Microalgae biorefineries: The Brazilian scenario in perspective

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ABSTRACT

Biorefineries have the potential to meet a significant part of the growing demand for energy, fuels, chemicals and materials worldwide. Indeed, the bio-based industry is expected to play a major role in energy security and climate change mitigation during the 21st century. Despite this, there are challenges related to resource consumption, processing optimization and waste minimization that still need to be overcome. In this context, microalgae appear as a promising non-edible feedstock with advantages over traditional land crops, such as high productivity, continuous harvesting throughout the year and minimal problems regarding land use. Importantly, both cultivation and microalgae processing can take place at the same site, which increases the possibilities for process integration and a reduction in logistic costs at biorefinery facilities. This review describes the actual scenario for microalgae biorefineries integration to the biofuels and Petrochemical industries in Brazil, while highlighting the major challenges and recent advances in microalgae large-scale production.

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1. Introduction

The fast-growing human population and the consequent growing demand for food, energy and water are and will continue to be serious worldwide challenges for the coming decades [1]. In addition, increasing concerns about anthropogenic climate change and excessive dependency on fossil fuels are directing investment towards more sustainable alternatives [2,3]. There is a consensus that new technologies will be required to allow the conciliation of economic growth and environmental sustainability in the long term.

Biorefineries are facilities that integrate biomass conversion processes into various marketable products and energy, while optimizing the use of resources and minimizing waste, thereby maximizing benefits and profitability [4,5]. The integration of emerging biorefineries with other industries is a potential solution to mitigate the threat of climate change and also provide means to support the demand for energy, fuels, chemicals and materials [6]. Although countries like USA, Brazil and Germany already have consolidated biorefineries, these are mainly based on the production of ethanol and biodiesel from edible feedstocks such as corn, sugarcane and soybean [5]. Thus, there is a need for innovative bio-based products in order to substitute petroleum derivatives, such as bulk chemicals and materials [1]. Furthermore, the use of alternative non-edible feedstocks is required to minimize the competition for energy and food resources in the global market [7].

Recently, the world has experienced the birth of second-generation ethanol commercial plants (using lignocellulosic feedstocks), with the construction of the first facilities in Italy, USA and Brazil. The use of lignocellulosic material resulting from agro-industrial activities, such as sugarcane bagasse and straw, will contribute to a more efficient use of land and proper environmental management [8]. However, there are concerns about logistics costs and the indirect competition for the use of soil [8–10]. The awareness of these constraints has driven attention and investment towards microalgae research and commercialization initiatives [11].

Microalgae are single-celled or colonial photosynthetic organisms that are naturally present in different aquatic/humid environments, including rivers, lakes, oceans and soils. They can be used as raw material for the production of a plethora of bioproducts, such as fuels, chemicals, materials, animal feed and food supplements [12]. Algal biomass has considerable advantages over traditional feedstocks: (i) High productivity—usually 10–100 times higher than land crops; (ii) Highly efficient carbon capture; (iii) High lipid or starch content, which can be used for biodiesel or ethanol production, respectively; (iv) Can be cultivated in seawater, brackish water or even wastewater and (v) Can be grown over non-arable land [10,13]. Microalgae can also be harvested continuously throughout the year [14], which is the reason for which they have the potential to form a continuous
biofuel production chain such as in traditional oil refineries [9]. In addition, both cultivation and microalgae processing can take place at the same site, a characteristic that favors the integrated and sequential production of various products and reduces logistic costs at biorefinery facilities [13]. Nonetheless, there are significant technological challenges to produce economically competitive algal-derived biofuel, such as: (i) Microalgae strains development (ii) Efficient methods for cultivation and crop protection; (iii) Improvement of harvesting and lipid extraction methods; (iv) Optimization of conversion/production processes of fuels and coproducts [15–19].

2. Potential for microalgae production in Brazil

Brazil possesses a large tropical coastal area, 10,959 km in total, has approximately 12% of the world’s freshwater supply, and receives average insolation levels of 8–22 MJ/m²/day. The country is also home to the world’s richest flora (40,989 species; 18,932 endemic) and possesses 3496 algae catalogued species [20]. There are currently over forty laboratories and institutions where algae cultures (microalgae, macroalgae and cyanobacteria) are kept in Brazil. Five of these facilities hold a considerable amount of strains (over 150 strains) [21,22].

