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REVIEW PAPER

Microalgae as a potential source for biodiesel production: techniques, methods, and other challenges

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SUMMARY

This paper reviews some of the most important aspects of microalgae as a potential source for biodiesel production. Microalgae are photosynthetic microorganisms that can grow rapidly in a variety of environments because of their unicellular or multicellular structure depending on the species. They have the advantage of self-reproduction using solar energy and converting it into chemical energy via photosynthesis. This process concludes a full cycle in a few days, obtaining higher lipid yields than terrestrial crops. This review shows several techniques and some methodologies used in the biodiesel production process from microalgae as well as the challenges that must be overcome for large-scale process and in bio-refineries. Copyright © 2016 John Wiley & Sons, Ltd.

KEY WORDS

microalgae; biodiesel; biofuel; lipids; bio-refinery

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1. INTRODUCTION

The International Energy Agency (2014) reported that 63.7% of the oil produced is consumed by the transportation sector, which means around 22% of the world CO_2 emissions are by this sector [1]. In Mexico, around 50% of the energy produced is consumed by the transportation sector, causing around half of the total CO_2 emissions by the energy sector in the country [2]. Biodiesel is one of the main alternatives to fossil fuels. The production of biodiesel has received much attention worldwide and was one of the first alternative fuels known to the public [3–6].

Microalgae, the third-generation biodiesel feedstock, has been developed as one of the most promising alternative sources of lipid for use in biodiesel production because of their high light to biomass conversion efficiency and higher growth rates and productivity compared with conventional energy crops like corn, hemp, soybean, camelina, canola, sunflower, castor, and palm oil [7–9].

Microalgae are autotrophic microorganisms with a high photosynthetic efficiency; they are important source of lipids, hydrocarbons, and other complex oils for biodiesel [10–12]; besides, they can produce other valuable fuels like bioethanol, biomethane, and biohydrogen [13–15].

Microalgae with high oil content has the oil yield potential up to 25 times higher than that of traditional biodiesel oilseeds, such as palm oil. Microalgae, with an oil production of at least 70% of oil by weight of dry biomass, require only 0.1 m^2 of land to produce 121,104 kg of biodiesel per year [16].

Success of microalgae biodiesel production depends on low-cost cultivation systems, efficient biomass harvesting, and separation methods as well as suitable oil extraction techniques [17]. In addition to these aspects, efficiency of microalgal biomass production is highly influenced by environmental conditions such as light, temperature, CO_2 concentration, nutrient composition, salinities, and mixing conditions [18].

Harvesting biomass is essential for mass production of biodiesel from microalgae. The major harvesting techniques applied include centrifugation, flocculation, chemical coagulation, filtration and screening, gravity sedimentation, flotation, and electrophoresis techniques [19–23].

Pure oil used in making biodiesel consists of triglycerides in which three fatty acid molecules are esterified with a molecule of glycerol. In biodiesel process, triglycerides are reacted with methanol in a reaction known as transesterification or alcoholysis. Transestrification produces methyl esters of fatty acids or biodiesel and glycerol. The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides, and finally to glycerol [24].

The aim of this review is to show the overall picture of microalgae production in the world. In a first analysis, this work exposes and compares methods and techniques that are currently being used related to strain selection, cultivation, and microalgae harvesting. Another analysis of this work focuses on the lipid extraction methods for biodiesel production and their challenges, especially scale-up to pilot plant or industrial level. Finally, it analyses a perspective of bio-refinery based on microalgae.

2. GENERAL ASPECTS OF BIODIESEL FROM MICROALGAE

2.1. Microalgae

Microalgae are one of the oldest organisms on earth. They are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions because of their unicellular or simple multicellular structure and are usually found in colonies. They can adapt to a variety of environments. The number of algae species are estimated between 1 and 10 million; about 200,000 to 800,000 are microalgae species, and just around 35,000 have been studied [25]. Around 15,000 have been used to obtain new compounds originating from algae biomass [26] like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins, and sterols [27]. Despite living in all latitudes, they are more populous in the northern hemisphere, having an annual production of around 1.5 million tons, which contributes to fix about 50% of global organic carbon [25,28,29].

They are classified into 11 divisions, 2 prokaryotes (*Cyanophyta*/cyanobacteria and *Prochlorophyta*), and 9 eukaryotes (*Glaucophyta, Rhodophyta, Heterokontophyta, Haptophyta, Cryptophyta, Dinoflagellates, Euglenids, Chlorarachniophyta,* and *Chlorophyta*) [30]. The US Aquatic Species Program [31] selects five groups of eukaryotic microalgae as high priority for biofuel production. They are diatoms (Bacillariophyceae), green algae (Chlorophyceae), golden brown algae (Chrysophyseae), prymnesiophytes, or haptophytes (including Prymnesiophyceae) and eustigmatophytes (Eustigmatophyceae). Many strains and genera of eukaryotic microalga are potential high-oil producers for large-scale culture [31–33]. The eukaryotic microalgae are categorized into different classes defined by their pigmentation, life cycle, and cell structure [34]. Microalgae composition has a varied concentration of proteins, carbohydrates, and lipids [35]; they have a high growth rate and can duplicate their cells several times in 1 day. During the exponential growth phase, it can be doubled at about 3.5 h [8,27], producing more biomass per unit area compared with terrestrial corps. Their size ranges from 2 to 200 μm depending on the species. Microalgae are called thallophytes that mean plant body lacking roots, embryos, vascular system, stems, and leaves. They use chlorophyll as primary pigment for photosynthesis [36] and can be cultured either autotrophic, heterotrophic, or mixotrophic [37].

2.2. Historical aspects

The use of microalgae is not new; the first report comes from China, where it was used as edible *Nostoc flagelliforme* as food for 2000 years [38]. In Mexico, the Aztecs used *Spirulina* (*Arthrospira platensis*) from Lake Texcoco as a food source according to the annals of the Spanish conquest early in the 16th century [39]. Since 1940, it has been used in aquaculture. After World War II, Germany began studies to obtain protein and fat from microalgae [40] likewise extended to countries like USA, Japan, Israel, and Italy. Since then, the idea of using it in wastewater treatment appeared. In 1960, Taiwan and Japan introduced to market an innovative and healthy food item using *Chlorella* species [41,42]. In this decade, interest began to develop support systems for space missions. In the decade of 1970, the main attention was given on the use of biomass for fuel and fertilizer. By 1980, the use of microalgae allowed the development of major chemicals for pharmaceutical and agrochemical industries [43,44]. The field of study of microalgae has been growing because of problems such as global warming, water scarcity, food security, and the constant increase in fuel prices [45]. Because of this, the current status of research is aimed at the fields of water treatment and pollution, atmospheric regeneration, natural health products, biofuels, and power generation [46–48].

2.3. Microalgae as a biofuel source

The use of microalgae as a source of biofuel has become relevant, particularly for biodiesel production [49–52], which is obtained from different raw materials, mainly animal fats and vegetable oils. Nowadays, biodiesel is produced from soybean, animal fats, palm oil, waste cooking oil, and jatropha oil. Although biodiesel from mircoalgae is not intensively produced yet, it has proven advantages over terrestrial crops [53]. Appropriate selection of the species is the first step for successful microalgae to biodiesel conversion because it depends on the amount of lipids within the cell, in particular, TAG content (triacylglyceride) (Figure 1). Researchers aim to increase both biomass productivity and lipid content, usually under stress conditions such as nitrogen starvation, phosphate limitation, and high Fe^{3+} concentrations. Another way is using wastewater as culture medium, finding specific species and growing them under local conditions [54–57].

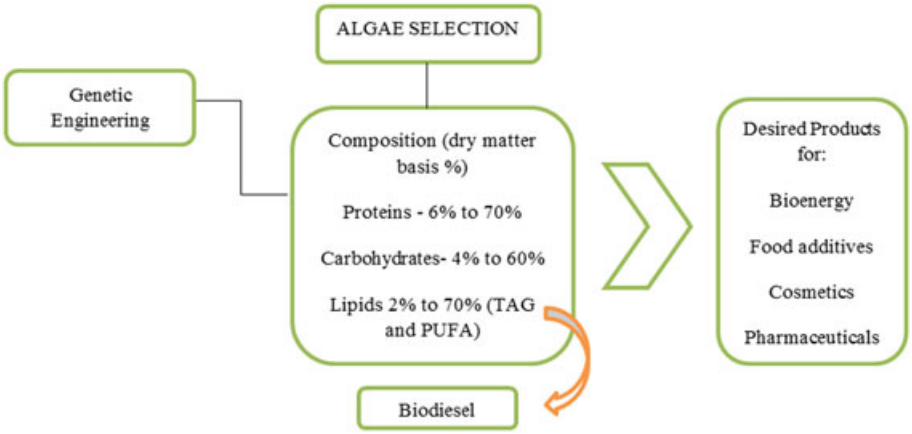

Figure 1. Algae selection based on its composition and some final products.

Some species have a lipid content between 1% and 70%; others can reach up to 90%, although 20–50% is a common value [58]. Assuming 30% as lipid content without optimizing the culture medium, production is estimated between 4.5 and 7.5 tons ha^{-1} $year^{-1}$, which is higher compared with terrestrial crops such as soybeans (0.4 tons ha^{-1} $year^{-1}$), rapeseed (0.68 tons ha^{-1} $year^{-1}$), palm oil (3.62 tons ha^{-1} $year^{-1}$), and jatropha (4.14 tons ha^{-1} $year^{-1}$) [59,60]. Biodiesel from microalgae contains no sulfur, thus allowing significant reduction of CO, SOx, and hydrocarbons, however, increases the amount of NOx in some engine types [61].

2.3.1. Microalgae biodiesel advantages

The advantages of microalgae-based biodiesel are fast growth rate (up to 100 times more than terrestrial crops) and high lipid content [62]; use of wasteland unsuitable for agriculture; no conflicts with use of food and its production [24]; efficient photosynthetic mechanism [7]; removal of large amounts of CO_2 emitted, contributing to GHG (Greenhouse Gases) mitigation [63]; removal of high concentrations of nitrogen, phosphorus, and heavy metals from wastewater [64,65]; production all over the year; can be grown in freshwater, saline water, and wastewater [66]; can produce 10 times more biodiesel per unit area of land compared with a typical terrestrial oleaginous; and others [24,31,67,68].

2.3.2. Problems facing microalgae biodiesel production

Problems related to microalgae-based biodiesel production are adapting cost-efficient technologies for production [69]; developing large-scale protocols and methodologies [70], although there are some proposed by Alfaro *et al.* [71]; analysis of the effects of several parameters that modify the amount of lipid productivities using microorganisms; and improving several modifications to both upstream (microalgae culture, harvesting, and drying biomass) and downstream processes (lipid extraction, biodiesel, and bioethanol conversion techniques) [72]. From the thermodynamic perspective, energy balance study is not clearly addressed [72]. But one of the main challenges is to make the correct selection of microalgae use for biodiesel production.

In the past, when large concentrations of carbon dioxide existed, microalgae were an important factor to give the atmosphere the proper conditions as we know them. Currently, they play an important biological role in the balance of oxygen and CO_2. On top of that, much of the currently extracted fossil fuel is also from microalgae. Therefore, because of advances in research and biotechnology, these organisms can be the key not only to continue fighting the climate issues but also to address energy problems that we are facing currently.

3. CULTURE SYSTEMS

Selection of microalgal cultivation system plays an important role in the biofuel production process. Such systems can be divided mainly into two groups mainly, open systems and closed systems (Figure 2), both with advantages and disadvantages depending on the desired final product. Despite of this, success for both systems at small scale to large scale depends on certain requirements in order to optimize the photosynthetic process and improve their own design, such as (i) effective light source intensity, (ii) optimal gas–liquid transfer (e.g., CO_2 and air), (iii) easy to operate, (iv) low contamination level, (v) low production costs, (vi) minimal land area, (vii) maximizing nutrients, and (viii) temperature control; all of these should be optimized for as many species as possible [73–78].

3.1. Open systems

Many large-scale systems used today are open systems, which have been used since 1950; some reasons for their preference are algae properties to adapt to the local environment, like climatic conditions, also the costs of land

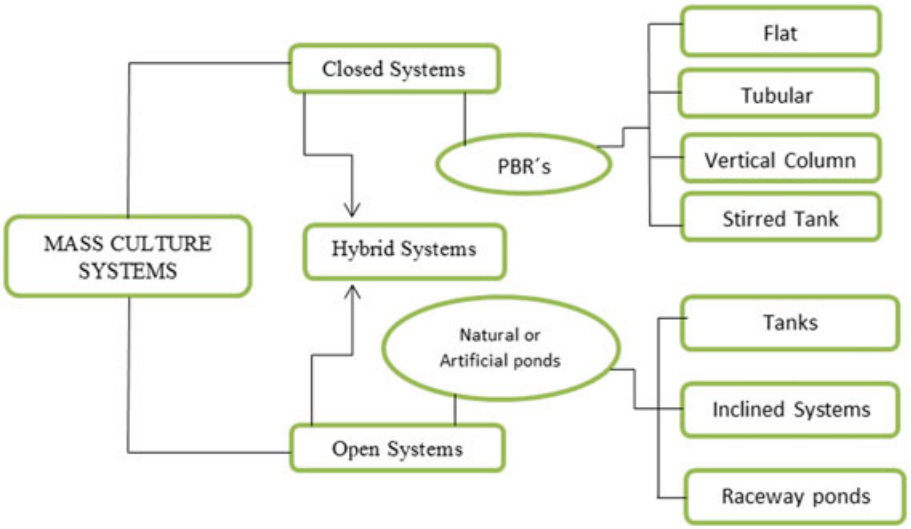

Figure 2. Mass culture system classification and the most common types.

and use of water. Some designs for this type can be categorized into natural waters (lakes, lagoons, and ponds) as well as artificial ponds; the latter could be shallow ponds, tanks, circular ponds, inclined type systems, and raceway ponds. Raceway ponds are currently used more commonly [79–81]. This type of system is generally shallow (20–50 cm) because the algae need to be exposed to sunlight and its depth is limited [82]. It has an oval shape, forming a closed circuit, mixed by a paddle wheel that prevents settling; nutrients are added in front of it and harvested from the other side; the flow is guided along a series of baffles [83,84]. In order to enhance CO_2 transfer, it is added by submerged aerators [85]; although to optimize such mass transfer, carbonation columns could be attached to improve the gas transfer to the liquid phase to at least 90% [86]. Nutrient source may come through runoff water from wastewater treatment plant [25], which allows reducing water use as well as decreasing nutrient consumption from fertilizers.

