	[69]
<i>Kappaphycus alvarezii</i>	acid	100	60	<i>Saccharomyces cerevisiae</i>	2.46	[70]
<i>Scenedesmus obliquus</i> ***	acid	120	30	-	-	[71]
<i>Spirogyra</i>	alkaline	-	120	<i>Saccharomyces cerevisiae</i>	20	[72]
	enzymatic	-	-	<i>Saccharomyces cerevisiae</i>	4.42	[73]
	enzymatic	-	-	<i>Zymomonas mobilis</i>	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 *Tetraselmis suecica* 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 bioethanol (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.

Converiti *et al.* [82] demonstrated this effect, reporting the increased production of total biogas at $0.39 \pm 0.02 \text{ m}^3 \text{ kg}^{-1}$ of dissolved organic carbon after 50 days of maturation and $0.30 \pm 0.02 \text{ m}^3$ 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 fritschii* [91], *Nannocloropsis 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 NO_x during combustion. NO_x is an undesirable emission that increases depending on the microalgae and their protein content; however, NO_x 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.

Acknowledgements

The National Council of Technological and Scientific Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq), the National Council for the Improvement of Higher Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES) and the University of Santa Cruz do Sul Research Foundation (Fundo de Apoio à Pesquisa da Universidade de Santa Cruz do Sul, FAP/UNISC)

Author details

Rosana C. S. Schneider, Thiago R. Bjerk, Pablo D. Gressler, Maiara P. Souza, Valeriano A. Corbellini and Eduardo A. Lobo

Environmental Technology Post-Graduation Program, University of Santa Cruz do Sul, UNISC, Brazil

References

- [1] Derner, R. B. et al. Microalgae, products and applications (Microalgas, produtos e aplicações). *Ciência Rural* 2006; 36(6) 1959-1967. <http://dx.doi.org/10.1590/S0103-84782006000600050>
- [2] Richmond, A. Handbook of microalgal culture: biotechnology and applied phycolgy. Oxford: Blackwell Science, 2004.
- [3] Borowitzka, M. A. Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology* 1999; 70,(1-3) 313-321. [http://dx.doi.org/10.1016/S0168-1656\(99\)00083-8](http://dx.doi.org/10.1016/S0168-1656(99)00083-8)
- [4] Gressler, P., Schneider, R. C S., Corbellini, V. A., Bjerk T., Souza M. P., Zappe A., Lobo, E. A. Microalgas: aplicações em biorremediação e energia. In English: Microalgae: Applications in bioremediation na energy. *Caderno de Pesquisa, Série Biologia*, 2012; 24, 1, 48-67. www.bioline.org.br/pdf?cp12004.
- [5] Chisti, Y. Biodiesel from microalgae. *Biotechnology Advances*, v. 25, n. 3, p. 294-306, <http://dx.doi.org/10.1016/j.biotechadv.2007.02.001>, 2007.
- [6] Harun, R. et al. Microalgal biomass as a cellulosic fermentation feedstock for bioethanol production. *Renewable and Sustainable Energy Reviews* 2010. <http://dx.doi.org/10.1016/j.rser.2010.07.071>
- [7] Jiang, L. et al. Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO₂. *Applied Energy* 2011; 88(10) 3336-3341. <http://dx.doi.org/10.1016/j.apenergy.2011.03.043>
- [8] Demirbas, M. F. Biofuels from algae for sustainable development. *Applied Energy* 2011;88(10) 3473-3480. <http://dx.doi.org/10.1016/j.apenergy.2011.01.059>
- [9] Marques A. E. et al. Biohydrogen production by *Anabaena* sp. PCC 7120 wild-type and mutants under different conditions: Light, nickel, propane, carbon dioxide and nitrogen. *Biomass and Bioenergy* 2011;35(10) 4426-4434. <http://dx.doi.org/10.1016/j.biombioe.2011.08.014>
- [10] Shuping, Z. et al. Production and characterization of bio-oil from hydrothermal liquefaction of microalgae *Dunaliella tertiolecta* cake. *Energy* 2010; 35(12) 5406-5411. <http://dx.doi.org/10.1016/j.energy.2010.07.013>,
- [11] Lopes, E. J. Carbon dioxide sequestration in photobioreactors (Seqüestro de dióxido de carbono em fotobiorreatores). 2007. Thesis. Graduate Program in Chemical Engineering (Programa de Pós-Graduação: Engenharia Química-Mestrado e Doutorado) – School of Chemical Engineering at Campinas State University (Faculdade de Engenharia Química da Universidade Estadual de Campinas), Campinas, 2007.
- [12] Brown, M. R. et al. Nutritional aspects of microalgae used in mariculture. *Aquaculture* 1997;151(1-4) 315-331. [http://dx.doi.org/10.1016/S0044-8486\(96\)01501-3](http://dx.doi.org/10.1016/S0044-8486(96)01501-3)