The Brazilian companies Petrobras and Embrapa are leading parallel initiatives aiming to characterize and domesticate productive native algal strains. While Petrobras’ primary focus is on marine microalgae for biodiesel production (See section “Integration of microalgae production to the oil industry”), Embrapa’s program is directed to continental microalgae genetic resource characterization and the establishment of a long term research program for biofuels and bioproducts production. Furthermore, studies focused on native strains have reported the isolation of Chlorella strains that present lipid productivity up to 200 mg/L/day, which is comparable to the most promising microalgae species isolated from other parts of the world, such as Nannochloropsis gadinata and N. salina [23,24].

3. Microalgae production integration to the Brazilian fuel industry

Considerable synergies do exist between microalgae production/processing and a wide range of industries. There are opportunities for the development of a sustainable microalgae-based industry whose productivity is independent of soil fertility and less dependent on water purity [19]. This section reviews the current scenario and possibilities for microalgae cultivation/processing in the fuel industry in Brazil. It is noteworthy, however, that there is an array of initiatives in the context of aquaculture and animal feed industries, rural and municipal wastewater treatment, coal-fired power stations, among others not covered here, that are currently being explored worldwide [5,13,19,25].

3.1. Integration of microalgae production to the sugarcane-ethanol industry

There are currently 399 sugarcane-ethanol production facilities in Brazil, mainly concentrated in the Northeastern and Southeastern regions of the country. They were responsible for processing 658,820 Mt of sugarcane for the production of 27.96 billion liters of ethanol, 37,880 Mt of sugar and 8870 MW of electricity in 2013. Additionally, two second-generation facilities began operations in 2014. These plants have the capacity to produce 82 million and 40 million liters of cellulosic ethanol, respectively, using sugarcane straw and bagasse as a feedstock (Fig. 1).

In 2014, the first industrial plant for the production microalgae oils started commercial operations in Brazil [26]. The company uses genetically modified microalgae cultivated in a closed heterotrophic system to produce oil for renewable chemicals.

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Fig. 1. Microalgae production and processing in the context of sugarcane biorefineries: Brazilian sugarcane biorefineries currently produce ethanol, sugar and electricity (gray lines/icons). Microalgae production and processing can be coupled to sugarcane biorefineries (black lines/icons), as shown in: (1) Renewable oils for industrial use and personal care can be produced from sucrose by heterotrophic microalgae; (2) Vinasse and CO2 can be used to grow starch-rich algae, raw material for ethanol and electricity production. In addition, these strains could be genetically modified to synthesize recombinant proteins such as cellulolytic enzymes. The sugarcane straw processing for cellulosic ethanol production was started in 2014 in Brazil** TRS: Total Recoverable Sugars. Based on information from references [4,25,31].

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(i.e.: Lubricants and cosmetics). The facilities, which have the capacity to produce 100 Mt of renewable oils per year, use sugarcane juice as carbon source for microalgae cultivation [26,34] (Fig. 1).

Initiatives towards the use of sugarcane plant by-products, such as vinasse and CO₂ for microalgae mixotrophic cultivation are also under way (Fig. 1). Vinasse is a liquid effluent generated at 10–15 L per liter of ethanol produced, with a high organic load and acidic pH. Carbon dioxide is produced in significant amounts during sugarcane juice fermentation and bagasse burning. The proper disposal and value aggregation of these effluents has been a challenge for the sector. Indeed, there are technologies for microalgae-based vinasse bioremediation and biomass production currently at pre-commercialization stages in Brazil [28] (Fig. 1). In addition, the genetic modification of microalgae strains for recombinant cellulase production and secretion to the culture medium might allow in house production of enzymes for cellulotic ethanol production, thereby favoring biorefinery processes integration and costs reduction [29,30] (Fig. 1). Alternatively, approaches focusing on the anaerobic treatment of vinasse prior to algae cultivation have been shown to enhance Chlorella vulgaris biomass productivity to 70 mg/L/d [31]. This approach can integrate the production of methane, derived from the anaerobic digestion process, to microalgae-based production of biofuels, such as biodiesel and ethanol [31].