3.1.1. Advantages

Construction materials are usually concrete with white plastic membrane. The main reason by which these systems are used is because of their low cost for large-scale deployment; large areas of land not necessary; energy usage is minimal in comparison with closed systems; and maintenance, cleaning, construction, and setup are easy [24,87,88]. There are some aspects that can be optimized, such as algal strain resistance to severe culture environment, (for example, *Dunaliella*, *Spirulina*, and *Chlorella* sp.) which are cultivated in high salinity, alkalinity, and nutrition, which results in high production rates [89–91]; prevention of the spreading of invasive species; and improving the control of culture medium, temperature, and contamination and, therefore, increases production rates; this is usually achieved using a transparent cover over the pond [25].

3.1.2. Limitations

Open systems still have some technological challenges to be solved, among the major problems they face are only a small number of algal species can be grown successfully, low productivity values are reported with respect to theoretical ones, difficult culture control, low depths produce high evaporation losses, and mixing is not efficient hence nutrients and light performance are not consumed. Besides, this faces threat of contamination from other algae, bacteria, and protozoa species; temperature fluctuates seasonally and produces low biomass concentrations rates in relation to large volumes that require large areas of land [36,68,83,92,93].

3.2. Closed systems

Control complexity of limiting parameters in open ponds can be achieved by the design and development of closed systems, commonly called photobioreactors [94], defined as reactors in which phototroph systems grow and perform photobiological reactions such as photosynthesis. The main feature is that light not only reaches the surface but also has to pass through the transparent walls to reach the greatest number of cells in the culture; therefore, it is essential to apply designs that reduce the light path, hence increasing the amount of light available to each cell [93].

While there are different types of photobioreactors, the main objective of all of them is to achieve high volumetric productivities and high light conversion efficiency. Special emphasis must be addressed on features such as surface-to-volume ratio; orientation; in some types slope angle, mixing, and degassing system; cleaning systems; temperature control; transparency and durability of materials; easy scale-up; and low installation and operating costs [95].

Tsoglin *et al.* [96] suggested some design parameters for closed systems. Those are

- The reactor should allow the cultivation of different microalgal species.
- Must have uniform illumination and rapid mass transfer (CO_2 generated and O_2 consumed).
- Design must prevent or minimize biofouling of the reactor.
- Reduce cell damage due to fluid dynamics.
- Photobioreactors must work under intense foam conditions, mainly on high rates of mass transfer reactors.
- Should have minimum non-illuminated part.

3.2.1. Advantages

Contamination control is higher than open systems; this leads to a better cell growth, improving the quality of biomass especially if high-value products are preferred [88,89,93,97,98]. Furthermore, it allows the use of a wide range of species. They need less space; they are able to achieve high production per volume reducing harvesting costs [92]. Photobioreactors lose less water by evaporation and enhance CO_2 fixation levels [99–101]. They are able to operate at high biomass concentrations because of their high surface area-to-volume ratio [102,103].

3.2.2. Limitations

Photobioreactors scale-up faces a great challenge, mainly because of their high construction costs, operation, and maintenance [92,104,105]. Use of glass may have advantages such as durability, higher light path efficiency, and easy to clean. However, high costs, weight addition, and safety risks are the limitations. Plastics are more economical; however, lifetime is shorter and may become opaque. Selection of materials is quite important, especially for large-scale purposes. Operation also faces challenges such as usage of artificial lighting, mixing systems, cooling systems, gas exchange system consumption (CO_2) versus dissolved (O_2), and batch or continuous production mode [32,83,94,105–110]. These parameters produce a negative impact on the energy balance because of energy consumption. A proposal is to integrate clean technologies such as solar and wind energy looking for sustainable biofuel production from microalgae [72].

Before designing the cultivation system, one should think of the type of microalgae that one wants to cultivate, because each species has special requirements of space, ventilation, lighting, and temperature and do not have the same metabolic behaviors. Although there are advantages and disadvantages in both types of systems, it is the combination of both of them that takes us to a constant and intensive production of microalgae. Normally, in their first stages of growth, closed systems are required to optimize the quality of the production and safety of the inoculum and subsequently stress them in open systems with large volume to produce the metabolites that we interested.

Culture systems are very important, because they carry out microalgal biomass aspects like productivity and quality. Therefore, it is important to design and implement methodologies to improve intensive production. Unfortunately, these standardizations are not well defined yet, because not all species are easily adapted to most geographical regions or simply there are differences in their metabolic behavior. Thus, design parameters have only been reported for specific regions and species. Table I shows the most relevant aspects for both systems, open ponds, and photobioreactors.

4. PHOTOBIOREACTOR TYPES

4.1. Tubular

Tubular photobioreactors consist of an array of glass or plastic transparent tubes, also called solar collectors, which capture energy from the sun and is attached to a reservoir, where nutrients are usually added, that is where the gas exchange takes place; algae culture circulates through the solar array and back to the reservoir [24]. The solar collector is normally cooled by spraying water, which generates considerable extra energy consumption. These arrangements can be horizontal, vertical, inclined, helical, conical, or torus [111–114]. Tubular designs have limitations on the length of the tubes, which is dependent on potential CO_2 depletion and O_2 accumulation; the latter must be removed to avoid the cell growth inhibition [107,115]. Tubular photobioreactors are considered suitable for outdoor cultures because of their large illuminated surface area and good biomass productivities [88,108,116,117] and others.

Table I. The major pros and cons of microalgae culture systems.

Open systems		Closed systems	
(+)	(−)	(+)	(−)
Cheaper to build	Low productivity	Higher control	Operational cost
Easy to operate	Less control	Higher biomass productivities	Scale up
Maintenance and cleaning	Evaporative losses	Light efficiency	Energy input
Less energy input	CO_2 transfer rate	Cell mixing/Gas transfer	Maintenance and cleaning
	Light limitation	Avoid contamination	
	Contamination		
	Weather dependence		

4.2. Flat

Flat photobioreactors [118–122] have cuboidal shapes usually constructed of glass, plexiglass, and polycarbonate; mixing system is provided by bubbling or mechanical rotation [123]. One peculiarity is that its geometry permits a high surface area-to-volume ratio with minimal light path, which produces high cell concentrations and hence high yields of biomass production [41]. Advantages over tubular design are the low accumulation of dissolved oxygen, high photosynthetic efficiency [124], low energy consumption, no dark volumes, and high CO_2 consumption rates [101,110,125,126], hence considered to be relatively easy to scale up [127]. The mixing and installation costs are still high [36,128].

4.3. Vertical column

This type of reactors typically consists of a transparent cylindrical container with a sparger at the bottom, which can be divided into bubble column reactors [77,129–133] and airlift column reactors [134–138]. Gas diffusion takes place from the bottom covering the entire volume of the bubble column reactor to the airlift column reactor which is divided by two interconnecting zones, 'raiser' where gas mixture is sparged and 'down comer' which is gas free. Mixing in both cases is carried out by bubbling the gas through the sparger without mechanical agitation [123]. They can be artificially illuminated either internally or externally [107,139]. The vertical column reactor offers many advantages so it has become very popular, due to its low cost, compact size, feasible scale-up, easy operation, efficient mixing with homogeneous distribution, very high mass transfer rate, absence of wall growth, and can be used for both outdoor and indoor cultures [106,107,139–142].

4.4. Stirred tank

It is the most conventional way for microalgae culture; agitation is provided mechanically by impellers of different sizes and shapes for mixing; CO_2 (from air) is bubbled at the bottom of the tank as carbon source; illumination can be by external or using optical fiber; the primary drawback consists in the low surface area-to-volume ratio, which decreases the light harvesting efficiency [143].

The microalgae culture systems can be combined in order to optimize the production process; hence, the majority of microalgal bio-factories work with the so-called hybrid systems. Hybrid systems mainly combine different kinds of culture systems, improving the overall product performance, also combine two growth stages. In the first stage, the growth conditions and contamination are controlled (use of closed systems), promoting cell division; the second stage is aimed to create cell stress conditions, causing the lipid synthesis (use of open systems) [144,145].

Photobioreactor development and new designs are a constant practice. They still have many technological challenges to solve, like energy consumption, growth–light dependency (for photoautotrophs crops), mixed gas and optimal cellular uptake, dissolved oxygen accumulation, equipment maintenance, and cleaning issue. As it scales up, the challenges increase for closed systems, mainly in manufacturing costs, because of instrumentation and control systems as well as material selection. Closed systems or photobioreactors are being adapted to demonstration plants with open systems to evaluate their efficiency and biomass production rates. Recently, outdoor operating photobioreactors have received more attention for large-scale applications as illustrated in Table II. Culture volume and biomass productivity are still very much varied, from 0.4 to 400 L and 0.03 to 0.5 g L^{-1} day^{-1}, respectively. As many reports confirm,

Table II. The biomass productivities, work volume, and scale of various reactor types and strains.

Strain	Reactor type	Biomass productivity ($g\,L^{-1}\,day^{-1}$)$_{DW}$	Volume (L)	Scale	Year	Reference
Consortium (*Chlorella* sp., *Scenedesmus* sp., and pennate diatom)	Helical tubular closed PBR (Biocoil)	0.025 AFDW		Outdoor	2016	[113]
Chlorella vulgaris FSP-E	Vertical tubular	0.2681	40	Outdoor	2016	[112]
Scenedesmus bijugatus	Vertical tubular	0.26	50	Outdoor	2015	[259]
Tetraselmis suecica	Tubular	0.52	250	Outdoor	2014	[260]
Nannochloropsis gaditana	Vertical flat panel	0.038	400	Outdoor	2016	[118]
Thermosynechococcus elongatus BP-1	Flat-panel airlift	2.9	40	Indoor	2016	[261]
Chlorella pyrenoidosa	Bubble column	0.34	0.4	Indoor	2016	[129]
Neochloris oleoabundans	Bubble column	0.4188	14.5	Indoor	2016	[130]
Chlorella sp.	Open raceway	0.0727	1000	Outdoor	2016	[262]
Chlorella luteoviridis	Open pond	1.78	150	Outdoor	2014	[263]
Parachlorella hussii	Open pond	1.83	150	Outdoor	2014	[263]
C. pyrenoidosa	Open raceway	0.114	8000	Outdoor	2016	[264]

AFDW, Ash-Free Dry Weight; PBR, Photobioreactor.

indoor systems have higher productivities compared with open systems, from 0.3 to nearly $3\,g\,L^{-1}\,day^{-1}$ and 0.07 to $1.8\,g\,L^{-1}\,day^{-1}$, respectively.

5. HARVESTING

In the process of microalgae biofuel production, harvesting plays an important role because of its scale-up capability into industrial production. Harvesting pretends to remove all the algal biomass from the aqueous medium; this process is significant because it represents 20–30% of the total production costs [146] mainly because of electrical energy consumption and separation equipment maintenance [142]. Removal of biomass also faces difficulties because nowadays, there is no universal methodology to allow a wide range of species; furthermore, the actual technology is still in the developmental stage [147]. Common biomass removal troubles are due to cell size, ranging from 2 to 200 µm for eukaryotic cells and 0.2 to 2 µm for cyanobacteria and the biomass to liquid ratio ($0.3–5\,kg\,m^{-3}$) resulting in low biomass concentrations with a very high volume management that impacts on energy consumption [95,148,149]. Biomass recovery depends on factors such as type and species selection, mainly size and density ($1020\,kg\,m^{-3}$ average) [150,151], the culture method (open or closed reactors), culture conditions, and final products [109,152,153]. The most common strategies currently employed are centrifugation, filtration, sedimentation, chemical coagulation, electrophoresis, and screening. Techniques such as ultrasound and organoclays are still in development; some of these are preceded by a flocculation process [36,142,154,155] (Figure 3). While these technologies have demonstrated advantages for harvesting microalgal biomass, it is true that they are required to minimize their drawbacks such as high costs and low recovery efficiencies of solid–liquid separation, based on a cost/effectiveness for both the whole production and the product quality in the downstream process [156].

5.1. Centrifugation

This method is based on the generation of centrifugal force that allows separation of algae biomass by density difference; it is probably the fastest and most reliable method for the recovery of microalgae; the equipment is easy to be cleaned and also permits a variety of species [157]. This method applies only for high-value products but not for large-scale operations [158,159]. Despite having high concentration efficiencies >95% [160], the scale-up process is restricted because of energy costs. However, Dassey *et al.* [19] observed that for high flow rates and thus large volumes, the energy decreases significantly, compensating it with low efficiencies making it applicable to large-scale production [19].