- [13] Spolaore, P. et al. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* 2006;101(2) 87-96. <http://dx.doi.org/10.1263/jbb.101.87>
- [14] Hirano, A. et al. Temperature effect on continuous gasification of microalgal biomass: theoretical yield of methanol production and its energy balance. *Catalysis Today* 1998; 45 (1-4) 399-404. [http://dx.doi.org/10.1016/S0920-5861\(98\)00275-2](http://dx.doi.org/10.1016/S0920-5861(98)00275-2).
- [15] Chen C. et al. Thermogravimetric analysis of microalgae combustion under different oxygen supply concentrations. *Applied Energy* 2011;88(9) 3189-3196.<http://dx.doi.org/10.1016/j.apenergy.2011.03.003>
- [16] Demirbas, A, FatihDemirbas, M. Importance of algae oil as a source of biodiesel. *Energy Conversion and Management* 2011;52(1)163-170. <http://dx.doi.org/10.1016/j.enconman.2010.06.055>
- [17] Harmelen, Van T, Oonk H. Microalgae biofixation processes: applications and potential contributions to greenhouse gas mitigation options.TNO:Eni Technologie;2006. <http://www.fluxfarm.com/uploads/3/1/6/8/3168871/biofixation.pdf> (accessed 18 July 2012).
- [18] Farhadian, M. et al. In situ bioremediation of monoaromatic pollutants in groundwater: a review. *Bioresource Technology* 2008;99(13) 5296-308. <http://dx.doi.org/10.1016/j.biortech.2007.10.025>,
- [19] Bashan, L. E. e Bashan, Y. Immobilized microalgae for removing pollutants: review of practical aspects. *Bioresource Technology* 2010;101(6) 1611-27. <http://dx.doi.org/10.1016/j.biortech.2009.09.043>,
- [20] Park, J. B. K. et al. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology* 2011;102(1) 35-42. <http://dx.doi.org/10.1016/j.biortech.2010.06.158>
- [21] Christenson, L, Sims, R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances* 2011;29(6) 686-702. <http://dx.doi.org/10.1016/j.biotechadv.2011.05.015>.
- [22] Gattullo, C. E. et al. Removal of bisphenol A by the freshwater green alga *Monoraphidiumbraunii* and the role of natural organic matter. *The Science of the Total Environment* 2012; 416, 501-506. <http://dx.doi.org/10.1016/j.biotechadv.2011.05.015>
- [23] Masroor, M. et al. An Overview of the Integration of Advanced Oxidation Technologies and Other Processes for Water and Wastewater Treatment. *International Journal of Engineering* 2009;3(2)120-146.
- [24] Fuchs, W. et al. Influence of standard wastewater parameters and pre-flocculation on the fouling capacity during dead end membrane filtration of wastewater treatment effluents. *Separation and Purification Technology*2006;52(1)46-52. <http://dx.doi.org/10.1016/j.seppur.2006.03.013>.