3.2. Integration of microalgae production to the biodiesel industry

Biodiesel production has been the most intensively studied area of microalgae-based biotechnology worldwide, especially in the last ten years [11,14,15–19]. In 2013, over 2.8 billion liters of biodiesel were produced in Brazilian biodiesel plants, using mainly soybean oil, animal tallow and cottonseed oil as feedstock [26]. Biodiesel production expansion in the last ten years has been driven by an increase in the domestic demand for diesel coupled to the gradual increment in the mandatory biodiesel blending. Considerable attention has been given to promising alternative feedstocks, such as oil palm, physic nut and microalgae, in order to meet the demand forecasted for the next decades [27].

The use of biodiesel production by-products, such as glycerol and POME (Palm Oil Mill Effluent), also offers attractive possibilities to integrate oleaginous microalgae cultivation systems to biodiesel plants. Cabanelas and collaborators [32] have shown that native strains of Chlorella vulgaris and Botryococcus terribilis presented considerable biomass productivities using domestic wastewater supplemented with glycerol (50 mM) as cultivation medium. In another study, Liang and collaborators [33] reported that the glycerol supplementation at a concentration of 100 mM increased C. vulgaris biomass productivity from 10 to 102 mg/L/day. Furthermore, the use of POME, either crude or anaerobically digested, for culturing microalgae is a promising alternative to treat this effluent while producing energy-rich biomass [34–37].

3.3. Integration of microalgae production to the oil industry

Brazil’s proven oil reserves, including large offshore fields located deep under pre-salt layers at the country’s coast, comprised 15.6 billion barrels in 2014 (around 0.9% of the world’s proven oil reserves). The exploration of these reserves might place the Brazilian oil industry as one of the ten top producers worldwide in the near future [38].

Produced water (PW) is the largest waste stream generated in oil and gas industries. This geologic water has varying concentrations of impurities such as inorganic salts, hydrocarbons and added elements used in the separation of water and oil in the production line [39]. The effect of discharging produced water on the environment is an issue that causes significant environmental concern [40]. The Brazilian oil company, Petrobras, has been making investments focused on microalgae-based treatment of produced water and CO₂ emissions capture at oil exploration platforms (Fig. 2). In 2012, the company’s first microalgae cultivation plant started pre-commercial operations at the Northeastern coastal region, taking advantage of its favorable climate and high number of sunny days. An native Nannochloropsis oculata strain has been adapted for growth in PW leading to a higher growth rate (μ = 0.22/d) in PW diluted at 50% in f/2 medium compared to the control (μ = 0.08/d) grown in 100% f/2 medium [41].

Recent advances in hydrothermal liquefaction (HTL) processes for whole microalgae biomass conversion into biocrude oil have opened new perspectives regarding the production of microalgae-based energy, chemicals, materials and biofuels (Fig. 2) [16]. This thermochemical technology can convert whole algal wet biomass directly, thereby reducing the cost of biomass drying and lipid extraction [16,42]. In the future, it might be possible to process algae biocrude oil together with petroleum in order to provide raw material for more sustainable alternatives to conventional oil derivatives (Fig. 2).

4. Challenges for microalgae large-scale production

In spite of the promising opportunities envisioned for microalgae-integrated biorefineries, the economic viability of large-scale algal biomass cultivation for low-value products (e.g.: Biofuels, bulk chemicals and biomaterials) has not been achieved yet. Indeed, even considering recent technologic advances, the costs for microalgae biofuel production are still at least 2-fold higher than for its fossil-based counterparts (Table 1). Therefore, continued enhancements are needed in algal biology, as well as in the areas of algal cultivation, harvesting and processing. In addition, after identifying economically viable biorefinery combinations, environmental risks and resources management analysis should be considered in order to determine the feasibility of the technologies and their combinations in a proposed area [46,52]. In this section the recent advances related to microalgae biorefineries are critically discussed in the light of their large-scale techno-economic feasibility.

4.1. Microalgae biomass cultivation

Microalgae are usually cultivated under photoautotrophic conditions, using the photosynthetic metabolic pathway to capture energy from light while fixing inorganic carbon (e.g.: CO₂) [53,54]. However, there are several microalgae species that can also grow in the absence of light using organic carbon compounds as both energy and carbon sources in a condition known as heterotrophic cultivation [53,55]. Additionally, some microalgae can grow using both photoautotrophic and heterotrophic modes combined (mixotrophic cultivation), where organic and inorganic carbon sources are metabolized in the presence of light [54]. All three growth modes have been reported for microalgae cultivation using oil/biofuel industries by-products as culture media substrates (Table 2).

