Oceanography

Gouveia, Oceanography 2014, 2:1
http://dx.doi.org/10.4172/2332-2632.1000120

Review Article
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From Tiny Microalgae to Huge Biorefineries

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Abstract

Microalgae are an emerging research field due to their high potential as a source of several biofuels in addition to the fact that they have a high-nutritional value and contain compounds that have health benefits. They are also highly used for water stream bioremediation and carbon dioxide mitigation. Therefore, the tiny microalgae could lead to a huge source of compounds and products, giving a good example of a real biorefinery approach.

This work shows and presents examples of experimental microalgae-based biorefineries grown in an autotrophic mode at a laboratory scale.

Keywords: Microalgae; Biorefinery

Introduction

Biofuel and bioproduct production from microalgae have several advantages when compared to the 1st and 2nd biofuel generation having: high areal productivity, minimal competition with conventional agriculture, environmental benefits by recycling nutrients (N and P) from waste waters and mitigating carbon dioxide from air emissions. In addition, all components of microalgae can be separated and transformed into different valuable products. The high metabolic versatility of microalgae and cyanobacteria metabolisms, offer interesting applications in several fields such as nutrition (human and animal), nutraceuticals, therapeutic products, fertilizers, plastics, isoprene, biofuels and environment (such as water stream bioremediation and carbon dioxide mitigation).

The high content of antioxidants and pigments (carotenoids such as fucoxanthin, lutein, betacarotene and/or astaxanthin and phycobilliproteins) and the presence of long-chain Polyunsaturated Fatty Acids (PUFAs) and proteins (essential amino acids methionine, threonine and tryptophan), makes microalgae an excellent source of nutritional compounds. Coextraction of other high-value products (PUFAs, such as Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA), and Arachidonic Acid (AA)) will also be evaluated since these compounds may enhance the nutritional or nutraceutical value of the microalgal oil.

Microalgae have also been screened for new pharmaceutical compounds with biological activity, such as antibiotics, antiviral, anticancer, enzyme inhibitory agents and other therapeutic applications. They have been reported to potentially prevent or reduce the impact of several lifestyle-related diseases [1-3] with antimicrobial (antibacterial, antifungal, antiprotozoal) and antiviral (including anti-HIV) functions and they also have cytotoxic, antibiotic, and anti-tumour properties as well as having biomodulatory effects such as immunosuppressive and anti-inflammatory roles [4,5]. *Chlorella* has also been used against infant malnutrition and neurosis [6], as well as being a food additive. Furthermore, algae are believed to have a positive effect on the reduction of cardio-circulatory and coronary diseases, atherosclerosis, gastric ulcers, wounds, constipation, anaemia, hypertension, and diabetes [6,7].

The microalgae compounds, such as carotenoids have also been associated and claimed to reduce the risk of: (1) certain cancers [8-11], (2) cardiovascular diseases [12,13], (3) macular degeneration and cataract formation [14,15] and possibly may have an effect on the immune system and may influence chronic diseases [16,17].

Besides nutritional, nutraceutical and therapeutic compounds, microalgae can also synthesize polysaccharides that can be used as an emulsion stabilizer or as biofloculants and polyhydroxyalkanoate, which are linear polyesters used in the production of bioplastics. Microalgae biomass has been demonstrated to improve the physical and thermal properties of plastic by replacing up to 25% of polymers, which increases the biodegradability of the final bioplastic. Microalgae can also produce isoprene, which is a key intermediate compound for the production of synthetic rubber and adhesives, including car and truck tires. It is also an important polymer building block for the chemical industry, such as for a wide variety of elastomers used in surgical gloves, rubber bands, golf balls, and shoes [18].

Furthermore, the aminoacids produced by microalgae can be used as biofertilizers and therefore assist higher plant growth. Amino-acid based fertilization supplies plants with the necessary elements to develop their structures by adding nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances [19-21]. Bio-fertilizers provide eco-friendly organic agroinput and are more cost-effective than chemical fertilizers.

Finally, regarding biofuels, they can be obtained from the microalgae biomass leftovers after the extraction of added-value compounds. According to the composition of the "waste" biomass, it can be used for the production of liquid biofuels (bioethanol, biodiesel, biobutano and bio-oil) [22,23] or gaseous biofuels (biomethane, biohydrogen, syngas etc.) [24-26]. The technology used to produce biofuels efficiently is not yet established, thus different biological and thermochemical processes still need to be studied and improved.

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Received Februay 12, 2014; **Accepted** March 24, 2014; **Published** March 26, 2014

Citation: Gouveia L (2014) From Tiny Microalgae to Huge Biorefineries. Oceanography 2: 120. doi:10.4172/2332-2632.1000120

Unfortunately, the economic viability of algae-based biofuels is still unfeasible. However, the high metabolic versatility of microalgae and cyanobacteria metabolisms allow the production of the several mentioned non-fuel products, which have a very high value and could play a major role in turning economic and energy balances more favorable. This versatility and huge potential of tiny microalgae could support a microalgae-based biorefinery and microalgae-based bioeconomy opening up vast opportunities in the global algae business.