5.2. Gravity sedimentation

Sedimentation is a simple process of solid–liquid separation under gravity, in this case the biomass from the medium. This process reduces operating costs because of its low electrical energy consumption, manpower, and strict control process requirements. This process can be enhanced by adding flocculants or lamella separators [149]. On the other hand, because of low final concentrations (0.5–3%), long settling times (0, 1–2, and $6\,cm\,h^{-1}$) and cell damage, this process has low reliability and efficiency (recovery 10–90%) [161]. It is highly influenced by the type of species, density, radius, size, cell mobility, and flow type (laminar or turbulent); thus, it is limited to strains

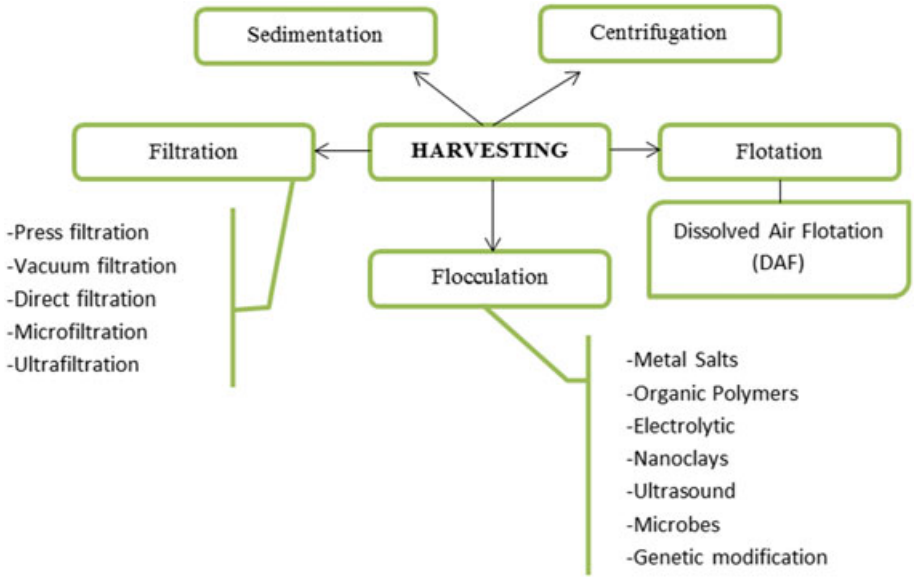

Figure 3. The different harvesting strategies used to recover microalgae biomass from culture media.

with high sedimentation rates. It can be used as a primary harvesting process, furthermore, requires large land areas for open ponds and sedimentation tanks. Gained biomass has high moisture content, which decreases efficiency on downstream processes. Despite its drawbacks and based on energy balance evaluation, sedimentation has positive techno-economic aspects that are sensitive for large-scale harvesting process [149,162].

5.3. Flocculation

Flocculation aims to increase biomass particle density, which has apparent negative charge; these aggregates also increase the particle size that makes the settlement easier [69,163]. Flocculants disturb the stability of the particles in suspension and allow them to aggregate, causing separation of the solid–liquid phases efficiently, improving the following processes such as centrifugation, sedimentation, and filtration. Usually, flocculants are inorganic multivalent metal salts, organic polymers, and microbes [20,23,164–167].

5.4. Filtration

Filtration process for harvesting microalgae needs mainly membranes, which allows only algal cells to pass through. The process is often abrasive for many microalgae causing its rupture as well as reduction in the quality of the cell content, so membrane design and structure must be based on species size used. This process requires backwashing to maintain the efficiency of the membranes; if fouling stores in the membrane, this process will require added time and cost [168]. For species with sizes larger than 70 μm, vacuum filtration is used. The French Press has been used for larger species like *Coelastrum proboscideum* and *Spirulina platensis*; direct filtration is used for species larger than 30 μm and microfiltration or ultrafiltration for smaller sizes [36]. The latter is efficient for fragile cells but is not appropriate for large-scale harvesting because of their low biomass recovery as well as the problem of membrane fouling [53,158,169]. Filtration processes are suited for small-scale systems. Large-scale systems require high maintenance and operating costs especially for membrane replacement or cleaning, also the energy used by the pumping system [21,170–172].

5.5. Flotation

Flotation is a separation process based on the attachment of air or gas bubbles to solid particles; the key of this process is the bubble size. The smaller the bubble size the higher the efficiency because of larger surface area per unit volume and lower buoyancy [173]. Bubble sizes can range from diameters smaller than 10 to 3000 μm according to the process [63,174–177]. The most common method is dissolved air flotation, a method used for the removal of sludge in the wastewater treatment, which consists of adding a flocculant to increase the particle size and then generate small bubbles that raise microalgal biomass. The advantage lies in the low cost and large-scale application; the drawback is the flocculent contamination [22,178–182].

Dissolved air flotation removes about 90–99% of algae and is more efficient than sedimentation [183]. Some parameters to be monitored in order to improve the process are tank pressure, recycle rate, hydraulic retention time, and floating rate of particles [155]. Bio-flotation uses oxygen, a product of photosynthesis, as flotation agent. Despite having good efficiencies in raceway ponds, day–night photosynthetic cycles produce discontinuous dissolved oxygen, making it not a feasible option [161].

5.6. Electrolytic methods

The negative charge of cells allows the use of the electric field in order to manipulate the movement of these charges out from the solution, at the same time this reaction generates hydrogen because of electrolysis of water causing solid molecules to be attached to gas bubbles and floated to the surface [184–186]. This method besides being effective, is of low costs, reduces operation time, and has low risk of contamination, because no chemical agents are added. One still has to develop equipment and strategies on a large scale in order to control the system temperature, energy consumption, and maintenance of cathode fouling.

5.7. Chemical coagulation

The chemical compound added to the culture to induce flocculation is a common practice in several countries and is very efficient for solid–liquid separation. This process is applicable to harvest cultures of several species of microalgae. The compounds may be inorganic or organic [187], one of the most used one is chitin, derived from exoskeletons of crustaceans.

5.8. Other developments

There are other methods recently studied in the process of microalgal biomass harvesting, including exposure of the microalgae culture to ultrasonic waves as cell aggregation method. This technique requires further study before it can be applied on a large scale. This technique coagulates not only microalgae biomass but also other sediments like heavy metals [188]. The use of nanoclays with Mg^{2+} or Fe^{3+}, capable to aggregate cells for settling, achieved a great interest because of their high efficiency and application to a variety of species and culture media, furthermore, its ability to be a recyclable agent. These techniques still need more research and technological development for their implementation [189,190].

Harvesting is a stage that requires further studies, mainly in scale-up, because harvesting generates higher cost by either current technology adaptation or energy consumption. Currently, different methodologies have been implemented in order to increase efficiencies of biomass concentration. In Table III, the efficiencies of different

Table III. The concentration efficiencies of various harvesting methods.

Strain	Harvesting method	Conditions	Concentration efficiency %	Year	Reference
Scenedesmus sp.	Flocculation	1.5 g L^{-1} of $Al_2(SO_4)_3$, pH 8.5	97.9	2016	[265]
	Centrifugation	2200 *g*	96		
Chlorella sp.	Flocculation	Magnetic iron oxide (Fe_3O_4) nanoparticles (MNPs) coating with amino-riched polyamidoamine (PAMAM) dendrime (80 mg L^{-1}), pH 8.0	95	2016	[266]
Chlorella sp.	Bio-flocculation	*Ankistrodesmus* sp., 50% (v/v), pH 7.1	82	2016	[267]
Aurantiochytrium sp. KRS101	Coagulation	$FeCl_3$ 1.00 g L^{-1}, 120 min	98.8	2015	[268]
	Electro-flotation	Anode: DSA, Cathode: A, 5.7 mA cm^{-2}, 40 min	59		
	Electro-coagulation–flotation	Anode: Al, Cathode: DSA, 11.4 mA cm^{-2}, 40 min	89.9		
	Centrifugation	1000 *g*, 30 min	87.2		
	Membrane filtration	Polyvinylidene fluoride (PVDF) 150 kDa, 240 min	99.9		
Prorocentrum lima	Double sedimentation–centrifugation	4 and 3 h, 12,000 *g* for 10 min	89	2015	[269]
Mixed algal cultures	Coagulation–flocculation/ Sedimentation	*Tanfloc* SG 20 mg L^{-1}, 4 days, pH 8.3	93.5	2016	[270]
Nannochloropsis salina	Flocculation–sedimentation	$Al_2(SO_4)_3$ 229 mg L^{-1},148 min	86.1	2016	[271]
Monoraphidium sp. FXY-10	Sedimentation	200 μM Fe3+, 12 h	90.74	2015	[272]
Phaeodactylum tricornutum	Submerged microfiltration	PVDF membranes	85–98	2013	[273]
Chlorella vulgaris	Submerged microfiltration	PVDF membranes	98–100		
Polyculture	Suspended air flotation SAF	Offshore PBR,depth 20 cm, 7 days	80–95	2016	[73]
C. vulgaris	Bioflocculation–flotation	*Cobetia marina* L03 20 mg L^{-1}, 5 mM $CaCl_2$	92.70	2015	[17]
Mixed algal cultures	Vacuum gas lift	Salinity of 40%, airflow rate of 10 L min^{-1} in microbubble air diffusion	49.5	2013	[274]
Phormidium sp.	Batch dissolved air flotation (BDAF)	Positively charged bubble flotation at a 30%, bubble rate at >16 mV, bubble formed at 6 bar	85	2015	[275]
Nannochloropsis sp.	Electro-coagulation–flocculation	Nickel electrodes, 4 V, 120 min	90	2014	[276]

harvesting techniques can be observed and some reports range from 80% to 100%. Microfiltration is a promising tool for larger scale systems but has the disadvantage of maintenance such as changing membranes periodically. The quality of biomass is an important point to be taken into account to obtain high value metabolites, so techniques such as flocculation or coagulation tend to contaminate the final biomass, which requires an additional process. Another recent option is to add other species to serve as a flocculant for better separation (bio-flocculation); filamentous species can be an alternative but still require further investigation.

Some works mention the immobilization of the biomass algal on supports. This can be a way less expensive for harvesting the algal biomass. Immobilization of cells within a matrix prevents the diffusion of the cells in the medium, but it allows the passage of metabolites and nutrients. It is one of the most widely used processes and can occur in synthetic polymers (resins, acrillamidas, and polyurethanes), proteins (collagen, gelatin, and egg albumin), natural polysaccharides (agars, alginates, or carragrenanos), or in the case of co-immobilization that occurs when working with alga–bacteria [191,192].

6. LIPID EXTRACTION

After reaching high biomass concentration using different types of culture systems, the next step is lipid extraction. The lipids are produced at the final stage of microalgal growth, commonly enhancing limiting nutrients like nitrogen [193]. The oil extraction process requires high amount of energy in the overall biodiesel production process, mainly because of the microalgal membrane, which has great chemical and mechanical resistance. Factors such as temperature and pressure have been used to achieve disruption of the cell membrane and then the extraction of high amounts of lipids as possible. There are various methods used for this purpose; some of them follow the same methodology adopted with terrestrial corps such as mechanical pressing, solvent extraction (hexane), or supercritical fluid extraction [95,194–199]. The choice of methodology is mainly based on the extraction efficiency, cost, and reproducibility (Figure 4). The precision cell disruption is the first step into the oil extraction process and may be carried out by mechanical, chemical, and biological processes.

6.1. Mechanical methods

Press/expeller method is the most common technique, initially applied for oil extraction from various oil seeds. By this method, oil from microalgal biomass can be removed nearly 70–75% of the dried biomass [200,201]. The remaining oil is attached to cell membrane so that it requires a second stage using solvents to increase the extraction efficiency [202]. High-frequency waves (microwave system) allow breaking weak hydrogen bonds of the cell wall. From another point of view, moisture of biomass causes the pressure increase, which promotes breakdown of the membrane [203–206] easier and faster access to its content; the final product quality is useful, but difficult to scale up. Ultrasound uses the cavitation effect; in consequence, bubbles collapse near cell walls allowing oil content release [207–209]. This method significantly improves oil extraction with high efficiency, short times, high yields, and low costs; the difficulty lies in scale-up [210]. Another common cell disruption method is bead milling, which consists of agitating very small beads at high speeds resulting in cell damage [103,211]. This method minimizes contamination from external sources and keeps chemical integrity of the cell content [212–214]; scale-up is complicated. The electroporation increases the cell wall permeability (pore size) and releases their contents by applying an external electromagnetic field [215]. Because of its simplicity, this method has acquired attention for industrial scale-up [216]. Other mechanical techniques are lyophilization, followed by grinding using liquid nitrogen [200], multipass homogenizers [217,218], and solvation by high salt solutions [219].

6.2. Chemical methods

The use of chemical reagents for lipid extraction reduces energy consumption compared with mechanical extractions, being this its main advantage. The use of solvents has the purpose to dissolve the lipids into the solvent and form a solution [220]. The most common solvents include benzene, cyclohexane, hexane, acetone, chloroform, diethyl ether, ethanol, and methanol [221,222] and combinations like hexane/methanol [223] and hexane/isopropanol [224]. The use of hexane and alcohol results less toxic and readily separating the phases in contact with water that improves the process [225]. The use of non-polar lipid solvents helps to break the hydrophobic interactions between neutral and non-polar lipids, while the polar solvents are involved in the disruption of hydrogen bonding between polar lipids [149].