Heterotrophic microalgae cultivation is usually conducted inside opaque fermenters [53] and tends to achieve higher productivities than photoautotrophic and mixotrophic modes (Table 2). Furthermore, since light independent growth is not diminished by the increase in the medium turbidity during microalgal cultivation, higher cell densities can be obtained leading to reduced harvesting costs [66]. However, issues related to the high substrate costs and high risks of contamination preclude large-scale heterotrophic microalgae cultivation for
biofuels [53,67,68]. Instead, heterotrophic microalgae have been commercially used only for the production of higher value products (i.e.: cosmetics and lubricants) (Fig. 1).

On the other hand, photoautotrophic cultivation is the most widely used for microalgae biomass cultivation [53,69–72]. It is performed in photobioreactors (PBRs), which can be of either open or closed types [53]. It is still not clear which production system is the best for commercial applications. However, due to their low capital and operational costs, open PBRs (e.g.: Open ponds and raceways) are often reported as less costly (Table 1). Indeed, open PBRs are cheaper and easier to build and operate, but present lower biomass productivity [14,53]. Furthermore, they are dependent on the climatic conditions and are continuously threatened by invading species, such as non-target algae, grazers and bacteria, indicating that further improvements will be required for large-scale biomass production [53]. Recently, a novel open cultivation system, named Algae Raceway Integrated Design (ARID), has been demonstrated to provide superior temperature and energy management than conventional raceways, which leads to significant cost reduction, especially at subtropical/temperate latitudes [46,73].

Closed PBRs (e.g.: Tubular reactors and flat panels) present relatively higher biomass productivities, better temperature, better contamination control and more efficient CO2 capture than open systems [15,18]. However, scalability limitations due to gas exchange requirements and the high installation and operation costs leads to a lower economic feasibility of closed systems [15,18,74,75]. Indeed, as demonstrated in Table 1, closed systems are comparatively more expensive than open pond/raceways. A detailed explanation of differences between these two modes has been extensively reviewed elsewhere [76–78] and is beyond the scope of this article. Hybrid systems, on the other hand, have presented promising results in large-scale production of marine microalgae as well as in economic viability analysis [79] (Table 1). Such systems take advantage of using closed PBRs to produce cell-dense microalgae inoculum in a contamination protected environment followed by large-scale mass production in low cost/easily operated open systems [79].

Significant cost reductions (>50%) can be achieved if CO2, nutrients and water for microalgae cultivation are obtained at low cost [51] (Table 1), which makes the biorefinery strategies described in here (see section “Microalgae production integration to the Brazilian fuel industry”) particularly attractive. Photoautotrophic microalgae cultivation using residues or wastewater is usually achieved by reducing the substrate organic load and increasing nutrient availability through previous substrate pretreatment (e.g.: Anaerobic digestion) [31]. This strategy is being currently explored worldwide, especially through the combination of urban/agriculture wastewater treatment and microalgae biomass production [reviewed in 80,81].

Regarding the use fuel industry by-products as media for microalgae growth, though, the most commonly reported cultivation mode is mixotrophy (Table 2). Mixotrophic microalgae have the potential to capture CO2 emissions while lowering the organic load of the medium during growth [54]. Although theoretically all PBR systems could be used for mixotrophic cultivation, the high organic load associated with the culture medium will probably hamper the use of large-scale systems based solely in open ponds/raceway. Thus, the use of a two-stage heterotrophic/mixotrophic and photoautotrophic culture strategy, where the microalgae seed is produced in fermenters (heterotrophic) or closed PBRs (mixotrophic) and subsequently inoculated in open photoautotrophic systems, appears to be the most promising approach to manage contaminants when using media with high organic load [68].
Table 1