The microalgae could play an important response to the worldwide biofuel demand, together with the production of high value-added products and assisting some other environmental issues such as water stream bioremediation and carbon dioxide mitigation.

Only the co-production of high added value products and environmental benefits could eventually off-set the high production costs of mass microalgae cultivation and support a microalgae-based bioeconomy. In fact, a microalgae-based biorefinery should integrate several processes and related industries, such as food, feed, energy, pharmaceutical, cosmetic, and chemical. Such an approach, in addition to the biomass, will take advantage of the various products synthesized by the microalgae. This adds value to the whole process which has a minimal environmental impact by recycling the nutrients and water, and by mitigating the CO_2 from the flue gases (Figure 1).

This review highlights the potential of the tiny autotrophic microalgae for the production of several products in an experimental (lab scale) Biorefinery. The production contains biofuel(s) and other high value-added compounds which could be used for different applications and markets.

From (Tiny) Microalgae to (Huge) Biorefineries

The main bottleneck of the biorefinery approach is to separate the different fractions without damaging one or more of the product fractions. There is a need for mild, inexpensive and low energy consumption separation techniques to overcome these bottlenecks [27,28]. They should also be applicable for a variety of end products which have a sufficient quality but are also available in large quantities [29,30].

Some of the biorefinery techniques appropriate for metabolite separation and extraction are ionic liquids or surfactants [28,31]. These techniques are relatively new and should therefore be studied thoroughly before commercial use will be possible.

Nannochloropsis sp. biorefinery

Nobre et al. [31] used *Nannochloropsis* sp. microalga and developed a Biorefinery with the extraction of carotenoids and fatty acids (mainly EPA) for food and the feed industry as well as lipids for biodiesel production. The biomass composition is present in Table 1.

The fractionated recovery of the different compounds was done by Supercritical Extraction using CO_2 and ethanol as an entrainer. From the biomass leftovers and using *Enterobacter aerogeneses* through dark fermentation, bioH_2 was also produced (Figure 2), yielding a maximum of 60.6 mL H_2/g alga [31].

The energy consumption and CO_2 emissions emitted during the whole process (microalgae cultivation, harvesting, dewatering, milling, extraction and leftover biomass fermentation), as well as the economic factors were evaluated [25]. The authors showed five pathways and two biorefineries which were analysed (Figure 3):

Path # 1) Oil extraction by soxhlet (oil SE);

Path #2) Oil and pigment extraction and fractionation through Supercritical Fluid Extraction (oil and pigment SFE);

Path #3) Hydrogen production through dark fermentation of the leftover biomass after soxhlet extraction (bioH_2 via SE);

Path #4) Hydrogen production by dark fermentation from the leftover biomass after Supercritical Fluid extraction (bioH_2 via SFE);

Path #5) Hydrogen production from the whole biomass through dark fermentation (bioH_2 using the whole biomass).

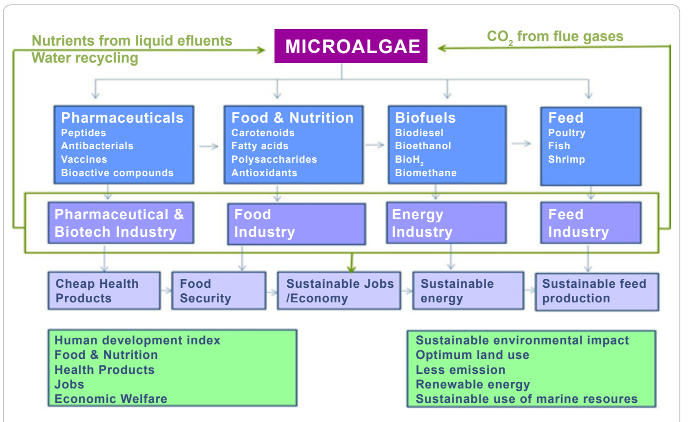

Figure 1: Example of a microalgae based biorefinery and how it integrates several related industries (adapted from Subhadra [46]).

Composition	(%)
Crude fat	41
Total sugars	17
Total minerals	13
Others	29

Table 1: *Nannochloropsis* sp. composition.

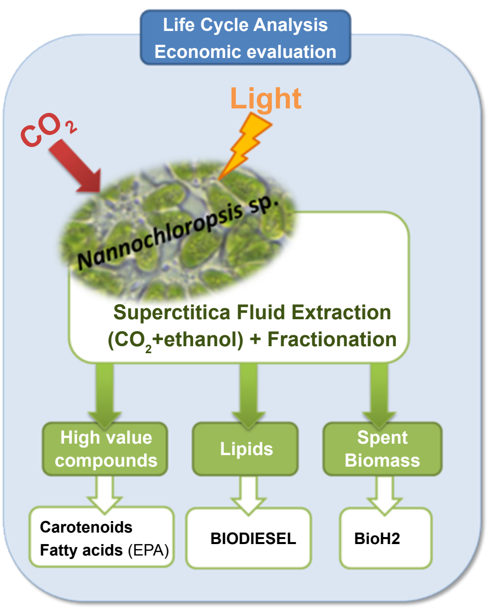

Figure 2: *Nannochloropsis* sp. biorefinery.