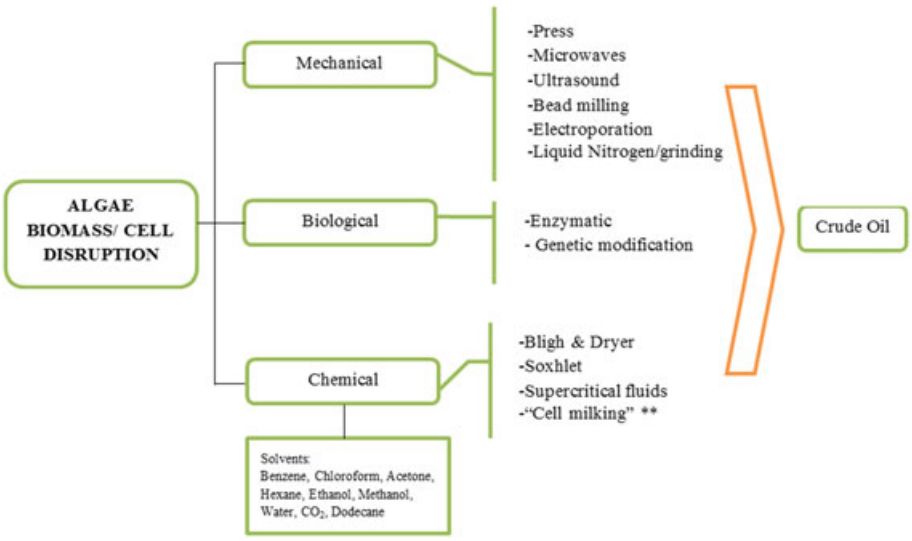

Figure 4. Biomass oil extraction methods. **Cell milking is not a cell disruption method.

This combination allows specific lipid extraction and separation from other products such as beta-carotenes and astaxanthin [158]. The advantage of these solvents is low cost and high efficiency therefore its use in oil extraction processes. The method used for extraction via solvent is the Bligh and Dyer method [205,226], which is nothing more than the methanol–chloroform–water combination [227]. It has efficiencies higher than 95% of total lipids, applicable to any cellular tissue and used in both dry and wet biomass [228]. The disadvantages are the large evaporation losses and toxicity; hence, it can be a limiting factor in industrial applications [229].

Soxhlet extraction is a technique of solid–liquid extraction; hexane is commonly used as the solvent, which is evaporated and re-condensed into the sample until reaching the complete removal. It has high efficiency even with long periods of energy extraction and evaporation, as well as their complexity of scale up [230,231].

The use of supercritical fluids is based on the solving skills promoted by fluids when they are above the critical point [232], which has proven its efficiency for oil extraction [194]. Currently, as a green solution advantage besides its properties, it is used as carbon dioxide solvent because of its critical properties (31,1 °C y 72,9 atm), in addition to its low toxicity and the fact that it is an inert gas [233]. The process begins with gas heating and compression to reach its critical point; it is then added to the harvested microalgae and acts as a solvent. After the extraction reaction has been completed, the solvent and product can be easily separated, once the temperature and pressure are lowed to atmospheric conditions. The CO_2 returns to its original state, achieving yields of up to 100% of oil. This technique is limited to energy consumption and scale-up. Among the advantages of the use of supercritical fluids are high efficiency, better fatty acid profile obtained compared with other methods, solvent-free extraction, separation is quick, the CO_2 can be obtained from industrial waste, and can be safely recycled, which positively impacts on the environment [234].

The use of water under its critical temperature and pressure high enough to keep the liquid state is another method used for oil extraction. At this temperature, water becomes less polar allowing it to dissolve the cellular content; when water is cooled back, it is no longer miscible with the extracted lipids and therefore separates easily [232]. Among its main advantages are reduced extraction time, high-quality products, low costs as a solvent, environmental compatibility, eliminating dewatering step, and drying [235]. The design is limited to large-scale units because of the rapid cooling systems. A very different approach to those already mentioned is called 'cell milking' in which a long-chain solvent like dodecane is used, which prevents cell damage and allows direct extraction of living cells, avoiding harvesting process and allowing culture to be reused for renewal of their lipid content [236,237]. It is limited to the type of algae because of the porosity of the cell wall and its low extraction efficiency, which demands further studies.

6.3. Biological methods

The use of enzymes to degrade the cell is a useful method for the extraction of oils contained within the cell. There are different types of enzymes; all of them can degrade specific bonds. Despite achieving good efficiencies, the cost of the enzymes as well as its reuse and reaction time are still limiting factors [238]. Genetic modification is intended to improve some species of algae to increase their lipid production, as well as their cell wall modification, achieving a weaker membrane, which may break at lower temperature and pressure. Some experiments have produced bacteria and yeast fatty acid excretions; however, yields are low and are not yet economically feasible [160,239].

The extraction with solvents is the most used method because of its high efficiency, selectivity between polar, non-polar lipids or both, and its easy application. However, at large scale, it still faces some challenges that have to do with the management, security, and the regulations that require the use of solvents. In Table IV, one can observe the yields of lipids by various techniques as well as species studied. As one could see, some mechanical techniques are complemented by chemical methods in order to increase the extraction yields; such is the case of the use of solvents with a system of microwave or ultrasound, hydraulic pressing, the latter with a great performance of extraction of more than 50%.

7. BIODIESEL PRODUCTION

Transesterification is the most commonly used method for biodiesel production; its purpose is to reduce the molecular weight of the oils to transform them into fatty acid alkyl esters when an alcohol is present (commonly ethanol or methanol) and a catalyst, the by-product is glycerin [149,240]. Some process parameters that modify the efficiency of the reaction are temperature, stirring, alcohol/oil molar ratio, moisture content, amount of free fatty acids, and the catalyst.

The catalysts include alkaline catalysts (sodium hydroxide, potassium hydroxide, and sodium methoxide); acid catalysts (hydrochloric acid, sulfuric acid, sulfonic acid, and phosphoric acid); enzymatic catalysts such as lipases; and inorganic heterogeneous catalysts (solid-phase catalysts) [241–246].

In order to improve the reaction process [247], an extraction–conversion process has been developed in one single step in which the solvent used in the extraction of oil also acts as reagent in the conversion into biodiesel fatty acids. The advantage is the reduction of stages in the process, costs, and time, having as limiting factors the moisture content and the product purification [248].

FAME's productivity is in fact little reported in the literature. Usually, it reports productivity of lipids and the profile of methyl esters. However, these data allow you

Table IV. The lipid yield and efficiencies of different extraction methods.

Strain	Extraction method	Conditions	Lipid yield %	Efficiency %	Year	Reference
Botryococcus braunii	Solvent mixtures	Chloroform–methanol mixture (75% v/v of methanol)	19.2		2016	[277]
	Solvent mixtures	Petroleum ether–methanol (75% v/v of methanol)	18.90			
Chlorella sp. KR-1	Chemical	Potassium persulfate 2 mM, 90 °C		95	2016	[278]
	Chemical	Hydrogen peroxide 0.5%, 90 °C		80		
Chlorella pyrenoidosa	Solvent—mechanical	Ethanol, hydraulic press, 30 °C, 600 kPa	11.3	72	2016	[279]
Chlorella sp. KR-1	Solvent—UV light	UV–Fenton-like reaction, 0.5% H_2O_2, 16 W of UV light		87	2015	[280]
Tetraselmis sp.	Solvent—microwave	Hara and Radin method + MW, 65 °C at 5 min	8.19		2014	[205]
Nannochloropsis sp.	Solvent—microwave	Folch *et al.* method + MW, 65 °C at 5 min	8.47			
Chlorella vulgaris	Solvent—microwave	Soxhlet, chloroform: methanol (2:1), 8 (h). MW 15 min at 100 °C	22.68		2016	[203]
C. vulgaris	Enzymatic hydrolysis -solvent	2% (v/v) Celluclast 1.5 L and 1% (v/v) Novozyme 188, 72 (h). chloroform : methanol = 2:1 (v/v)	~10		2013	[281]
Scenedesmus sp.	Supercritical carbon dioxide (SC-CO_2)	53 °C, 500 bar, and 1.9 g min^{-1}	7.41		2014	[282]
C. vulgaris	Solvent—ultrasound	Bligh and Dyer method, ultrasonic bath 40 kHz, intensity of 29.7 W L^{-1}	52.5		2013	[283]
C. vulgaris	Electroporation	21 × 100 µs pulses, 4 kV, 10 Hz	22		2014	[284]

to understand and compare the performance of the different species studied for the production of biodiesel as the end product of the process. In Table V, it is presented some of the species reported recently and their productivity. It is still necessary the information with regard to demonstration plants at large scale showing the technical benefits of the process.

8. MICROALGAE AND THE BIO-REFINERY CONCEPT

The strategy by which microalgae will become a reality on a large scale is under the concept of bio-refinery. It means the integration of various conversion processes and equipment to produce energy, power, and high value-added

Table V. The FAME productivity of some algae strains.

Species	FAME productivity (mg L^{-1} day^{-1})	Year	Reference
Chlorella vulgaris	66.1	2016	[285]
C. vulgaris	22.42	2014	[286]
Hindakia PKU AC 169	26	2013	[287]
Scenedesmus obliquus NIES-2280	55.9	2015	[288]
Chlorella sorokiniana	1.32	2012	[289]
Chlorella minutissima UTEX2341	180.68	2011	[290]
Scenedesmus rubescens-like	107.8	2012	[291]
Nannochloropsis salina	44.12	2015	[292]
Chlorella zofingiensis	11.85–30.14	2013	[293]
C. vulgaris NIES-227	57–212	2015	[294]
S. obliquus	24.2	2014	[295]

chemicals from the microalgal biomass produced. The aim of the bio-refinery is to obtain a wide variety of products and at the same time generate little or 'zero' waste. These facilities contribute to the preservation of the environment and sustainable development because biofuels are renewable, carbon neutral and are also available in all regions [249].

Some studies have reported that microalgal biomass can produce various kinds of products such as bioethanol, bio-hydrogen, bio-oils, bio-methane, aviation fuel, pigments, bio-fertilizers, plastics, nutrients, animal food, antioxidants, cosmetics, pharmaceuticals, vaccines, EPA, and DHA, developing methodologies that lead to the implementation of bio-refineries in terms of their cost-effectiveness, size facilities, and environmental sustainability [14,15,250–258].

In Figure 5. it is presented a bio-refinery concept for microalgae. In a first stage, it is necessary to have in mind the supplies of input to the process, the energy, the materials, and the selection of the species. In the second stage, it is important the cultivation of microalgae and configuration of both open systems and photobioreactor systems. The third stage has to do with the selection of the harvest methods divided mainly between those who consume mostly energy (active) and those who dispense energy or not use it (passive). Later stage involves biomass harvesting and water reuse coming from the microalgae culture systems, either to external uses or the same process. The last stages of products and by-products mainly are aimed at bioenergy, food or food industry, and high value added products or fine chemicals, because of the last the profitability of a bio-refinery may be more promising in a near future.

However, there are still a variety of technological barriers, markets, and policies that obstruct economic viability and competitiveness of such fuels. Among some technical considerations suggested by researchers are (i) perform scaling from laboratory to pilot plant avoiding extrapolation of these results, (ii) conduct further research for the use of wastewater as a culture medium for microalgae, and the use of CO_2 from the air and/or industrial exhaust gases as a carbon source for microalgae culture, (iii) use of polluted water bodies such as lakes or rivers that serve as a source of nutrients while phytoremediation techniques are used, (iv) demonstrate the feasibility of harvesting technologies at large scale and propose development of new ones at industrial levels, (v) study the algal biology and the feasibility of genetic engineering for specific purposes like high value-added products, and (vi) renewable energies must be used as

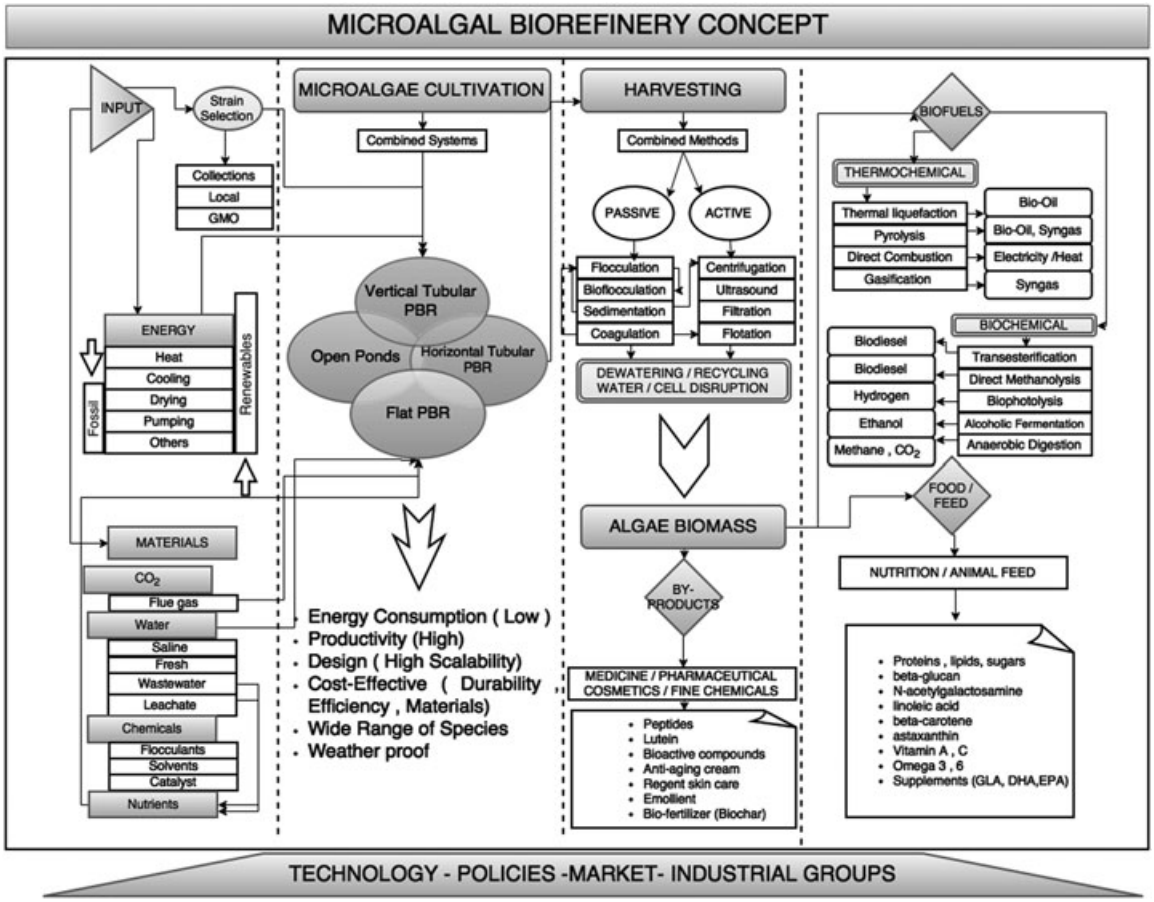

Figure 5. The important stages of the microalgal bio-refinery concept.