<table>
<thead>
<tr>
<th>Cultivation System</th>
<th>Harvesting</th>
<th>Extraction/Conversion</th>
<th>Production cost</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Ponds</td>
<td>Filter press dewatering</td>
<td>Wet solvent lipid extraction + Oil hydrotreating + Anaerobic digestion of residues</td>
<td>8.79 US$/gallon of algal biodiesel</td>
<td>[43]</td>
</tr>
<tr>
<td>Hybrid (Closed PBR/Open ponds)</td>
<td>Flotation + Centrifugation</td>
<td>Hydrolysis + In situ transesterification + Flash separation</td>
<td>13.94 US$/gallon of algal biodiesel</td>
<td>[44]</td>
</tr>
<tr>
<td>Hybrid (Closed PBR/Open ponds)</td>
<td>Filter press dewatering</td>
<td>Wet solvent lipid extraction + Oil hydrotreating + Anaerobic digestion of residues</td>
<td>14.12 US$/gallon of algal biodiesel</td>
<td>[43]</td>
</tr>
<tr>
<td>Closed PBR</td>
<td>Flotation + Centrifugation</td>
<td>Hydrolysis + In situ transesterification + Distillation</td>
<td>8.714 US$/gallon of algal biodiesel</td>
<td>[44]</td>
</tr>
<tr>
<td>Hybrid (Closed PBR/Open ponds)</td>
<td>Filter press dewatering</td>
<td>Hydrothermal liquefaction + Catalytic hydrothermal gasification + Solids to animal feed</td>
<td>7.30 US$/gallon of algal biocrude</td>
<td>[45]</td>
</tr>
<tr>
<td>Algae Raceway Integrated Design + GMO strain</td>
<td>Electrocoagulation</td>
<td>Hydrothermal liquefaction + Catalytic hydrothermal gasification</td>
<td>7.50 US$/gallon of algal biocrude</td>
<td>[46]</td>
</tr>
<tr>
<td>Hybrid (Closed PBR/Open ponds)</td>
<td>Filter press dewatering</td>
<td>Hydrolysis</td>
<td>9.04 US$/gallon of algal biocrude</td>
<td>[45]</td>
</tr>
<tr>
<td>Open Ponds</td>
<td>Dissolved air flotation + Centrifugation</td>
<td>Wet solvent lipid extraction + Oil hydrotreating + Anaerobic digestion of residues</td>
<td>8.52 US$/gallon of algal TAG</td>
<td>[47]</td>
</tr>
<tr>
<td>Open Ponds</td>
<td>Flocculation + Centrifugation</td>
<td>Hot algal lipid stream extraction + Anaerobic digestion of residues</td>
<td>10.87 US$/gallon of algal TAG</td>
<td>[48]</td>
</tr>
<tr>
<td>Open Ponds</td>
<td>Dissolved air flotation + Centrifugation</td>
<td>Wet solvent lipid extraction + Oil hydrotreating + Anaerobic digestion of residues</td>
<td>12.7 US$/gallon of algal TAG</td>
<td>[49]</td>
</tr>
<tr>
<td>Closed PBR</td>
<td>Dissolved air flotation + Centrifugation</td>
<td>Wet solvent lipid extraction + Oil hydrotreating + Anaerobic digestion of residues</td>
<td>18.1 US$/gallon of algal TAG</td>
<td>[47]</td>
</tr>
<tr>
<td>Closed PBR</td>
<td>Dissolved air flotation + Centrifugation</td>
<td>Wet solvent lipid extraction + Oil hydrotreating + Anaerobic digestion of residues</td>
<td>31.6 US$/gallon of algal TAG</td>
<td>[49]</td>
</tr>
<tr>
<td>Closed PBR</td>
<td>Dissolved air flotation + Centrifugation</td>
<td>Wet solvent lipid extraction + Oil hydrotreating + Anaerobic digestion of residues</td>
<td>77.00 US$/gallon of algal TAG</td>
<td>[50]</td>
</tr>
<tr>
<td>Open Ponds</td>
<td>Flocculation + Centrifugation</td>
<td>N. A.</td>