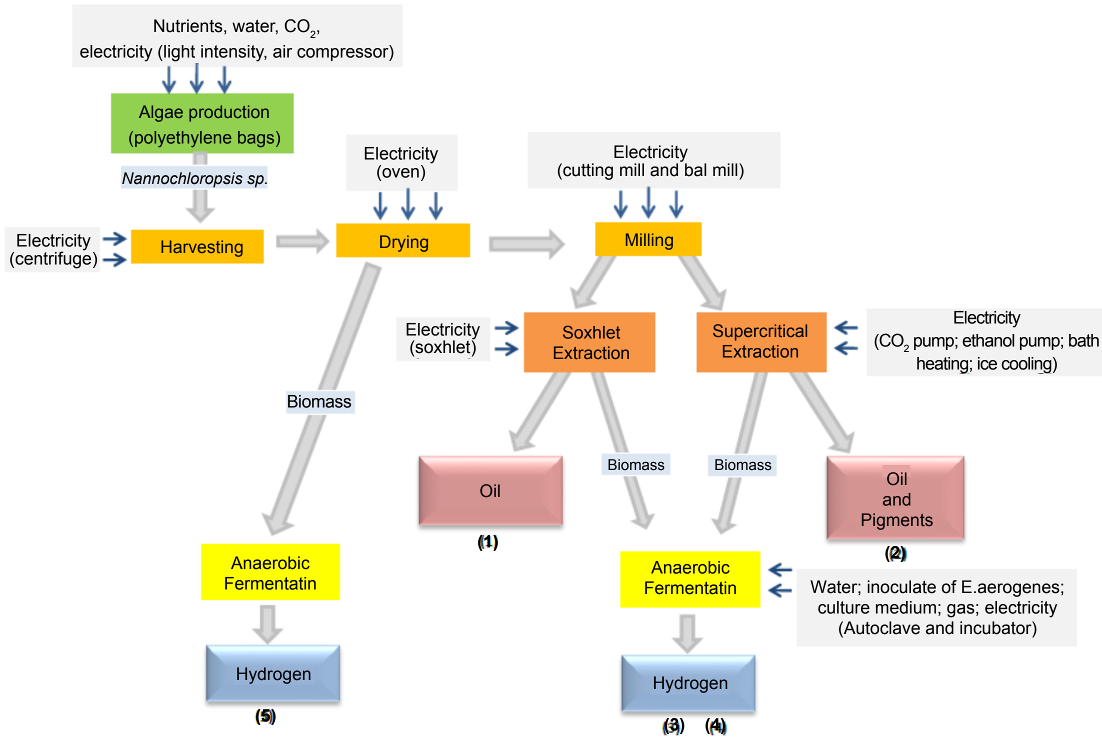

Figure 3: *Nannochloropsis* sp. biorefinery (including all steps, material and energy, and different pathways) to the production of oil, pigments and bioHydrogen) (adapted from Ferreira, et al. [50]).

Where path #1 and path #3 are the Biorefinery 1, path #2 and path #4 are the Biorefinery 2 and path #5 is the direct bioH_2 production.

The analysis of pathways #1, #2 and #5 considers a system boundary from the *Nannochloropsis* sp. microalgal culture to the final product output (oil, pigments, or bioH_2, respectively). For pathways #3 and #4, the bioH_2 production from the leftover biomass from SE and SFE respectively was evaluated.

The authors concluded that the oil production pathway by SE shows the lowest energy consumption, 176-244 MJ/MJ_{prod}, and CO_2 emissions, 13-15 kg CO_2/MJ_{prod}.

However, economically the most favourable biorefinery was the one producing oil, pigments and H_2 via Supercritical Fluid Extraction (SFE).

From the net energy balance and the CO_2 emission analysis, Biorefinery 1 (biodiesel SE + bioH_2) presented the better results. Biorefinery 2 (biodiesel SFE + bioH_2) showed results in the same range of those in Biorefinery 1. However, the use of SFE produced high-value pigments in addition to the fact that it is a clean technology which does not use toxic organic solvents.

Therefore, Biorefinery 2 was the best in terms of energy/CO_2/ and it being the most economically advantageous solution.

Anabaena sp. biorefinery

The experimental biohydrogen production by photoautotrophic cyanobacterium *Anabaena* sp. was studied by Marques et al. [24]. Hydrogen production from the *Anabaena* biomass leftovers was also achieved by fermentation through the *Enterobacter aerogenes* bacteria and was reported by Ferreira et al. [32] (Figure 4).