Table VI. A strengths, weaknesses, opportunities, and threats analysis for biodiesel from microalgae and the bio-refinery concept.

	Strengths	Weaknesses	Opportunities	Threats
Algae culture	Open ponds, mostly used for large-scale applications because of their low capital cost, low energy use, and ease operation. Photobioreactors are useful because of their high productivity, biomass quality for high added value products, CO_2–O_2 transfer, and low risk of contamination; in some selected cases, the high area to volume ratio, light distribution, and photosynthetic efficiency.	Open ponds, even they do not need suitable land for agriculture purposes, the required surface area for large applications is considerable. They have low productivities, high risk of contamination, and water losses as wells as poor gas mixing ratio. Photobioreactors (PBRs) are difficult to scale up; the operation, energy demand and maintenance costs are still high.	In the scale-up process, use of both systems could help to increase productivity and quality of the desired products. There are still design challenges about solar distribution efficiencies, dissolved oxygen control, heating and cooling control, 'multi-trophy' microalgae cultivation and best strain selection, and/or the idea of polyculture systems.	Seasonal aspects of each country are different. Therefore, algae must adapt to these conditions as well as the bioreactor design (either open or closed systems). More data from demonstrative-scale culture systems are needed. The keys of design culture systems parameters are neither defined nor standardized.
Harvesting	No energy requirements: flocculation, bio-flocculation, sedimentation, and coagulation. Use of these methods is usually low cost, simple, and low concentration needed; in some cases, culture media are minor to no econtamination, which allow culture recycling. Energy requirements: centrifugation, filtration, electrical-based processes, and flotation. These methods are suitable for a wide range of species; they are fast, with high biomass recovery and noninvasive.	Chemical flocculants, coagulants, bio-flocculants, and sedimentation: Risk of contamination, toxicity, and changes in cellular composition or deterioration, pH dependency, and long reaction time. High recovery cost and culture recycling limited. Centrifugation, filtration, electrical-based processes, and flotation: High energy requirements. Sonication needs more studies. Electrode replacement and membrane replacement (filtration) increase operation cost. Sometimes flotation needs chemical flocculants.	Developments in dissolved air flotation, electrophoresis, and auto-flocculation systems are required for large-scale process. Energy efficiency centrifuge design must be introduced. Electrical-based processes are promising, but they need more demonstrative assessments. Bio-flocculants and metabolic manipulation need intensive studies and cost-effective analysis in order to demonstrate its efficiency.	Flocculants and its environmental impact should be understood, especially water-recovery process, pollution, and toxicity. Biomass composition after using chemicals or metabolic manipulation must be analyzed in order to reach the desirable products like oils, proteins, sugars, or specific metabolites. Microalgae harvesting is one of the most energy-consuming step that needs attention in the whole process.
Lipid extraction	Solvent extraction, good for small-scale, solvent recovery, simple and low cost methods. Supercritical CO_2, solvent-free extraction, low toxicity, high efficiency, and reduced time. Mechanical methods, high efficiency except for the	High cost is the main barrier to all these methods for large scale production. Scale up technology requires high investment cost. Energy consumption is another factor; heating and cooling systems are required for	Chemical extraction depends on different factors that must be defined for industrial processes: strain selection, type of solvents, volume/biomass ratio, reaction time, and solvent recovery cycles as wells as biomass quality. Energy required for mechanical methods could be solved by the addition of	Use of chemical solvents need environmental and security regulations. Solvent extraction limits potential uses of the biomass recovery because of its contamination or toxicity. Mechanical methods can cause cell damage and poor quality by-products

(*Continues*)

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Table VI. (Continued)

	Strengths	Weaknesses	Opportunities	Threats
	expeller press, reduce solvent consumption. Electroporation requires less energy input and low solvent usage, hence, saves energy required for drying algae; lipid extraction requires only one step. Biological methods avoid solvents and possible biomass contamination or toxicity.	biomass and/or solvent recovery (chemical, microwave, and sonication). Supercritical CO_2 could be a promising process; unless, high pressure and control temperature reactors are needed; thus, it requires a large amount of energy. Enzymatic extraction is still in the developmental stage and needs more data to prove its efficiency. Costs for commercial enzymes are still not suitable for large scale.	renewable energies to the process followed by a detailed profitability analysis. Sonication, electroporation, and supercritical CO_2 have shown high efficiencies; however, more pilot-scale and demonstrative studies have to be carried out.	or co-products, affecting the cost-effective analysis of the desirable outputs.
Transesterification	One step transesterification may reduce the overall process cost and improve the high heating value of biodiesel. However, base catalyst is the most used process because of its fast reaction, reproducibility, and high efficiency. On the other hand, enzymatic catalyst has some advantages like less energy usage and easier biodiesel separation and purification compared with base catalyst.	One step transesterification efficiency and recovery is highly water dependent. Esters are difficult to remove from water after the process. Processes like biomass drying—oil extraction—transesterification could be more expensive at large scale. Enzymatic catalyst is still not feasible at large scale because of diverse factors such as enzyme production cost, reaction time (slower than base catalyst), and enzyme recovery. Activation and inactivation conditions are not defined yet. Base catalyst is limited by free fatty acids and is suitable if Acid Value (AV) is lower than 2%. Biodiesel and glycerol purification requires process energy.	Glycerol is a good by-product but needs an extra process that increases the capital cost. Techno-economical analysis must be carried out. Reuse of methanol reduces overall process cost. Use of lipases could be competitive in the near future if the cost is more accessible.	Washing biodiesel can cause water contamination and the use of large amounts of this resource. Use of chemical solvents needs environmental and security regulations. Biodiesel could not to be as 'green' as it seems because of the use of solvents and catalyst from fossil industry. This dependency must be studied.

(*Continues*)

Table VI. (Continued)

	Strengths	Weaknesses	Opportunities	Threats
		Acid catalyst needs more attention at large scale because of their corrosive nature as well as the environmental impact in their recovery processes.		
Biofuels from microalgae	Algae facilities can use CO_2 from gas industry, producing oxygen instead. Water impact decreases because of algae, which can grow in different types of water bodies: fresh water, saline water, wastewater, and leachates, reducing the greenhouse gases (GHG) emissions and water treatment. Industrial fertilizer dependency could reduce or eliminate using wastewater as an algae nutrient source (mainly nitrogen and phosphorus). They can grow in areas unsuitable for agricultural purposes. Resultant biomass, depending on its composition and culture conditions, could be used as biodiesel, bioethanol, biogas, and biohydrogen source. Other compounds like sugars, lipids, proteins, polyunsaturated fatty acids, vitamins, bio-active compounds, pigments, fertilizers, and other high added value products result from microalgae industry. Algae-based biofuels could reduce oil dependency in the mid-term.	Strain selection is not easy; the goal is to find a strain or group of strains with high lipid content, best suited for different regions and weather conditions, with fast growth rate, high CO_2 absorption, tolerant, and easy to harvest and disrupt. Energy balance is still not defined; in some cases, it is negative because of the operation process, which includes culturing, harvesting, drying, and fractioning products and by-products. Fossil fuels production is still more feasible than extractions from algae biomass. Data from lab scale are not comparable with drawbacks presented in pilot plant or large scale.	Life cycle analysis data are needed in order to know the environmental sustainability and profitability of algae-based biofuels. Algae industry could replace fuels, food, feed, and other commercial applications in sectors like nutrition, cosmetics, health, and pharmaceutical. To turn into economically feasible industry, developments must be connected in four specific sectors: research and development, policies, market, and industry. CO_2 recovery from power plants or geothermal wells could be a source for algae growth.	Achieving a stable biomass productivity is the biggest problem because of algae growth limited by climate changes, solar irradiance, rains, temperature, and humidity. Water loss and strain contamination is another risk for open systems, thus, the productivity and biomass quality. Use of genetically modified organism (GMOs) needs measures of sterilization methods and their environmental impact.

complements, for mixing the culture, illumination, dewatering, processing, and drying.

9. STRENGTH, WEAKNESSES, OPPORTUNITIES, AND THREATS ANALYSIS

There are different routes for the implementation of a production plant for biodiesel and more for a bio-refinery. The methods and techniques to be followed at each stage of the process will be the challenge to overcome in the near future. It is for this reason that in Table VI it is presented a strength, weaknesses, opportunities, and threats analysis to condense and better understand the main stages in the process of production and extraction of lipids from algae as well as some key ideas for the development of a bio-refinery. The objective is to have a clearer idea about the planning and design that allow the development and deployment of this technology on a large scale.

10. CONCLUDING REMARKS

It is a reality that currently the use of microalgae as source not only of bioenergy but also of other metabolites of high value is developing in an intensive way in several countries of the world such as the USA, China, Germany, and Spain, only to mention a few. The energy transition can be a mechanism that also impulses the development of algae-based third-generation biofuels and bio-refineries. Among the points that require further development to make this happen are (i) the selection of species is recommended to be obtained locally because geographical conditions may favor their handling and production. The genetical modification still has problems to be resolved on productivity and environmental safety because of their proliferation; (ii) the use of combined systems (open system and photobioreactors) is a measure that improves both the quality and the productivity of the species; the geographical location and their environmental conditions are determinants for efficient production; (iii) the non-invasive harvest methods (supercritical CO_2, sonication, and filtration) have the advantage of acquiring the best quality biomass, however face large-scale technological barriers as well as high energy consumption; (iv) making the method of extraction and conversion to biodiesel actually renewable and therefore environmentally friendly, they have to look for catalysts as well as solvents that also come from sustainable and renewable systems, for example, bio-ethanol or bio-methanol for the transeterfication process. In the future, these solvents also may come from microalgae; (v) to ensure the implementation of bio-refineries favorable, it requires an in-depth life cycle analysis, evaluating mainly the inputs, processes, and products as well as the possibility to recirculate or reuse wastes like biomass, heat, gas, and water. It is important to achieve the synergy among concepts of zero waste, industrial symbiosis, and the combination of renewable energies. It is fundamental to achieve a new sustainable industrial concept that is responsible with the environment and the society.