<td>109.00 US$/gallon of algal TAG</td>
<td>[50]</td>
</tr>
<tr>
<td>Open Ponds + High productivity + Free nutrients/CO₂</td>
<td>Flocculation + Centrifugation</td>
<td>N. A.</td>
<td>0.44 US$/kg of algal biomass</td>
<td>[51]</td>
</tr>
<tr>
<td>Open Ponds + High productivity + Free nutrients/CO₂</td>
<td>Flocculation + Centrifugation</td>
<td>N. A.</td>
<td>1.98 US$/kg of algal biomass</td>
<td>[51]</td>
</tr>
<tr>
<td>Closed PBR + High productivity + Free nutrients/CO₂</td>
<td>Flocculation + Centrifugation</td>
<td>N. A.</td>
<td>4.19 US$/kg of algal biomass</td>
<td>[51]</td>
</tr>
<tr>
<td>Closed PBR</td>
<td>Flocculation + Centrifugation</td>
<td>N. A.</td>
<td>11.02 US$/kg of algal biomass</td>
<td>[51]</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Microalgae growth mode</th>
<th>Substrate/Medium</th>
<th>Microalgae Species</th>
<th>Biomass Productivity</th>
<th>Lipid productivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterotrophic</td>
<td>Pretreated sugarcane molasses</td>
<td><em>Chlorella zofingenis</em></td>
<td>1.55 gL⁻¹ d⁻¹</td>
<td>0.710 gL⁻¹ d⁻¹</td>
<td>[56]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Corn ethanol thin stillage supernatant</td>
<td><em>Chlorella vulgaris</em></td>
<td>2.5 gL⁻¹ d⁻¹</td>
<td>11 gL⁻¹ d⁻¹</td>
<td>[57]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Diluted crude glycerol</td>
<td><em>Botryococcus braunii</em></td>
<td>0.271 gL⁻¹ d⁻¹</td>
<td>0.044 gL⁻¹ d⁻¹</td>
<td>[58]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Diluted technical glycerol</td>
<td><em>Scenedesmus sp.</em></td>
<td>0.229 gL⁻¹ d⁻¹</td>
<td>0.031 gL⁻¹ d⁻¹</td>
<td>[59]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Diluted technical glycerol</td>
<td><em>Nanochloropsis sp.</em></td>
<td>0.074 gL⁻¹ d⁻¹</td>
<td>0.155 gL⁻¹ d⁻¹</td>
<td>[60]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Diluted sugarcane molasses</td>
<td><em>Botryococcus braunii</em></td>
<td>0.184 gL⁻¹ d⁻¹</td>
<td>0.068 gL⁻¹ d⁻¹</td>
<td>[61]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Diluted sugarcane vinasse</td>
<td><em>Scenedesmus sp.</em></td>
<td>0.064 gL⁻¹ d⁻¹</td>
<td>N.A.</td>
<td>[62]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Diluted beet vinasse</td>
<td><em>Spirulina maxima</em></td>
<td>0.240 gL⁻¹ d⁻¹</td>
<td>N.A.</td>
<td>[63]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Glycerol diluted in domestic wastewater</td>
<td><em>Botryococcus terribilis</em></td>
<td>0.282 gL⁻¹ d⁻¹</td>
<td>0.035 gL⁻¹ d⁻¹</td>
<td>[32]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Produced water</td>
<td><em>Cyanobacteria (Oscillatoriales)</em></td>
<td>4.8 g m⁻² d⁻¹</td>
<td>N.A.</td>
<td>[64]</td>
</tr>
<tr>
<td>Mixotrophic</td>
<td>Treated produced water</td>
<td><em>Microalgae polyculture</em></td>
<td>11 g m⁻² d⁻¹</td>
<td>N.A.</td>
<td>[65]</td>
</tr>
<tr>
<td>Photoautotrophic</td>
<td>Anaerobically digested vinasse</td>
<td><em>Chlorella vulgaris</em></td>
<td>0.070 gL⁻¹ d⁻¹</td>
<td>0.017 gL⁻¹ d⁻¹</td>
<td>[31]</td>
</tr>
</tbody>
</table>