Different culture conditions and gas atmospheres were tested in order to maximize the autotrophic bioH_2 yield *versus* the energy consumption and CO_2 emissions. The authors stated that the best conditions included an Ar+CO_2+20% N_2 gas atmosphere and medium light intensity (384 W) [32]. The yielded H_2 could be increased using the biomass leftovers through a fermentative process; however this would mean higher energy consumption as well as an increase in CO_2 emissions.

Chlorella vulgaris biorefineries

Quite a few reported works describe biorefineries from *Chlorella vulgaris* and these are stated below:

Cv1 – An integrating process for lipid recovery from the biomass of *Chlorella vulgaris* and methane production from the remaining biomass (after lipid extraction) was worked on by Collet et al. [33]. The authors demonstrated that, in terms of Life Cycle Assessment (LCA), the methane from algae (algal methane) is the worst case, compared to algal biodiesel and diesel, in terms of abiotic depletion, ionizing radiation, human toxicity, and possible global warming. These negative results are mainly due to a strong demand for electricity. For the land use category, algal biodiesel also had a lesser impact compared to algal methane. However, algal methane is a much better option in terms of acidification and eutrophication.

Cv2 - Another work concerning the simultaneous production of biodiesel and methane in a biorefinery concept was done by Ehimen et al. [34]. The authors obtained biodiesel from a direct transesterification on the *Chlorella* microalgal biomass, and from the biomass residues they obtained methane through anaerobic digestion. For a temperature of 40°C and a C/N mass ratio of 8.53, a maximum methane concentration of 69% (v/v) with a specific yield of 0.308 m^3 CH_4/kg VS was obtained. However, in this work the biodiesel yield was not reported.

Cv3 - In another work, the *Chlorella vulgaris* biorefinery approach was studied by Gouveia, et al. [35] and it included a Photosynthetic Algal Microbial Fuel Cell (PAMFC), where the microalga *Chlorella vulgaris* are present in the cathode compartment (Figure 5). The study demonstrated the simultaneous production of bioelectricity and added-value pigments, with possible wastewater treatment. The authors proved that the light intensity increases the PAMFC power and augments the carotenogenesis process in the cathode compartment. The maximum power produced was 62.7 mW/m^2 with a light intensity of 96 µE/(m^2.s).

Cv4 - A bioethanol-biodiesel-microbial fuel cell was reported by Powel and Hill [36] and basically consisted in an integration of photosynthetic *Chlorella vulgaris* (in the cathode) that captured CO_2 emitted by yeast (in the anode) fermenters, creating a microbial fuel cell. The study demonstrated the possibility of electrical power generation and oil for biodiesel, in a bioethanol production facility. The remaining biomass after oil extraction could also be used in animal feed supplement [36].

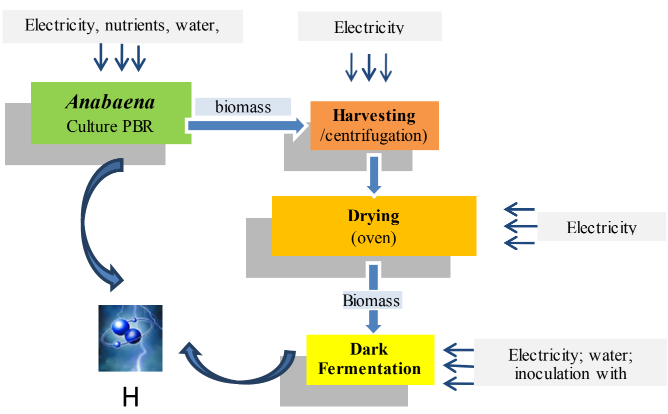

Figure 4: *Anabaena* sp. biorefinery (including both H_2 production: autotrophically and by dark fermentation (through *Enterobacter aerogenes*) (adapted from Ferreira, et al. [32]).

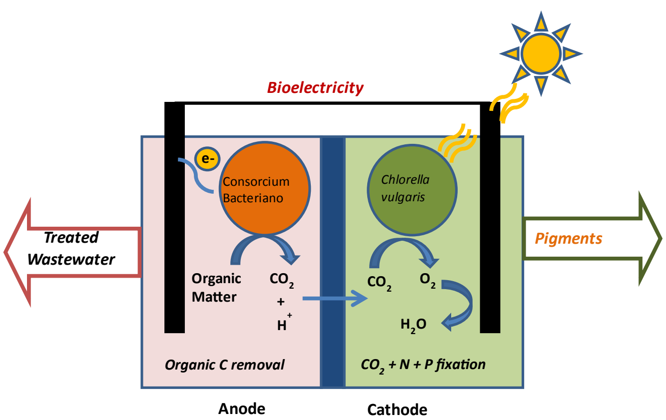

Figure 5: *Chlorella vulgaris* biorefinery (Photosynthetic Algal Microbial Fuel Cell) (adapted from da Silva TL, et al. [30]).