REFERENCES

1. International Energy Agency. 2014 Key World Energy STATISTICS, 2014.
2. Secretariat of Energy (Mexico). Energy information system, 2015. [Online]. Available: http://sie.energia.gob.mx/.
3. Song C, Liu Q, Ji N, Deng S, Zhao J, Li S, Kitamura Y. Evaluation of hydrolysis–esterification biodiesel production from wet microalgae. *Bioresource Technology* 2016; **214**:747–754.
4. Sen Gupta S, Shastri Y, Bhartiya S. Model-based optimisation of biodiesel production from microalgae. *Computers and Chemical Engineering* 2016; **89**:222–249.
5. He Q, Yang H, Hu C. Culture modes and financial evaluation of two oleaginous microalgae for biodiesel production in desert area with open raceway pond. *Bioresource Technology* 2016; **218**:571–579.
6. Mohseni S, Pishvaee MS, Sahebi H. Robust design and planning of microalgae biomass-to-biodiesel supply chain: a case study in Iran. *Energy* 2016; **111**:736–755.
7. Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, Smith AG. Biodiesel from algae: challenges and prospects. *Current Opinion in Biotechnology* 2010; **21**(3):277–286.
8. Metting FB. Biodiversity and application of microalgae. *Journal of Industrial Microbiology and Biotechnology* 1996; **17**(5–6):477–489.
9. Minowa T, Yokoyama S, Kishimoto M, Okakura T. Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction. *Fuel* 1995; **74**(12):1735–1738.
10. Vidyashankar S, VenuGopal KS, Swarnalatha GV, Kavitha MD, Chauhan VS, Ravi R, Bansal AK, Singh R, Pande A, Ravishankar GA, Sarada R. Characterization of fatty acids and hydrocarbons of chlorophycean microalgae towards their use as biofuel source. *Biomass and Bioenergy* 2015; **77**:75–91.
11. Du Z, Hu B, Ma X, Cheng Y, Liu Y, Lin X, Wan Y, Lei H, Chen P, Ruan R. Catalytic pyrolysis of microalgae and their three major components: carbohydrates, proteins, and lipids. *Bioresource Technology* 2013; **130**:777–782.
12. Santillan-Jimenez E, Pace R, Marques S, Morgan T, McKelphin C, Mobley J, Crocker M. Extraction,

characterization, purification and catalytic upgrading of algae lipids to fuel-like hydrocarbons. *Fuel* 2016; **180**:668–678.
13. Juantorena AU, Lastres O, Hernández G, Bustos A, Sebastian PJ, Eapen D. Hydrogen production by microorganisms and its application in a PEMFC. *International Journal of Energy Research* 2012; **36**(8):902–910.
14. de Farias Silva CE, Bertucco A. Bioethanol from microalgae and cyanobacteria: a review and technological outlook. *Process Biochemistry* 2016.
15. Zhen G, Lu X, Kobayashi T, Kumar G, Xu K. Anaerobic co-digestion on improving methane production from mixed microalgae (*Scenedesmus* sp., *Chlorella* sp.) and food waste: kinetic modeling and synergistic impact evaluation. *Chemical Engineering Journal* 2016; **299**:332–341.
16. Ahmad AL, Yasin NHM, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: a review. *Renewable and Sustainable Energy Reviews* 2011; **15**(1):584–593.
17. Lei X, Chen Y, Shao Z, Chen Z, Li Y, Zhu H, Zhang J, Zheng W, Zheng T. Effective harvesting of the microalgae *Chlorella vulgaris* via flocculation–flotation with bioflocculant, 2015.
18. Milano J, Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V. Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews* 2016; **58**:180–197.
19. Dassey AJ, Theegala CS. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. *Bioresource Technology* 2013; **128**:241–245.
20. Hansel PA, Guy Riefler R, Stuart BJ. Efficient flocculation of microalgae for biomass production using cationic starch. *Algal Research* 2014; **5**:133–139.
21. Nurra C, Clavero E, Salvadó J, Torras C. Vibrating membrane filtration as improved technology for microalgae dewatering. *Bioresource Technology* 2014; **157**:247–253.
22. Kurniawati HA, Ismadji S, Liu JC. Microalgaeharvesting by flotation using natural saponin and chitosan. *Bioresource Technology* 2014; **166**:429–434.
23. Chatsungnoen T, Chisti Y. Harvesting microalgae by flocculation–sedimentation. *Algal Research* 2016; **13**:271–283.
24. Chisti Y. Biodiesel from microalgae. *Biotechnology Advances* 2007; **25**(3):294–306.
25. Ratha SK, Prasanna R. Bioprospecting microalgae as potential sources of 'Green Energy'—challenges and perspectives (review). *Applied Biochemistry and Microbiology* 2012; **48**(2):109–125.
26. Cardozo KHM, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E. Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology Toxicology & Pharmacology CBP* 2007; **146**(1–2):60–78.
27. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* 2006; **101**(2):87–96.
28. Frac M, Jezierska-tys S, Tys J. Microalgae for biofuels production and environmental applications : a review. *Journal of Biotechnology* 2010; **9**(54):9227–9236.
29. Richmond A, Hu Q. *Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition* (2nd). Wiley Blackwell: Oxford, UK, 2013.
30. Graham LE, Wilcox LW. *Algae*. Prentice-Hall: Upper Saddle River NJ, 2000.
31. Sheehan J. A look back at the U.S. Department of Energy's Aquatic Species Program: biodiesel from algae. *Renewable Energy* 1998; **328** July:1–328.
32. Chini Zittelli G, Rodolfi L, Biondi N, Tredici MR. Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. *Aquaculture* 2006; **261**(3):932–943.
33. Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering* 2009; **102**(1):100–112.
34. Khan SA, Hussain MZ, Prasad S, Banerjee UC. Prospects of biodiesel production from microalgae in India. *Renewable and Sustainable Energy Reviews* 2009; **13**(9):2361–2372.
35. Pulz O, Gross W. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology* 2004; **65**(6):635–648.
36. 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.
37. Lee RE. *Phycology*. Cambridge University Press: New York, 1980.
38. Gao K. Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. *Journal of Applied Phycology* 1998; **10**(1):37–49.
39. Richmond A. *Spirulina*. In Micro-algal Biotechnology, Borowitzka MA, Borowitzka LJ (eds.), Cambridge U.P.: Cambridge, 1988; 85–121.

40. Burlew J. *Algal Culture from Laboratory to Pilot Plant*. Carnegie Institution of Washington: Washington DC, 1953.
41. Hu Q, Kurano N, Kawachi M, Iwasaki I, Miyachi S. Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor. *Applied Microbiology and Biotechnology* 1998; **49**(6):655–662.
42. Soong P. *Production and development of Chlorella and Spirulina in Taiwan*. In Algae biomass, Shelef G, Soeder CJ (eds.). Elsevier/North Holland Biomedical Press: Amsterdam, 1980; 97–113.
43. Goldman JC. Outdoor algal mass cultures—I. Applications. *Water Research* 1979; **13**(1):1–19.
44. Soeder CJ. Massive cultivation of microalgae: results and prospects. *Hydrobiologia* 1980; **72**(1):197–209.
45. Gavrilescu M, Chisti Y. Biotechnology—a sustainable alternative for chemical industry. *Biotechnology Advances* 2005; **23**(7–8):471–499.
46. Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM. Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences* 2012; **19**(3):257–275.
47. González LE, Cañizares RO, Baena S. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. *Bioresource Technology* 1997; **60**(3):259–262.
48. Sydney EB, Sturm W, de Carvalho JC, Thomaz-Soccol V, Larroche C, Pandey A, Soccol CR. Potential carbon dioxide fixation by industrially important microalgae. *Bioresource Technology* 2010; **101**(15):5892–5896.
49. Yu N, Dieu LTJ, Harvey S, Lee D-Y. Optimization of process configuration and strain selection for microalgae-based biodiesel production. *Bioresource Technology* 2015; **193**:25–34.
50. Ahn Y-C, Lee I-B, Lee K-H, Han J-H. Strategic planning design of microalgae biomass-to-biodiesel supply chain network: multi-period deterministic model. *Applied Energy* 2015; **154**:528–542.
51. Taleb A, Pruvost J, Legrand J, Marec H, Le-Gouic B, Mirabella B, Legeret B, Bouvet S, Peltier G, Li-Beisson Y, Taha S, Takache H. Development and validation of a screening procedure of microalgae for biodiesel production: application to the genus of marine microalgae *Nannochloropsis*. *Bioresource Technology* 2015; **177**:224–232.
52. Santander C, Robles PA, Cisternas LA, Rivas M. Technical–economic feasibility study of the installation of biodiesel from microalgae crops in the Atacama Desert of Chile. *Fuel Processing Technology* 2014; **125**:267–276.
53. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews* 2010; **14**(1):217–232.
54. Gouveia L, Graça S, Sousa C, Ambrosano L, Ribeiro B, Botrel EP, Neto PC, Ferreira AF, Silva CM. Microalgae biomass production using wastewater: treatment and costs: scale-up considerations. *Algal Research* 2016; **16**:167–176.
55. Komolafe O, Velasquez Orta SB, Monje-Ramirez I, Noguez IY, Harvey AP, Orta Ledesma MT. Biodiesel production from indigenous microalgae grown in wastewater. *Bioresource Technology* 2014; **154**:297–304.
56. Guo Z, Liu Y, Guo H, Yan S, Mu J. Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. *Journal of Environmental Sciences* 2013; **25**: S85–S88.
57. Cabanelas ITD, Arbib Z, Chinalia FA, Souza CO, Perales JA, Almeida PF, Druzian JI, Nascimento IA. From waste to energy: microalgae production in wastewater and glycerol. *Applied Energy* 2013; **109**:283–290.
58. Patil V, Tran K-Q, Giselrød HR. Towards sustainable production of biofuels from microalgae. *International Journal of Molecular Sciences* 2008; **9**(7):1188–95.
59. Tsukahara K, Sawayama S. Liquid fuel production using microalgae. *Journal of the Japan Petroleum Institute* 2005; **48**(5):251–259.
60. Kee Lam M, Lee KT. Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win–win strategies toward better environmental protection. *Biotechnology Advances* 2011; **29**(1):124–141.
61. Johnson MB, Wen Z. Development of an attached microalgal growth system for biofuel production. *Applied Microbiology and Biotechnology* 2010; **85**(3):525–534.
62. Tredici MR. Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. *Biofuels* 2010; **1**(1):143–162.
63. Packer M. Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. *Energy Policy* 2009; **37**(9):3428–3437.
64. Mulbry W, Kondrad S, Buyer J. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. *Journal of Applied Phycology* 2008; **20**(6):1079–1085.
65. De Godos I, Blanco S, García-Encina PA, Becares E, Muñoz R. Long-term operation of high rate algal

ponds for the bioremediation of piggery wastewaters at high loading rates. *Bioresource Technology* 2009; **100**(19):4332–9.
66. Wu X, Ruan R, Du Z, Liu Y. Current status and prospects of biodiesel production from microalgae. *Energies* 2012; **5**(8):2667–2682.
67. Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Current Opinion in Biotechnology* 2008; **19**(5):430–6.
68. Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B. Second generation biofuels: high-efficiency microalgae for biodiesel production. *BioEnergy Research Journal* 2008; **1**(1):20–43.
69. Chen C-Y, Yeh K-L, Aisyah R, Lee D-J, Chang J-S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource Technology* 2011; **102**(1):71–81.
70. Halim R, Gladman B, Danquah MK, Webley PA. Oil extraction from microalgae for biodiesel production. *Bioresource Technology* 2011; **102**(1):178–85.
71. Juantorena Ugás A, Alfaro Vives OG, de Armas Perez Borroto E. Escalado del cultivo de la microalga Chlorella vulgaris en cultivadores de película descendente. Aplicación de la teoría de los modelos para el escalado. *Tecnología Química* 2008; **28**(2):11–19.
72. Lam MK, Lee KT. Microalgae biofuels: a critical review of issues, problems and the way forward. *Biotechnology Advances* 2012; **30**(3):673–690.
73. Novoveská L, Zapata AKM, Zabolotney JB, Atwood MC, Sundstrom ER. Optimizing microalgae cultivation and wastewater treatment in large-scale offshore photobioreactors. *Algal Research* 2016; **18**:86–94.
74. Wang J, Rosov T, Wensel P, McGowen J, Curtis WR. A preliminary implementation of metabolic-based pH control to reduce CO2 usage in outdoor flat-panel photobioreactor cultivation of *Nannochloropsis oceanica* microalgae. *Algal Research* 2016; **18**:288–295.
75. Wang L, Liu J, Zhao Q, Wei W, Sun Y. Comparative study of wastewater treatment and nutrient recycle via activated sludge, microalgae and combination systems. *Bioresource Technology* 2016; **211**:1–5.
76. Schoepp NG, Stewart RL, Sun V, Quigley AJ, Mendola D, Mayfield SP, Burkart MD. System and method for research-scale outdoor production of microalgae and cyanobacteria. *Bioresource Technology* 2014; **166**:273–281.
77. Khoo CG, Lam MK, Lee KT. Pilot-scale semi-continuous cultivation of microalgae *Chlorella vulgaris* in bubble column photobioreactor (BC-PBR): hydrodynamics and gas–liquid mass transfer study. *Algal Research* 2016; **15**:65–76.
78. Wolf J, Stephens E, Steinbusch S, Yarnold J, Ross IL, Steinweg C, Doebbe A, Krolovitsch C, Müller S, Jakob G, Kruse O, Posten C, Hankamer B. Multi-factorial comparison of photobioreactor geometries in parallel microalgae cultivations. *Algal Research* 2016; **15**:187–201.
79. Fernández I, Acién FG, Guzmán JL, Berenguel M, Mendoza JL. Dynamic model of an industrial raceway reactor for microalgae production. *Algal Research* 2016; **17**:67–78.
80. de Godos I, Mendoza JL, Acién FG, Molina E, Banks CJ, Heaven S, Rogalla F. Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. *Bioresource Technology* 2014; **153**:307–314.
81. Prussi M, Casini D, Tredici MR, Rodolfi L, Bassi N, Zittelli GC, Bondioli P. Review of energy balance in raceway ponds for microalgae cultivation: re-thinking a traditional system is possible. *Applied Energy* 2013; **102**:101–111.
82. Singh RN, Sharma S. Development of suitable photobioreactor for algae production—a review. *Renewable and Sustainable Energy Reviews* 2012; **16**(4):2347–2353.
83. Chisti Y. Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology* 2008; **26**(3):126–131.
84. Stephenson AL, Kazamia E, Dennis JS, Howe CJ, Scott SA, Smith AG. Life-cycle assessment of potential algal biodiesel production in the united kingdom: a comparison of raceways and air-lift tubular bioreactors. *Energy and Fuels* 2010; **24**(7):4062–4077.
85. Terry KL, Raymond LP. System design for the autotrophic production of microalgae. *Enzyme and Microbial Technology* 1985; **7**(10):474–487.
86. Putt R, Singh M, Chinnasamy S, Das KC. An efficient system for carbonation of high-rate algae pond water to enhance CO_2 mass transfer. *Bioresource Technology* 2011; **102**(3):3240–3245.
87. Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering* 2009; **102**(1):100–112.
88. Ugwu CU, Aoyagi H, Uchiyama H. Photobioreactors for mass cultivation of algae. *Bioresource Technology* 2008; **99**(10):4021–4028.
89. Harun R, Singh M, Forde GM, Danquah MK. Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews* 2010; **14**(3):1037–1047.