Indeed, contamination control and crop protection pose a major challenge for microalgae large-scale cultivation, especially if the use of waste streams rich in organic compounds is considered [54,82–84]. In line with this assumption, industrial residues/effluents are often pretreated in order to favor microalgae growth over contaminants. The pretreatment processes used can be simple dilutions, pH corrections and suspended solids settling as well as clarification, hydrolysis, ultraviolet irradiation, anaerobic digestion, filtration, heavy metals removal, among others [31,32,56–65,84] (Table 2). In addition, a number of strategies can be deployed to mitigate contamination during the microalgae cultivating course [82,85,86]. For example, cyanobacteria and
rotifers invaders can be reduced in *Nannochloropsis salina* cultures by setting salinity to 22 ppt and using ammonium chloride as a nitrogen source [65]. Pesticides such as parathion and dichlorodiphenyltrichloroethane (DDT) have also been successfully used in *Chlorella* cultures to control infestation by Copepoda crustaceans with no significant toxicity for the algae [87]. Furthermore, the management of contaminants in large-scale production systems using microscopic and molecular methods for real-time tracking of pests combined with specific crop protection strategies (e.g.: pesticide application) have been recently demonstrated [85,88].

It is important to highlight though, that maintaining axenic conditions (algal monocultures without other microorganism’s) in algal culturing systems would neither be practical nor economic viable, since a large diversity of microbial organisms will inevitably be present, even in treated cultures/substrates [83]. Therefore, the selection of robust microalgae strains capable of achieving and maintaining high growth rates in exposed cultivation systems even in the presence of a small percentage of contaminants will be required. These strains will probably be specific for each substrate/location as indicated by the higher productivities obtained with indigenous microalgae grown in produced water [64,65] shown in Table 2. In addition, in order to maximize microalgae culture resilience and productivity, the use of engineered polycultures where diverse genotypes are grown together in an ergonomic manner should be considered [89]. Also promising is the use of breeding and genetic engineering (GMO) techniques to develop strains with traits that provide crop protection and fast efficient growth [reviewed in 15,90]. Indeed, a two-fold increase in the biomass productivity has been reported in transgenic *Chlamydomonas reinhardtii* strains capable of self-adjusting the ratios of their chlorophyll binding proteins in response to changing light levels or culture densities [65]. Financial modeling based on these strains productivities indicated that 240% increase in receipts and 85% reduction in the cost algal biocrude would be achieved compared to the non-GMO baseline case [46].

Finally, since spills of cultured microalgae are expected to eventually occur from biomass production facilities, the risk of releases into natural ecosystems must be considered for large-scale cultivation. In addition, as massive spills might also happen due to extreme climatic events (e.g.: Storms, floods and earthquakes), strategies to mitigate cultured strains leakage using physical containment and genetic modifications that preclude cultured microalgae from competing in nature should be applied either to transgenic or nontransgenic algae [52,91]. The mitigation strategy employed should be carefully evaluated case-by-case considering the microalgal strain chances of surviving and reproducing in nature as well as its capability of causing irreversible, or slowly reversible, impacts in the environment [52]. For example, a strain carrying traits that enhance competitive fitness both in artificial systems and in the wild, such as improved nutrient uptake or prevention of contamination by toxin production [92], should be classified as high risk. On the other hand, traits that enhance capture of light [93,94], which is usually not a limiting factor in nature, or transgenic herbicide resistance, as long as the herbicide is not generally used in the environment, could be classified as low risk [52]. Additionally, assessing the environmental risks of uncontrolled non-native strains releases can be especially challenging, since large numbers of species with varying degrees of fitness co-exist in natural ecosystems in an unpredictable balance [95,96]. Thus the most stringent mitigation systems are needed both for non-native species and for selected/engineered strains that possess enhanced fitness into the wild. In such cases, genetic modifications that allow cultivation only in artificial systems and are lethal to the algae in nature (e.g.: disruption/reduction of carbon capturing or nitrate utilization) will probably be required [52].

4.2. Microalgae biomass harvesting and conversion

After cultivation, the algal biomass must be harvested and converted into biofuels and bioproducts. The choice of the downstream processes used greatly influences the economic viability, efficiency, operating costs and energy requirements of microalgae biorefineries [97] (Table 1). Harvesting is generally a multistage process resulting in the concentration of algae in water, being responsible for up to 30% of the total production costs. It generally involves one or more steps of solid-liquid phase separation, such as flocculation, filtration, flotation, settling and/or centrifugation [98]. Centrifuges are a proven technology that has been used as a means for harvesting algae for many years [74]. However, the high energy requirements and the costs to centrifuge large amounts of water reduce the economic viability of this process [97]. Therefore, harvesting methods with low-costs and high processivity are required to dewater algal biomass prior to lipid extraction or biomass conversion.

Harvesting techniques based on the use of chemical flocculants and dissolved air flotation are low-cost methods that have been successfully applied either to wastewater treatment or to cultured microalgae [99–101]. Especially promising are methods based on the use of non-toxic flocculants such as mixed calcium and magnesium hydroxide [102]. In addition, recent economic assessments indicate that methods based on membrane filtration, ultrasound and electrocoagulation harvesting have the potential to achieve relatively low costs. Among them, electrocoagulation is the most mature technology with commercial applications existing in the wastewater treatment industry to remove a variety of charged ionic contaminants from solution [97,103].