- [25] Chang, I.-S, Kim, S.-N. Wastewater treatment using membrane filtration—effect of biosolids concentration on cake resistance. *Process Biochemistry* 2005; 40(3-4) 1307-1314. <http://dx.doi.org/10.1016/j.procbio.2004.06.019>
- [26] Almeida, E. et al. Industrial effluent treatment via oxidation in the presence of ozone. (Tratamento de efluentes industriais por processos oxidativos na presença de ozônio.) *Quim. Nova* 2004; 279(5) 818-824. <http://dx.doi.org/10.1590/S0100-40422004000500023>
- [27] Daly, R. I. et al. Effect of chlorination on *Microcystisaeruginosa* cell integrity and subsequent microcystin release and degradation. *Environmental Science & Technology* 2007;41(12) 4447-4453.
- [28] Caron, D. A et al. Harmful algae and their potential impacts on desalination operations off southern California. *Water research* 2010;44;(2) 385-416. <http://dx.doi.org/10.1016/j.watres.2009.06.051>, 2010
- [29] Chinnasamy S, Bhatnagar A, Claxton R. Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth médium. *Bioresource Technology* 2010;101(17) 6751–6760. <http://dx.doi.org/doi:10.1016/j.biortech.2010.03.094>
- [30] Brennan, L, Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews* 2010;(14)2 557-577. <http://dx.doi.org/10.1016/j.rser.2009.10.009>
- [31] Pittman, J. K. et al. The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology* 2010;102(1) 17-25. <http://dx.doi.org/10.1016/j.biortech.2010.06.035>
- [32] Mulbry, W. et al. Toward scrubbing the bay: Nutrient removal using small algal turf scrubbers on Chesapeake Bay tributaries. *Ecological Engineering* 2010;36(4), 536-541. <http://dx.doi.org/10.1016/j.ecoleng.2009.11.026>
- [33] Chiu, S.-Y. et al. Reduction of CO₂ by a high-density culture of *Chlorella* sp. in a semicontinuousphotobioreactor. *Bioresource Technology* 2008;99(9) 3389-3396. <http://dx.doi.org/10.1016/j.biortech.2007.08.013>
- [34] Chiu, S.-Y. et al. The air-lift photobioreactors with flow patterning for high-density cultures of microalgae and carbon dioxide removal. *Engineering in Life Sciences* 2009;9(3) 254-260. <http://dx.doi.org/10.1002/elsc.200800113>
- [35] Muñoz, R. et al. Biofilm photobioreactors for the treatment of industrial wastewaters. *Journal of Hazardous Materials* 2009;161(1) 29-34. <http://dx.doi.org/10.1016/j.jhazmat.2008.03.018>
- [36] Bjerck, T. R. Microalgae cultivate in fotobiorreator and joint reactor objectiving the bio-remediation and production of biofuels (In Portuguese: Cultivo de microalgasemfo-

- tobiorreator e reatormistovisando a biorremediação e produção de biocombustíveis). Universidade de Santa Cruz do Sul, Santa Cruz do Sul-Rio Grande do Sul. 2012.
- [37] Gressler, P. D. Efficiency Evaluation of the *Desmodesmus subspicatus* (R. Chodat) E. Hegewald & A. Schmidt (Chlorophyceae) to grown in tubular photobioreactor with effluent from ETE-Unisc for bioremediation and energy generation (In Portuguese: Avaliação da eficiência de *Desmodesmus subspicatus* (R. Chodat) E. Hegewald & A. Schmidt (Chlorophyceae) cultivada em Fotobiorreator tubular com efluente da ETE-unisc, visando biorremediação e obtenção de energia). Universidade de Santa Cruz do Sul, Santa Cruz do Sul-Rio Grande do Sul. 2011.
- [38] Ai, W. et al. Development of a ground-based space micro-algae photo-bioreactor. *Advances in Space Research* 2008;41(5) 742–747. <http://dx.doi.org/10.1016/j.asr.2007.06.060>.
- [39] Krichnavaruk, S. et al. Optimal growth conditions and the cultivation of *Chaetoceros calcitrans* in airlift photobioreactor. *Chemical Engineering Journal* 2005;105(3) 91-98. <http://dx.doi.org/10.1016/j.cej.2004.10.002>
- [40] Vasumathi K.K. et al. Parameters influencing the design of photobioreactor for the growth of microalgae. *Renewable and Sustainable Energy Reviews* 2012;16(7) 5443-5450. <http://dx.doi.org/10.1016/j.rser.2012.06.013>
- [41] Demirbas, A. Use of algae as biofuel sources. *Energy Conversion and Management* 2010; 51(12) 2738-2749. <http://dx.doi.org/10.1016/j.enconman.2010.06.010>.
- [42] Xin, L. et al. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus* sp. LX1, growing in secondary effluent. *New Biotechnology* 2010;27(1) 59-63. <http://dx.doi.org/10.1016/j.nbt.2009.11.006>
- [43] Fierro, S. et al. Nitrate and phosphate removal by chitosan immobilized *Scenedesmus*. *Bioresource Technology* 2008;99(5) 1274-1279. <http://dx.doi.org/10.1016/j.biortech.2007.02.043>
- [44] Wu L.F. et al. The feasibility of biodiesel production by microalgae using industrial wastewater. *Bioresource Technology* 2012, 113 :14-18 .
- [45] Ruiz-Marin, A. et al. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology* 2010;101(1) 58-64. <http://dx.doi.org/10.1016/j.biortech.2009.02.076>
- [46] Martínez, M. E. et al. Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresource Technology* 2000;73(3) 263-272. [http://dx.doi.org/10.1016/S0960-8524\(99\)00121-2](http://dx.doi.org/10.1016/S0960-8524(99)00121-2)
- [47] Harun R. et al. Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews* 2010; 14(3) 1037–1047. <http://dx.doi.org/10.1016/j.rser.2009.11.004>.
- [48] Sheehan, J. et al. A Look Back at the US Department of Energy's Aquatic Species Program: biodiesel from algae. US Department of Energy's, Office of Fuels development:

- NREL/TP-580-24190.1998. <http://www.nrel.gov/docs/legosti/fy98/24190.pdf> (accessed 01 august 2012).
- [49] Soares, D. Assessment of cell growth and lipids productivity of marine microalgae in different cropping systems (In Portuguese: Avaliação do crescimento celular e da produtividade de lipídeos de microalgas marinhas em diferentes regimes de cultivo. 2010). 107 f. Thesis. Graduate Program in Biochemical Sciences. (Curso de Pós-Graduação em Ciências: Bioquímica – Mestrado e Doutorado) – Federal University of Paraná (Universidade Federal do Paraná), Curitiba, 2010.
- [50] Song, D. et al. Exploitation of oil-bearing microalgae for biodiesel. *Chinese Journal of Biotechnology* 2008; 24(3) 341-348. [http://dx.doi.org/10.1016/S1872-2075\(08\)60016-3](http://dx.doi.org/10.1016/S1872-2075(08)60016-3)
- [51] Qin J. Bio-hydrocarbons from algae—impacts of temperature, light and salinity on algae growth. Barton, Australia: Rural Industries Research and Development Corporation; 2005.
- [52] MATA, T. M. et al. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* 2010;14(1) 217-232. <http://dx.doi.org/10.1016/j.rser.2009.07.020>
- [53] Radmann, E. M., Costa, J. A. V. Lipid content and fatty acid composition of microalgae exposed to CO₂, SO₂ and NO (Conteúdo lipídico e composição de ácidos graxos de microalgas expostas aos gases CO₂, SO₂ e NO). *Química Nova* 2008;31(7) 1609–1612. <http://dx.doi.org/10.1590/S0100-40422008000700002>.
- [54] Bicudo, C., E., M., Menezes, M. Genus of algae from inland waters of Brazil, identification and description key (Gênero de algas de águas continentais do Brasil, chave para identificação e descrição). 2. ed. São Carlos-SP: RIMA, 2006.
- [55] Porte A. F, Schneider R. C. S, Kaercher J. A, Klamt R. A, Schmatz W. L, Silva W. L. T, Filho W. A. S. Sunflower biodiesel production and application in family farms in Brazil. *Fuel* 2010; 89(12) 3718–3724. <http://dx.doi.org/10.1016/j.fuel.2010.07.025>.
- [56] Gouveia L, Oliveira A. C. Microalgae as a raw material for biofuels Production. *J Ind Microbiol Biotechnol* 2009;36 269–274. <http://dx.doi.org/10.1007/s10295-008-0495-6>.
- [57] Guschina, I. A , Harwood, J. L. Lipids and lipid metabolism in eukaryotic algae. *Progress in Lipid Research* 2006;45(2) 160-186. <http://dx.doi.org/10.1016/j.plipres.2006.01.001>, 2006.
- [58] Doan, T. T. Y. et al. Screening of marine microalgae for biodiesel feedstock. *Biomass and Bioenergy* 2011;35(7) 2534-2544. <http://dx.doi.org/10.1016/j.biombioe.2011.02.021>.
- [59] Khan, S. A. et al. Prospects of biodiesel production from microalgae in India. *Renewable and Sustainable Energy Reviews* 2009;13(9) 2361–2372. <http://dx.doi.org/10.1016/j.rser.2009.04.005>