90. Lee Y. Microalgal mass culture systems and methods: their limitation and potential. *Most* 2001; **13**(4):307–315.
91. Prasanna R, Sood A, Jaiswal P, Nayak S, Gupta V, Chaudhary V, Joshi M, Natarajan C. Rediscovering cyanobacteria as valuable sources of bioactive compounds (review). *Applied Biochemistry and Microbiology* 2010; **46**(2):119–134.
92. Carvalho AP, Meireles LA, Malcata FX. Microalgal reactors: a review of enclosed system designs and performances. *Biotechnology Progress* 2006; **22**(6):1490–1506.
93. Borowitzka MA. Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology* 1999; **70**(1–3):313–321.
94. Zittelli GC, Lavista F, Bastianini A, Rodolfi L, Vincenzini M, Tredici MR. Production of eicosapentaenoic acid by *Nannochloropsis* sp. cultures in outdoor tubular photobioreactors. *Journal of Biotechnology* 1999; **70**(1–3):299–312.
95. Richmond A. Maintenance of mass culture. In *Handbook of Microalgal Culture Biotechnology and Applied Phycology*, Richmond A (ed.). Blackwell Publishing Ltd: Oxford, UK, 2004; xviii; 566.
96. Tsoglin LN, Gabel BV, Fal'kovich TN, Semenenko VE. Closed photobioreactors for microalgal cultivation. *The Russian Journal of Plant Physiology Transl Fiziol Rast Moscow* 1996; **43**(1):131–136.
97. Pulz O. Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology* 2001; **57**(3):287–293.
98. Grobbelaar JU. Factors governing algal growth in photobioreactors: the 'open' versus 'closed' debate. *Journal of Applied Phycology* 2008; **21**(5):489–492.
99. Merchuk JC, Gluz M, Mukmenev I. Comparison of photobioreactors for cultivation of the red microalga *Porphyridium* sp. *Journal of Chemistry* 2000; **1126**:1119–1126.
100. Grima EM, Acie FG, Chisti Y. Photobioreactors: light regime, mass transfer, and scaleup. *Journal of Biotechnology* 1999; **70**(1–3):231–247.
101. Sierra E, Acién FG, Fernández JM, García JL, González C, Molina E. Characterization of a flat plate photobioreactor for the production of microalgae. *Chemical Engineering Journal* 2008; **138**(1–3):136–147.
102. Davis R, Aden A, Pienkos PT. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy* 2011; **88**(10):3524–3531.
103. Amaro HM, Guedes AC, Malcata FX. Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy* 2011; **88**(10):3402–3410.
104. Amin S. Review on biofuel oil and gas production processes from microalgae. *Energy Conversion and Management* 2009; **50**(7):1834–1840.
105. Pulz O, Scheibenbogen K, Institut IGV, Scheunert-allee A, Rehbriicke B. Photobioreactors: design and performance with respect to light energy input. *Managing* 1998; **59**:123–152.
106. Bitog JP, Lee IB, Lee CG, Kim KS, Hwang HS, Hong SW, Seo IH, Kwon KS, Mostafa E. Application of computational fluid dynamics for modeling and designing photobioreactors for microalgae production: a review. *Computers and Electronics in Agriculture* 2011; **76**(2):131–147.
107. Eriksen NT. The technology of microalgal culturing. *Biotechnology Letters* 2008; **30**(9):1525–1536.
108. Molina E, Fernández J, Acién FG, Chisti Y. Tubular photobioreactor design for algal cultures. *Journal of Biotechnology* 2001; **92**(2):113–131.
109. Gong Y, Jiang M. Biodiesel production with microalgae as feedstock: from strains to biodiesel. *Biotechnology Letters* 2011; **33**(7):1269–1284.
110. Pires JCM, Alvim-Ferraz MCM, Martins FG, Simões M. Carbon dioxide capture from flue gases using microalgae: engineering aspects and biorefinery concept. *Renewable and Sustainable Energy Reviews* 2012; **16**(5):3043–3053.
111. Dogaris I, Welch M, Meiser A, Walmsley L, Philippidis G. A novel horizontal photobioreactor for high-density cultivation of microalgae. *Bioresource Technology* 2015; **198**:316–324.
112. Chen C-Y, Chang Y-H, Chang H-Y. Outdoor cultivation of *Chlorella vulgaris* FSP-E in vertical tubular-type photobioreactors for microalgal protein production. *Algal Research* 2016; **13**:264–270.
113. Nwoba EG, Ayre JM, Moheimani NR, Ubi BE, Ogbonna JC. Growth comparison of microalgae in tubular photobioreactor and open pond for treating anaerobic digestion piggery effluent. *Algal Research* 2016; **17**:268–276.
114. Ifrim GA, Titica M, Barbu M, Boillereaux L, Cogne G, Caraman S, Legrand J. Multivariable feedback linearizing control of *Chlamydomonas reinhardtii* photoautotrophic growth process in a torus photobioreactor. *Chemical Engineering Journal* 2013; **218**:191–203.
115. Vonshak G, Torzillo A. Environmental stress physiology. In *Microalgal Culture: Biotechnology and Applied Phycology*, Richmond A (ed.). Blackwell Publishing Ltd: Oxford, UK, 2004.
116. Cuaresma M, Janssen M, Vílchez C, Wijffels RH. Productivity of *Chlorella sorokiniana* in a short light-path (SLP) panel photobioreactor under high irradiance. *Biotechnology and Bioengineering* 2009; **104**(2):352–359.

117. Richmond A, Boussiba S, Vonshak A, Kopel R. A new tubular reactor for mass production of microalgae outdoors. *Journal of Applied Phycology* 1993; **5**(3):327–332.
118. San Pedro A, González-López CV, Acién FG, Molina-Grima E. Outdoor pilot production of *Nannochloropsis gaditana*: influence of culture parameters and lipid production rates in flat-panel photobioreactors. *Algal Research* 2016; **18**:156–165.
119. Huang J, Feng F, Wan M, Ying J, Li Y, Qu X, Pan R, Shen G, Li W. Improving performance of flat-plate photobioreactors by installation of novel internal mixers optimized with computational fluid dynamics. *Bioresource Technology* 2015; **182**:151–159.
120. Ruiz J, Álvarez-Díaz PD, Arbib Z, Garrido-Pérez C, Barragán J, Perales JA. Performance of a flat panel reactor in the continuous culture of microalgae in urban wastewater: prediction from a batch experiment. *Bioresource Technology* 2013; **127**:456–463.
121. Wang L, Tao Y, Mao X. A novel flat plate algal bioreactor with horizontal baffles: structural optimization and cultivation performance. *Bioresource Technology* 2014; **164**:20–27.
122. Kandilian R, Tsao T-C, Pilon L. Control of incident irradiance on a batch operated flat-plate photobioreactor. *Chemical Engineering Science* 2014; **119**:99–108.
123. Kumar K, Dasgupta CN, Nayak B, Lindblad P, Das D. Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. *Bioresource Technology* 2011; **102**(8):4945–4953.
124. Richmond A. Microalgal biotechnology at the turn of the millennium : A personal view, *Journal of Applied Phycology* 2000; **12**(3–5): 441–451.
125. Doucha J, Straka F, Lívanský K. Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. *Journal of Applied Phycology* 2005; **17**(5):403–412.
126. Chiu S-Y, Kao C-Y, Chen C-H, Kuan T-C, Ong S-C, Lin C-S. Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. *Bioresource Technology* 2008; **99**(9):3389–96.
127. Stewart C. A study of methods of carbon dioxide capture and sequestration—the sustainability of a photosynthetic bioreactor approach. *Energy Conversion and Management* 2005; **46**(3):403–420.
128. Norsker N-H, Barbosa MJ, Vermuë MH, Wijffels RH. Microalgal production—a close look at the economics. *Biotechnology Advances* 2011; **29**(1):24–7.
129. Ding Y-D, Zhao S, Liao Q, Chen R, Huang Y, Zhu X. Effect of CO_2 bubbles behaviors on microalgal cells distribution and growth in bubble column photobioreactor. *International Journal of Hydrogen Energy* 2016; **41**(8):4879–4887.
130. Peng L, Zhang Z, Cheng P, Wang Z, Lan CQ. Cultivation of Neochloris oleoabundans in bubble column photobioreactor with or without localized deoxygenation. *Bioresource Technology* 2016; **206**:255–263.
131. López-Rosales L, García-Camacho F, Sánchez-Mirón A, Martín Beato E, Chisti Y, Molina Grima E. Pilot-scale bubble column photobioreactor culture of a marine dinoflagellate microalga illuminated with light emission diodes. *Bioresource Technology* 2016; **216**:845–855.
132. Wang S-K, Hu Y-R, Wang F, Stiles AR, Liu C-Z. Scale-up cultivation of *Chlorella ellipsoidea* from indoor to outdoor in bubble column bioreactors. *Bioresource Technology* 2014; **156**:117–122.
133. Nauha EK, Alopaeus V. Modeling method for combining fluid dynamics and algal growth in a bubble column photobioreactor. *Chemical Engineering Journal* 2013; **229**:559–568.
134. Guo X, Yao L, Huang Q. Aeration and mass transfer optimization in a rectangular airlift loop photobioreactor for the production of microalgae. *Bioresource Technology* 2015; **190**:189–195.
135. Fernandes BD, Mota A, Ferreira A, Dragone G, Teixeira JA, Vicente AA. Characterization of split cylinder airlift photobioreactors for efficient microalgae cultivation. *Chemical Engineering Science* 2014; **117**:445–454.
136. Pirouzi A, Nosrati M, Shojaosadati SA, Shakhesi S. Improvement of mixing time, mass transfer, and power consumption in an external loop airlift photobioreactor for microalgae cultures. *Biochemical Engineering Journal* 2014; **87**:25–32.
137. Jones SMJ, Harrison STL. Aeration energy requirements for lipid production by *Scenedesmus* sp. in airlift bioreactors. *Algal Research* 2014; **5**:249–257.
138. Yen H-W, Liao Y-T, Liu YX. Cultivation of oleaginous *Rhodotorula mucilaginosa* in airlift bioreactor by using seawater. *Journal of Bioscience and Bioengineering* 2016; **121**(2):209–212.
139. Suh IS, Lee C-G. Photobioreactor engineering: design and performance. *Biotechnology and Bioprocess Engineering* 2003; **8**:313–321.
140. Vunjak-Novakovic G, Kim Y, Wu X, Berzin I, Merchuk JC. Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies. *Industrial and Engineering Chemistry Research* 2005; **44**(16):6154–6163.
141. Kaewpintong K, Shotipruk A, Powtongsook S, Pavasant P. Photoautotrophic high-density cultivation of vegetative cells of *Haematococcus pluvialis*