Electrocoagulation uses metallic electrodes to produce positively charged ions that induce coagulation of microalgal cells, which are negatively charged. The settled coagulated cells can then be removed by conventional sedimentation methods [97]. Electrocoagulation systems can achieve 95% recovery of algae using only 25% of the energy compared to centrifuge technology [16,97,103]. However, the after harvesting microalgae solution generated possesses up to 6% total solids when starting with an algae solution of approximately 0.1% total solids [97,103]. Consequently, electrocoagulation may serve as a primary harvesting methodology that can be used in combination with further concentrating methods (e.g.: Membrane filtration or centrifugation) to achieve higher concentration factors or higher throughput.

The technological pathway traditionally envisioned for downstream processing of microalgal biomass involves biomass harvesting and drying followed by the extraction of the lipidic fraction and conversion to biofuels (i.e.: biodiesel). The remaining lipid extracted algae (LEA) fraction would be directed to the production of fertilizers, animal feed or other bioproducts [16,19]. This model, however, has been challenged by recent advances in extraction/conversion processes capable of handling wet feedstocks, as well as, converting whole algal biomass into oil [16,46,97,104] (Table 1).

The prohibitive costs and energy requirements of algal biomass drying have led to the development of processes capable of extracting lipids from wet biomass, such as the wet solvent extraction process. This process pretreats harvested algae biomass in water with conditioning chemicals to enable non-polar solvent to remove lipids (Valicor Renewables, LLC). The extracted algal mass is then phase separated and lipids are recovered from the nonpolar solvent by distillation [97]. The economic viability of using only the lipidic fraction for the production of biofuels while converting the LEA fraction into other products (i.e: fertilizers and animal feed) is controversial though [19,45,46]. Furthermore, the use of wastewater for cultivating microalgae might render algal
biomass unsuitable for the production of animal feed or food supplements.

Recent studies indicate that whole wet algal biomass conversion into oil through hydrothermal liquefaction and catalytic hydrothermal gasification (HTL-CHG) might be the most promising downstream processing pathway [16,46,97,104] (Table 1). Indeed, Richardson and coworkers [97] reported that the revenues for a scenario using HTL-CHG are 69% higher than using wet solvent extraction. In addition, the combination of electrocoagulation harvesting and HTL-CHG can lead to 90% lower lipid total cost compared to centrifugation followed by wet solvent extraction [46,97].

The HTL-CHG, which functions both as an extraction and as a conversion technology, converts not only the algal lipids, but also part of proteins and carbohydrates into oil that can be upgraded to biofuels (e.g.: renewable diesel, jet fuel, gasoline) [104]. The total oil yield from algae is generally in the range from 35% to 65% as measured by weight of oil as a percent of feedstock dry weight, which is considerably higher than the yields obtained with methods that use only the lipidic fraction of algal biomass [97,105]. HTL-CHG processing converts algal slurries with approximately 20% of solids in water into oil by using temperatures and pressures just below the supercritical point of water. No acid pretreatment, solvent, or separations of acid and solvent are needed [105]. The wet feedstock is first processed via HTL to produce oil leaving an aqueous effluent containing organic matter. This effluent is then processed via CHG to gasify the remaining organic matter producing methane gas and to recycle water and nutrients, such as nitrogen, potassium, phosphorous and micro-nutrients [97,105].

However, even though HTL-CHG technology looks promising, future works focused on large scale demonstrations are still required. Furthermore, for carbohydrate-rich algae biomass, it might be more efficiently to biochemically convert feedstock into ethanol and bulk chemicals instead. In such cases, processing of algal biomass through hydrolysis followed by ethanol fermentation or mixed organic acid fermentation followed by ketonization and catalytic upgrading to aromatics should be considered [16].

5. Conclusions

The biofuel and petrochemical refineries provide promising possibilities for the exploration of microalgae feedstock integration to existing industrial facilities. Although still in its infancy, microalgae large-scale production might provide valuable resources for future industrial ecology, bringing many environmental deliverables such as reduction of fossil fuel usage, mitigation of green-house gas emissions, better land use and improvement in food security. However, in order to achieve commercial viability, several innovative technological solutions are still needed to overcome the issues related to microalgae strains development, cultivation systems, crop protection, harvesting, processing, environmental risks and resources management. Therefore, we emphasize the need for large scale demonstrations to increase the confidence in algae-based and integrated biorefineries. Hopefully, the present and future R&D investments, policy initiatives and public-private partnerships should push forward the development of economically viable microalgae biorefineries setting the course towards a future carbon-smart society.

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