- [60] Kaiwan-arporn P, Hai P. D, Thu N. T, Annachhatre A. P. Cultivation of cyanobacteria for extraction of lipids. *Biomass and Bioenergy* 2012; 44 142-149. <http://dx.doi.org/10.1016/j.biombioe.2012.04.017>
- [61] John R. P, Anisha G.S, Nampoothiri K. M, Pandey A. Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresource Technology* 2011; 102(1) 186-193. <http://dx.doi.org/10.1016/j.biortech.2010.06.139>
- [62] Harun, R, Danquah, M. K. Enzymatic hydrolysis of microalgae biomass for bioethanol production. *Chemical Engineering Journal* 2011b;168(3) 1079-1084. <http://dx.doi.org/10.1016/j.cej.2011.01.088>.
- [63] Harun, R, Danquah, M. K. Influence of acid pre-treatment on microalgal biomass for bioethanol production. *Process Biochemistry* 2011a;46(1) 304-309. <http://dx.doi.org/10.1016/j.procbio.2010.08.027>
- [64] Balat, M. Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. *Energy Conversion and Management* 2010;52(2) 858-875. <http://dx.doi.org/10.1016/j.enconman.2010.08.013>
- [65] Goh, C. S, Lee, K. T. A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia. as an underlay for renewable and sustainable development. *Renewable and Sustainable Energy Reviews* 2010;14(2) 842-848. <http://dx.doi.org/10.1016/j.rser.2009.10.001>
- [66] Nguyen MT, Choi SP, Lee J, Lee JH, S. S. Hydrothermal acid pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production. *J Microbiol Biotechnol*. 2009;19(2) 161-166. <http://dx.doi.org/10.4014/jmb.0810.578>
- [67] Harun, R., Jason, W. S. Y., Cherrington, T., & Danquah, M. K. Exploring alkaline pretreatment of microalgal biomass for bioethanol production. *Applied Energy* 2010;88(10) 3464-3467. <http://dx.doi.org/10.1016/j.apenergy.2010.10.048>
- [68] Harun R, Liu B, Danquah M. K. Analysis of Process Configurations for Bioethanol Production from Microalgal Biomass. *Progress in Biomass and Bioenergy Production: In Tech*; 2011. DOI: 10.5772/17468
- [69] Yazdani, P., Karimi, K., & Taherzadeh, M. J. Improvement of Enzymatic Hydrolysis of A Marine Macro-Alga by Dilute Acid Hydrolysis Pretreatment. *Bioenergy Technology* 2011, 186-191. . <http://dx.doi.org/10.3384/ecp11057186>
- [70] Khambhaty, Y., Mody, K., Gandhi, M. R., Thampy, S., Maiti, P., Brahmabhatt, H., Eswaran, K., et al. (). *Kappaphycus alvarezii* as a source of bioethanol. *Bioresource Technology* 2012;103(1) 180-185. . <http://dx.doi.org/10.1016/j.biortech.2011.10.015>
- [71] Miranda, J. R., Passarinho, P. C., & Gouveia, L. Pre-treatment optimization of *Scenedesmus obliquus* microalga for bioethanol production. *Bioresource Technology* 2012;104 342-8. <http://dx.doi.org/doi:10.1016/j.biortech.2011.10.059>

- [72] Eshaq, F. S.; Ali, M. N.; Mohd, M. K. Spirogyra biomass a renewable source for bio-fuel (bioethanol) production. *International Journal of Engineering Science and Technology* 2010;2(12)7045-7054.
- [73] Mushlihah, S., Sunarto, E., Irvansyah, M. Y., &Utami, R. S. Ethanol Production from Algae Spirogyra with Fermentation by *Zymomonasmobilis* and *Saccharomyces cerevisiae*. *J. Basic. Appl. Sci. Res* 2011;1(7), 589-593.
- [74] Chen, R. et al. Use of an algal hydrolysate to improve enzymatic hydrolysis of ligno-cellulose. *Bioresource Technology* 2012;108(1) 149-154. <http://dx.doi.org/10.1016/j.biortech.2011.12.143>
- [75] Yoon, M. et al. Improvement of saccharification process for bioethanol production from *Undaria* sp. by gamma irradiation. *Radiation Physics and Chemistry* 2012;81(8) 999-1002. <http://dx.doi.org/10.1016/j.radphyschem.2011.11.035>
- [76] Elliott D.C., Sealock Jr. L.J., Chemical processing in high-pressure aqueous environments: Low temperature catalytic gasification. *Chemical Engineering Research and Design* 1996; 74(5): 563-566.
- [77] Tsukahara K., Sawayama S. Liquid fuel production using microalgae. *Jpn Petrol Inst* 2005; 48(5) 251–259.
- [78] Miao X. et al. Fast pyrolysis of microalgae to produce renewable fuels. *J Anal Appl Pyrolysis* 2004; 71(2) 855–863.
- [79] Amin S. Review on biofuel oil and gas production processes from microalgae. *Energy Conversion and Management* 2009; 50(7) 1834-1840. <http://dx.doi.org/10.1016/j.enconman.2009.03.001>.
- [80] Bently J.; Derby R. Ethanol. fuel cells: converging paths of opportunity. *Renewable-FuelsAssociation* 2008; http://www.ethanolrfa.org/objects/documents/129/rfa_fuel_cell_white_paper.pdf>. (Accessed 24 July, 2012).
- [81] Xu, H. et al. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *Journal of Biotechnology* 2006;126(4) 499–507. <http://dx.doi.org/10.1016/j.jbiotec.2006.05.002>
- [82] Converti, A. et al. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing: Process Intensification* 2009;48(6) 1146-1151. <http://dx.doi.org/10.1016/j.cep.2009.03.006>
- [83] Chen S., Chen B., Song D. Life-cycle energy production and emissions mitigation by comprehensive biogas–digestate utilization *Bioresource Technology* 2012; 114: 357-364. <http://dx.doi.org/10.1016/j.biortech.2012.03.084>.
- [84] Ehimen E.A., Holm-Nielsen J.-B., Poulsen M., Boelsmand J.E. Influence of different pre-treatment routes on the anaerobic digestion of a filamentous algae. *Renewable Energy* 2013; 50: 476-480. <http://dx.doi.org/10.1016/j.renene.2012.06.064>.