Chlorella protothecoids biorefinery

The biorefinery stated by Campenni et al. [37] used *Chlorella protothecoides* as a source of lipids and carotenoids, it was grown autotrophically and with nitrogen deprivation and the addition of a 20 g/l NaCl solution (Figure 6).

The total carotenoid content was 0.8% (w/w) (canthaxanthin (23.3%), echinenone (14.7%), free astaxanthin (7.1%) and lutein/ zeaxanthin (4.1%)) which can be used for food applications. Furthermore, the total lipid content reached 43.4% (w/w), with a fatty acid composition of C18:1 (33.6%), C16:0 (23.3%), C18:2 (11.5%), and C18:3 (less than 12%), which is needed to fulfil the biodiesel EN 14214 quality specifications [38] and can be used for the biofuel (biodiesel) industry.

The leftover biomass is still available for hydrogen or bioethanol production in a biorefinery approach, as the residue still contains sugar taking advantage of all the *C. protothecoids* gross composition.

Chlorella reinhardtii biorefinery

The production of biohydrogen and the consequent biogas (methane) production by anaerobic fermentation of the residue of *Chlorella reinhardtii* biomass were achieved by Mussgnug et al. [39].

The authors reported that using the biomass, after the hydrogen production cycle instead of using the fresh biomass, would increase the biogas production by 123%. The authors attributed these results to the storage compounds, such as starch and lipids with a high fermentative potential which is the key in the microalgae-based integrated process and could be used for more value-added applications.

Dunaliella salina biorefinery

Sialve et al. [40] attested the production of methane from the leftover biomass of *Dunaliella salina* after the oil extraction to make biodiesel. The authors found a much higher yield (around 50%) for a shorter hydraulic retention time (HRT, 18 days), than the corresponding values reported by Collet et al. [33] using the *Chlorella vulgaris* biomass.

Dunaliella tertiolecta biorefinery

The chemoenzymatic saccharification and bioethanol fermentation of the residual biomass of *Dunaliella tertiolecta* after lipid extraction (for biodiesel production purposes) were investigated by Kim et al. [41]. The bioethanol was produced from the enzymatic hydrolysates without pretreatment by *S. cerevisiae,* resulting in yields of 0.14 g ethanol/g residual biomass and 0.44 g ethanol/g glucose produced from the residual biomass.

According to these authors, the residual biomass generated during microalgal biodiesel production, could be used for bioethanol production in order to improve the economic feasibility of a microalgae-based integrated process.

Arthrospira (*Spirulina*) biorefinery

Olguin [42] highlighted that the biorefinery strategy offers new opportunities for a cost-effective and competitive production of biofuels along with nonfuel compounds. The author studied an integrated system where the production of biogas, biodiesel, hydrogen and other valuable products (e.g. PUFAs, phycocyanin, and fish feed) could be possible.

Spirogyra sp. biorefinery

Pacheco et al. [43] pointed a biorefinery from *Spirogyra* sp., a sugar-rich microalga, for bioH_2 production as well as pigments (Figure 7). The economic and life cycle analysis of the whole process, allowed the authors to conclude that it is crucial to increase the sugar content of the microalgae to increase the bioH_2 yield. Furthermore, it is important to reduce the centrifugation needs and use alternative methods for pigment extraction other than using acetone solvents. The electrocoagulation and solar drying were used for harvesting and dewatering, respectively, and were able to reduce energy requirements by 90%. Overall, centrifugation of the microalgal biomass and heating of the fermentation vessel are still major energy consumers and CO_2 contributors to this process. Pigment production is necessary to improve the economic benefits of the biorefinery, but it is mandatory to reduce its extraction energy requirements that are demanding 62% of the overall energy.

Mostafa et al. [44] evaluated the growth and lipid, glycerol, and carotenoid content of nine microalgae species (green and blue green microalgae) grown in domestic wastewater obtained from the Zenein Wastewater Treatment Plant in the Giza governorate in Egypt (Figure 8). The authors cultivated the different species under different conditions, such as without treatment after sterilization, with nutrients and sterilization, and with nutrients without sterilization, at 25 ± 1°C, under continuous shaking (150 rpm) and illumination (2,000 lx), for 15 days. The highest biodiesel production from algal biomass cultivated in wastewater was obtained by *Nostoc humifusum* (11.80%) when cultivated in wastewater without treatment and the lowest (3.8%) was recorded by *Oscillatoria* sp. when cultivated on the sterilized domestic wastewater. The authors concluded that cultivating microalgae on domestic wastewater, combines nutrient removal and algal lipid production which has a high potential in terms of biodiesel feedstock. This methodology is suitable and non-expensive compared to the conventional cultivation methods for sustainable biodiesel and glycerol.

According to Subhadra and Edwards [45] (Figure 9), an integrated Renewable Energy Park (IREP) approach can be envisaged by combining different renewable energy industries, in resource-specific regions, for synergetic electricity and liquid biofuel production, with zero net carbon emissions. Choosing the appropriate location, an IREP design, combining a wind power plant with solar panels and algal growth facilities to harness additional solar energy, could greatly optimize land. Biorefineries configured within these IREPs can produce about 50 million gallons of biofuel per year, providing many other value-added co-products and having almost no environmental impact [46] (Figure 9).

Clarens et al. [47] suggested that the results from algae-to-energy systems can be either net energy positive or negative depending on the specific combination of cultivation and conversion processes used addressed the shortcoming "well-to-wheel", including the conversion of each biomass into transportation energy sources. The algal conversion pathway resulted in a combination of biodiesel and bioelectricity production for transportation, evaluated by Vehicle

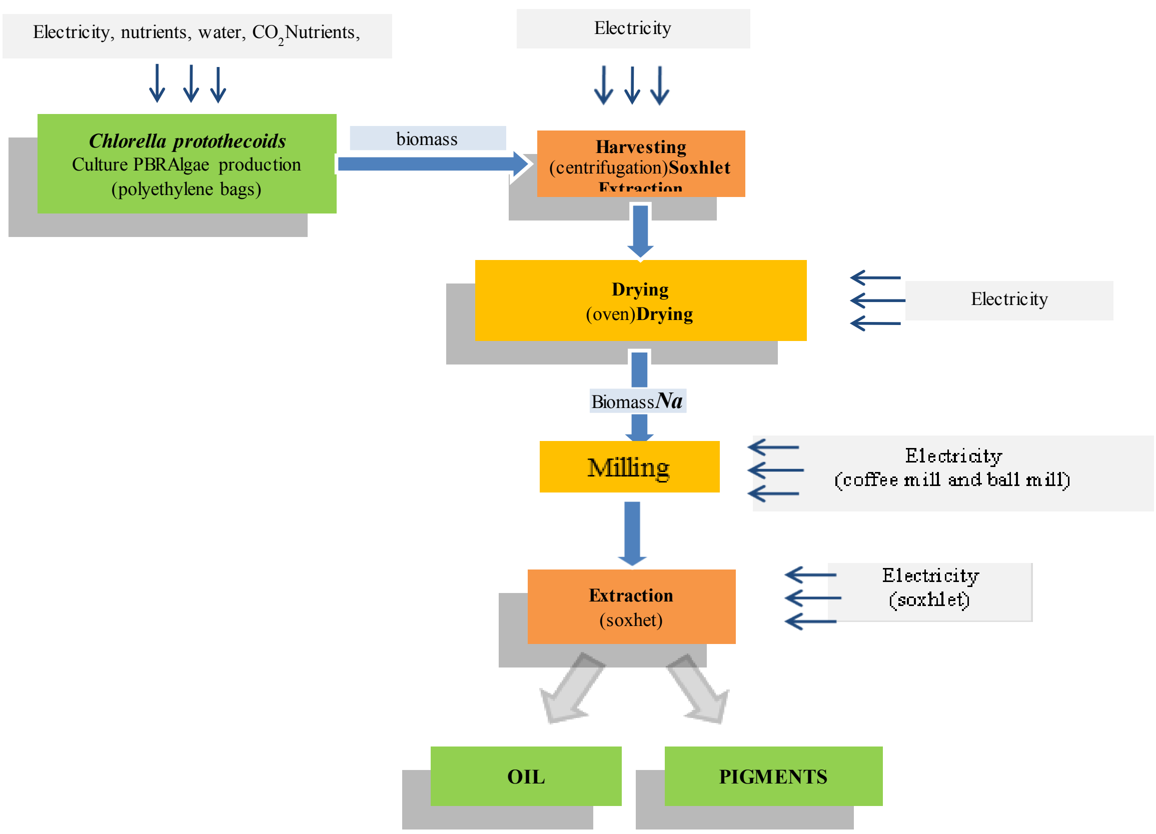

Figure 6: *Chlorella protothecoids* biorefinery (adapted from Campenni', et al. [37]).