- [85] Bernard, O. Hurdles and challenges for modelling and control of microalgae for CO₂ mitigation and biofuel production. *Journal of Process Control* 2011; 21(10): 1378-1389. doi:10.1016/j.jprocont.2011.07.012
- [86] Abdel-Raouf N., Al-Homaidan A.A., Ibraheem I.B.M. Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences* 2012; 19(3): 257-275. <http://dx.doi.org/10.1016/j.sjbs.2012.04.005>
- [87] Harun, R. et al. Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. *Biomass and Bioenergy* 2010; 35(1) 741-747. <http://dx.doi.org/10.1016/j.biombioe.2010.10.007>
- [88] Tamagnini P., Leitão E., Oliveira P., Ferreira D., Pinto F., Harris D., Cyanobacterial hydrogenases. Diversity, regulation and applications. *FEMS Microbiol Rev* 2007; 31:692-720.
- [89] Ferreira A.F., Marques A. C., Batista A.P., Marques P. A.S.S., Gouveia L., Silva C. M. Biological hydrogen production by *Anabaena* sp. e Yield,energy and CO₂ analysis including fermentative biomass recovery. *International Journal of Hydrogen Energy* 2012; 37: 179 -190. doi:10.1016/j.ijhydene.2011.09.056
- [90] Bhatnagar A., Chinnasamy S., Singh M., Das K.C., Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. *Applied Energy* 2011; 88 (10): 3425-3431. <http://dx.doi.org/10.1016/j.apenergy.2010.12.064>
- [91] Biller P., Ross A.B., Skill S.C., Lea-Langton A., Balasundaram B., Hall C., Riley R., Llewellyn C.A. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process *Algal Research* 2012; 1: 70-76. <http://dx.doi.org/10.1016/j.algal.2012.02.002>
- [92] Tsukahara K., Kimura T., Minowa T., Sawayama S., Yagishita T., Inoue S., Hanaoka T., Usui Y., Ogi T., Microalgal cultivation in a solution recovered from the low-temperature catalytic gasification of the microalga. *Journal of Bioscience and Bioengineering* 2001; 91 (3) 311-313. [http://dx.doi.org/10.1016/S1389-1723\(01\)80140-7](http://dx.doi.org/10.1016/S1389-1723(01)80140-7)
- [93] Du Z., Mohr M., Ma X., Cheng Y., Lin X., Liu Y., Zhou W., Chen P., Ruan R. Hydrothermal pretreatment of microalgae for production of pyrolytic bio-oil with a low nitrogen content. *Bioresource Technology* 2012; 120: 13-18 <http://dx.doi.org/10.1016/j.biortech.2012.06.007>.
- [94] Jena U., Vaidyanathan N., Chinnasamy S., Das K.C., Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass, *Bioresource Technology* 2011; 102 (3): 3380-3387. doi:10.1016/j.biortech.2010.09.111