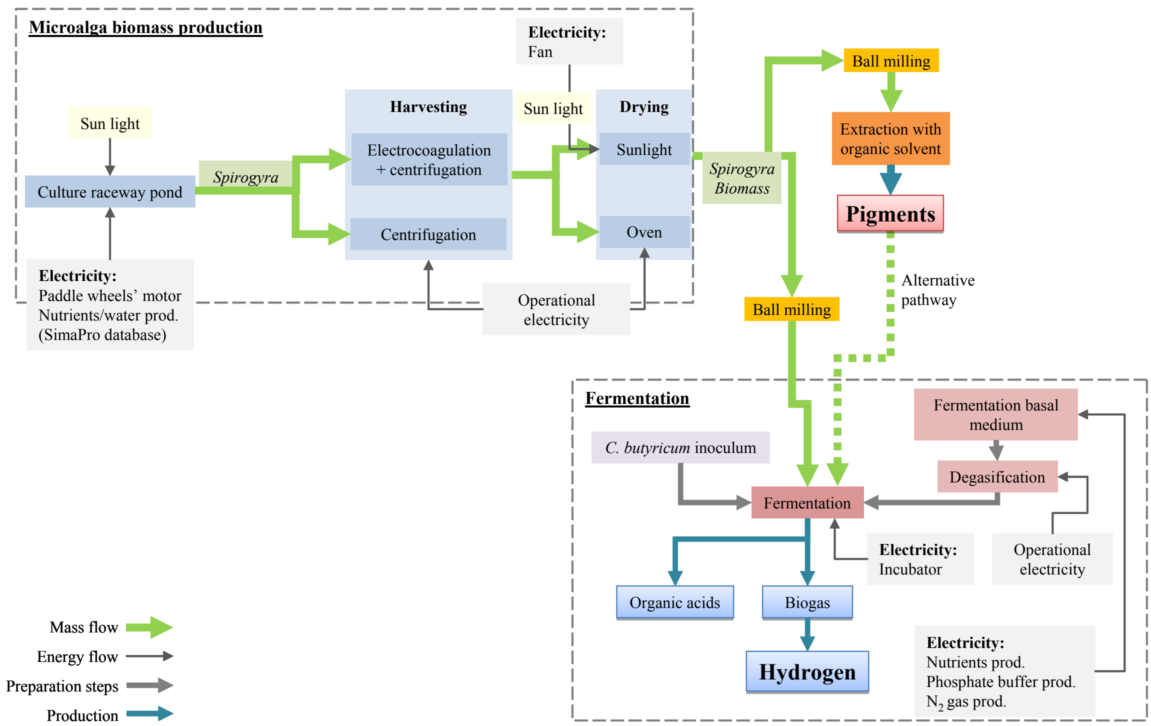

Figure 7: *Spirogyra* sp. biorefinery (adapted from Pacheco, et al. [43]).

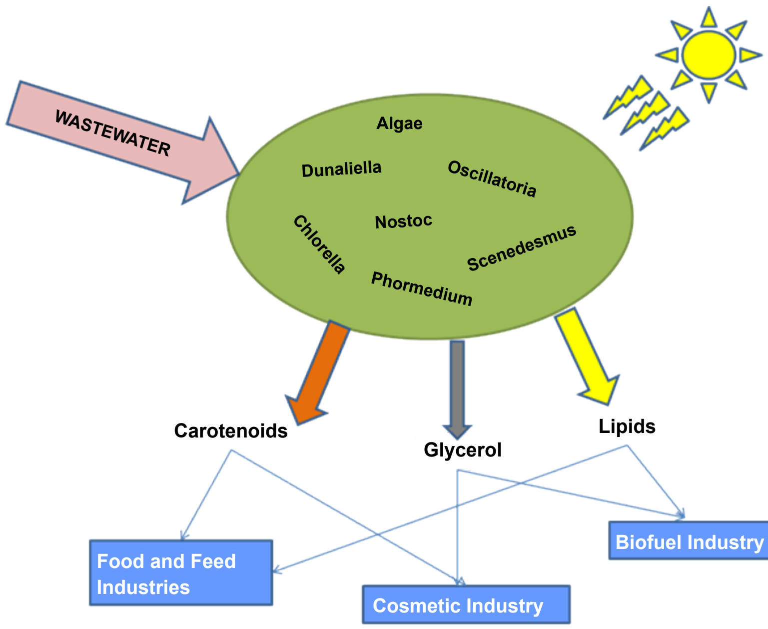

Figure 8: Biorefinery from several microalgae using w astew ater w ith lipid, carotenoid and glycerol production (adapted from Mostafa, et al. [44]).

Kilometers Traveled (VKT) per hectare. In this study, it was assumed that bioelectricity and biodiesel are used in commercially available Battery Electric Vehicles (BEVs) and Internal Combustion Vehicles (ICVs), respectively. The authors depicted four pathways:

A. Methane-derived bioelectricity from the bulk algae biomass by anaerobic digestion

B. Biodiesel from the algae lipids and methane-derived bioelectricity from the residual biomass by anaerobic digestion

C. Biodiesel from the algae lipids and bioelectricity from the residual biomass by direct combustion

D. Bioelectricity from the bulk algae biomass by direct combustion

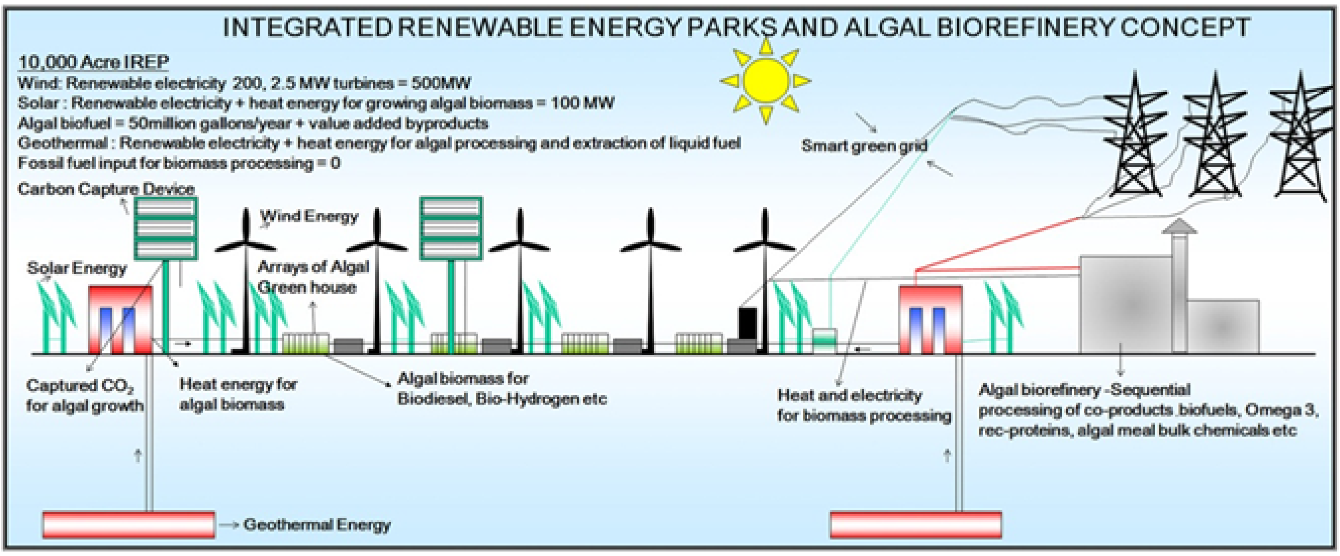

Figure 9: Integrated renewable energy and an Algal Biorefinery concept: a framework for the production of biofuel and high-value products with zero net carbon emissions (from Subhadra and Edwards [45].

The four pathways follow various nutrient sources (e.g., virgin commercial CO_2, CO_2 from a coal-fired power plant, compressed CO_2 from flue gas, commercial fertilizers, and wastewater supplementation).

The authors found that algae-to-energy systems depend on the combination of cultivation and conversion processes used. They concluded that the conversion pathways involving direct combustion for the production of bioelectricity generally outperformed systems involving anaerobic digestion and biodiesel production. They ranked the four pathways as D>A>C>B in terms of energy return on investment.

The authors found an algae bioelectricity (D) generation of 1,402,689 MJ/km and algae biodiesel + bioelectricity (C) generation of 1,110 MJ/km. These algae-to-energy systems generate 4 and 15 times as VKT per hectare as switch grass or canola, respectively [47].

Subhadra and Edwards [48] analyzed the water footprint of two simulated algal biorefineries for the production of biodiesel, algal meal, and omega-3 fatty acids. The authors highlighted the advantages of multiproducts to attain a high operational profit with a clear return on investment. The energy return of algal biodiesel for different scenarios ranged between 0.016-0.042 MJ.

Park, et al. [49,50] also studied algae which are grown as a by-product of High-Rate Algal Ponds (HRAPs) operated for wastewater treatment. In addition to significantly better economics, algal biofuel production from wastewater treatment HRAPs has a much smaller environmental footprint compared to commercial algal production HRAPs which consume freshwater and fertilizers.

Conclusions

Biomass, as a renewable source, is attracting worldwide attention to satisfy the so called bioeconomy demand. Microalgae could be the appropriate feedstock as they did not compete with food and feed production, in terms of either land or water. Furthermore, microalgae remove/recycle nutrients from wastewater and flue-gases providing additional environmental benefits.

Due to their efficient sunlight utilization, microalgae are projected as living-cell factories with simple growth requirements. Their potential for energy and value-added products production is widely recognized.

Nevertheless, to be economically sustainable the tiny microalgae should supply a huge biorefinery. Technical advances combined with the several advantages such as CO_2 capture, wastewater bioremediation and the extraction of value added-products will greatly increase algal bioproduct profitability.

The versatility and the huge potential of the tiny microalgae could support a microalgae biorefinery and microalgae-based bioeconomy, opening up a huge increase of opportunities in the global algae business.

Acknowledgements

The author would like to thank all the collaborators and co-authors of the all manuscripts mentioned in this paper, and Stephanie Seddon-Brown for English proof reading of this manuscript.

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Citation: Gouveia L (2014) From Tiny Microalgae to Huge Biorefineries. Oceanography 2: 120. doi:10.4172/2332-2632.1000120

This article was originally published in a special issue, **Seaweeds, Micro- and Macro-algae** handled by Editor. Dr. Jeffrey Craig Bailey, University of North Carolina, USA