in airlift bioreactor. *Bioresource Technology* 2007; **98** (2):288–295.
142. Suali E, Sarbatly R. Conversion of microalgae to biofuel. *Renewable and Sustainable Energy Reviews* 2012; **16**(6):4316–4342.
143. Ogbonna JC, Soejima T, Tanaka H. An integrated solar and artificial light system for internal illumination of photobioreactors. *Journal of Biotechnology* 1999; **70**(1–3):289–297.
144. Park Y-T, Lee H, Yun H-S, Song K-G, Yeom S-H, Choi J. Removal of metal from acid mine drainage using a hybrid system including a pipes inserted microalgae reactor. *Bioresource Technology* 2013; **150**:242–248.
145. Adesanya VO, Cadena E, Scott SA, Smith AG. Life cycle assessment on microalgal biodiesel production using a hybrid cultivation system. *Bioresource Technology* 2014; **163**:343–355.
146. Hall DO, Fernández FGA, Guerrero EC, Rao KK, Grima EM. Outdoor helical tubular photobioreactors for microalgal production: modeling of fluid-dynamics and mass transfer and assessment of biomass productivity. *Biotechnology and Bioengineering* 2003; **82**(1):62–73.
147. Gudin C, Chaumont D. Cell fragility—the key problem of microalgae mass production in closed photobioreactors. *Bioresource Technology* 1991; **38**(2–3):145–151.
148. Chorus I, Bartram J. Toxic cyanobacteria in water : a guide to their public health consequences, monitoring and management, 18. E & FN Spon, 1999.
149. Rawat I, Ranjith Kumar R, Mutanda T, Bux F. Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. *Applied Energy* 2013; **103**:444–467.
150. Pieterse AJH, Cloot A. Algal cells and coagulation, flocculation and sedimentation processes. *Water Science and Technology* 1997; **36**(4):111–118.
151. Li Q, Du W, Liu D. Perspectives of microbial oils for biodiesel production. *Applied Microbiology and Biotechnology* 2008; **80**(5):749–756.
152. Gudin C, Thepenier C. Bioconversion of solar energy into organic chemicals by microalgae. *Advances in Biotechnological Processes* 1986; **6**:73–110.
153. Demirbas A. Use of algae as biofuel sources. *Energy Conversion and Management* 2010; **51**(12):2738–2749.
154. Lee Y-C, Kim B, Farooq W, Chung J, Han J-I, Shin H-J, Jeong SH, Park J-Y, Lee J-S, Oh Y-K. Harvesting of oleaginous *Chlorella* sp. by organoclays. *Bioresource Technology* 2013; **132**:440–5.
155. Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of Renewable and Sustainable Energy* 2010; **2**:1.
156. Garcia-Mier L, Jimenez-Garcia SN, Chapa-Oliver AM, Mejia-Teniente L, Ocampo-Velazquez RV, Rico-García E, Feregrino-Pérez AA, Guevara-Gonzalez R. *Biosystems Engineering: Biofactories for Food Production in the Century XXI*. 2014.
157. Shelef G, Sukenik A. Microalgae harvesting and processing: a literature review, 0, p. 65, 1984.
158. Molina Grima E, Belarbi E-H, Acién Fernández FG, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances* 2003; **20**(7–8):491–515.
159. Heasman M, Diemar J, O'Connor W, Sushames T, Foulkes L. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary. *Aquaculture Research* 2000; **31**(8–9):637–659.
160. Greenwell HC, Laurens LML, Shields RJ, Lovitt RW, Flynn KJ. Placing microalgae on the biofuels priority list: a review of the technological challenges. *Journal of the Royal Society Interface* 2010; **7**(46):703–726.
161. Christenson L, Sims R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances* 2011; **29**(6):686–702.
162. Coward T, Lee JGM, Caldwell GS. Development of a foam flotation system for harvesting microalgae biomass. *Algal Research* 2013; **2**(2):135–144.
163. Ghernaout D, Ghernaout B. On the concept of the future drinking water treatment plant: algae harvesting from the algal biomass for biodiesel production—a review. *Desalination and Water Treatment* 2012; **49**(1–3):1–18.
164. Kawaroe M, Prartono T, Sunuddin A, Saputra D. Marine microalgae *Tetraselmis suecica* as flocculant agent of bio-flocculation method. *HAYATI Journal of Biosciences* 2016.
165. Liu J, Tao Y, Wu J, Zhu Y, Gao B, Tang Y, Li A, Zhang C, Zhang Y. Effective flocculation of target microalgae with self-flocculating microalgae induced by pH decrease. *Bioresource Technology* 2014; **167**:367–375.
166. 't Lam GP, Vermuë MH, Olivieri G, van den Broek LAM, Barbosa MJ, Eppink MHM, Wijffels RH, Kleinegris DMM. Cationic polymers for successful flocculation of marine microalgae, 2014.
167. Gerde JA, Yao L, Lio J, Wen Z, Wang T. Microalgae flocculation: impact of flocculant type, algae species and cell concentration. *Algal Research* 2014; **3**:30–35.

168. Kim J, Yoo G, Lee H, Lim J, Kim K, Kim CW, Park MS, Yang JW. Methods of downstream processing for the production of biodiesel from microalgae. *Biotechnology Advances* 2013; **31**(6):862–876.
169. Rossignol N, Vandanjon L, Jaouen P, Quéméneur F. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. *Aquacultural Engineering* 1999; **20**(3):191–208.
170. Hwang K-J, Lin S-J. Filtration flux–shear stress–cake mass relationships in microalgae rotating-disk dynamic microfiltration. *Chemical Engineering Journal* 2014; **244**:429–437.
171. Pavez J, Cabrera F, Azócar L, Torres A, Jeison D. Ultrafiltration of non-axenic microalgae cultures: energetic requirements and filtration performance. *Algal Research* 2015; **10**:121–127.
172. Hwang T, Kotte MR, Han J-I, Oh Y-K, Diallo MS. Microalgae recovery by ultrafiltration using novel fouling-resistant PVDF membranes with *in situ* PEGylated polyethyleneimine particles. *Water Research* 2015; **73**:181–192.
173. Koren JPF, Syversen U. State-of-the-art electroflocculation. *Filtration & Separation* 1995; **32**(2):153–146.
174. Levin GV, Clendenning JR, Gibor A, Bogar FD. Harvesting of algae by froth flotation. *Applied Microbiology* 1962; **10**(2):169–175.
175. Edzwald J. Principles and applications of dissolved air flotation. *Water Science and Technology* 1995; **31**(3–4):1–23.
176. Rubio J, Souza ML, Smith RW. Overview of flotation as a wastewater treatment technique. *Minerals Engineering* 2002; **15**(3):139–155.
177. Csordas A, Wang J-K. An integrated photobioreactor and foam fractionation unit for the growth and harvest of *Chaetoceros* spp. in open systems. *Aquacultural Engineering* 2004; **30**(1–2):15–30.
178. Garg S, Wang L, Schenk PM. *Effective harvesting of low surface-hydrophobicity microalgae by froth flotation*. 2014.
179. Coward T, Lee JGM, Caldwell GS. Harvesting microalgae by CTAB-aided foam flotation increases lipid recovery and improves fatty acid methyl ester characteristics. *Biomass and Bioenergy* 2014; **67**:354–362.
180. Coward T, Lee JGM, Caldwell GS. Development of a foam flotation system for harvesting microalgae biomass. *Algal Research* 2013; **2**(2):135–144.
181. Zhou W, Gao L, Cheng W, Chen L, Wang J, Wang H, Zhang W, Liu T. Electro-flotation of *Chlorella* sp. assisted with flocculation by chitosan. *Algal Research* 2016; **18**:7–14.
182. Zhang X, Wang L, Sommerfeld M, Hu Q. Harvesting microalgal biomass using magnesium coagulation-dissolved air flotation. *Biomass and Bioenergy* 2016; **93**:43–49.
183. Henderson R, Parsons SA, Jefferson B. The impact of algal properties and pre-oxidation on solid–liquid separation of algae. *Water Research* 2008; **42**(8–9):1827–45.
184. Koopman B, Lincoln E. Autoflotation harvesting of algae from high-rate pond effluents. *Agriculture Wastes* 1983; **5**(4):231–246.
185. Kumar HD, Yadava PK, Gaur JP. Electrical flocculation of the unicellular green alga *Chlorella vulgaris* Beijerinck. *Aquatic Botany* 1981; **11** null:187–195.
186. Mollah MYA, Morkovsky P, Gomes JAG, Kesmez M, Parga J, Cocke DL. Fundamentals, present and future perspectives of electrocoagulation. *Journal of Hazardous Materials* 2004; **114**(1–3):199–210.
187. Luísa G. Microalgae as a feedstock for biofuels, 2011.
188. Bosma R, Van Spronsen WA, Tramper J, Wijffels RH, Ultrasound, a new separation technique to harvest microalgae Nomenclature, *Journal of Applied Phycology* 2003; **15**, m: 143–153.
189. Trujillo FJ, Juliano P, Barbosa-Cánovas G, Knoerzer K. Separation of suspensions and emulsions via ultrasonic standing waves—a review. *Ultrasonics Sonochemistry* 2014; **21**(6):2151–2164.
190. Lee Y-C, Huh YS, Farooq W, Chung J, Han J-I, Shin H-J, Jeong SH, Lee J-S, Oh Y-K, Park J-Y. Lipid extractions from docosahexaenoic acid (DHA)-rich and oleaginous *Chlorella* sp. biomasses by organic-nanoclays. *Bioresource Technology* 2013; **137**:74–81.
191. Talukder MMR, Das P, Wu JC. Immobilization of microalgae on exogenous fungal mycelium: a promising separation method to harvest both marine and freshwater microalgae. *Biochemical Engineering Journal* 2014; **91**:53–57.
192. Shi J, Podola B, Melkonian M. Application of a prototype-scale twin-layer photobioreactor for effective N and P removal from different process stages of municipal wastewater by immobilized microalgae. *Bioresource Technology* 2014; **154**:260–266.
193. Singh P, Guldhe A, Kumari S, Rawat I, Bux F. Combined metals and EDTA control: an integrated and scalable lipid enhancement strategy to alleviate biomass constraints in microalgae under nitrogen limited conditions. *Energy Conversion and Management* 2016; **114**:100–109.
194. Mendes RL, Fernandes HL, Coelho J, Reis EC, Cabral JMS, Novais JM, Palavra AF. Supercritical CO_2 extraction of carotenoids and other lipids from

Chlorella vulgaris. *Food Chemistry* 1995; **53**(1):99–103.
195. Medina AR, Giménez AG, Camacho FG, Pérez JAS, Grima EM, Gómez AC. Concentration and purification of stearidonic, eicosapentaenoic, and docosahexaenoic acids from cod liver oil and the marine microalga *Isochrysis galbana*. *Journal of the American Oil Chemists Society* 1995; **72**(5):575–583.
196. Belarbi E, Molina E, Chisti Y. A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. *Enzyme and Microbial Technology* 2000; **26**(7):516–529.
197. Rodolfi L, Zittelli GC, Barsanti L, Rosati G, Tredici MR. Growth medium recycling in *Nannochloropsis* sp. mass cultivation. *Biomolecular Engineering* 2003; **20**(4–6):243–248.
198. Sazdanoff N. Modeling and simulation of the algae to biodiesel fuel cycle, 2006.
199. Demirbas A. Competitive liquid biofuels from biomass, **88**, 1, 2011, 17–28.
200. Abbassi A, Ali M, Watson IA. Temperature dependency of cell wall destruction of microalgae with liquid nitrogen pretreatment and hydraulic pressing. *Algal Research* 2014; **5**:190–194.
201. Yu X, Gouyo T, Grimi N, Bals O, Vorobiev E. Pulsed electric field pretreatment of rapeseed green biomass (stems) to enhance pressing and extractives recovery. *Bioresource Technology* 2016; **199**:194–201.
202. Cravotto G, Boffa L, Mantegna S, Perego P, Avogadro M, Cintas P. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. *Ultrasonics Sonochemistry* 2008; **15**(5):898–902.
203. Sharma AK, Sahoo PK, Singhal S, Joshi G. Exploration of upstream and downstream process for microwave assisted sustainable biodiesel production from microalgae *Chlorella vulgaris*. *Bioresource Technology* 2016; **216**:793–800.
204. Teo CL, Idris A. Evaluation of direct transesterification of microalgae using microwave irradiation. *Bioresource Technology* 2014; **174**:281–286.
205. Teo CL, Idris A. *Enhancing the Various Solvent Extraction Method via Microwave Irradiation for Extraction of Lipids from Marine Microalgae in Biodiesel Production*. 2014.
206. Wahidin S, Idris A, Shaleh SRM. Rapid biodiesel production using wet microalgae via microwave irradiation. *Energy Conversion and Management* 2014; **84**:227–233.
207. McMillan JR, Watson IA, Ali M, Jaafar W. Evaluation and comparison of algal cell disruption methods: microwave, waterbath, blender, ultrasonic and laser treatment. *Applied Energy* 2013; **103**:128–134.
208. Wang M, Yuan W. Microalgal cell disruption in a high-power ultrasonic flow system. *Bioresource Technology* 2015; **193**:171–177.
209. Ma G, Hu W, Pei H, Jiang L, Song M, Mu R. *In situ* heterogeneous transesterification of microalgae using combined ultrasound and microwave irradiation. *Energy Conversion and Management* 2015; **90**:41–46.
210. Mercer P, Armenta RE. Developments in oil extraction from microalgae. *European Journal of Lipid Science and Technology* 2011; **113**(5):539–547.
211. Doucha J, Lívanský K. Influence of processing parameters on disintegration of *Chlorella* cells in various types of homogenizers. *Applied Microbiology and Biotechnology* 2008; **81**(3):431–440.
212. Postma PR, Miron TL, Olivieri G, Barbosa MJ, Wijffels RH, Eppink MHM. Mild disintegration of the green microalgae *Chlorella vulgaris* using bead milling. *Bioresource Technology* 2015; **184**:297–304.
213. Günerken E, D'Hondt E, Eppink M, Elst K, Wijffels R. Influence of nitrogen depletion in the growth of *N. oleoabundans* on the release of cellular components after beadmilling. *Bioresource Technology* 2016; **214**:89–95.
214. Günerken E, Gonzalez LG, Elst K, Eppink M, Wijffels R. Disruption of *Neochloris oleoabundans* by bead milling as the first step in the recovery of intracellular metabolites. *Current Opinion in Biotechnology* 2013; **24**:S43.
215. Singh R, Kumar A. Pulsed electric fields, processing and application in food industry. *European Journal of Food Research & Review* 2011; **1**(2):71–93.
216. Garoma T, Janda D. Investigation of the effects of microalgal cell concentration and electroporation, microwave and ultrasonication on lipid extraction efficiency. *Renewable Energy* 2016; **86**:117–123.
217. Chisti Y, Moo-Young M. Disruption of microbial cells for intracellular products. *Enzyme and Microbial Technology* 1986; **8**(4):194–204.
218. Kleinig AR, Middelberg APJ. On the mechanism of microbial cell disruption in high-pressure homogenisation. *Chemical Engineering Science* 1998; **53**(5):891–898.
219. Lee J-Y, Yoo C, Jun S-Y, Ahn C-Y, Oh H-M. Comparison of several methods for effective lipid extraction from microalgae. *Bioresource Technology* 2010; **101**(1):S75–S77.
220. Cooney M, Young G, Nagle N. Extraction of bio-oils from microalgae. *Separation & Purification Reviews* 2013; **38**:291–325 2009.
221. Cheng J, Huang R, Li T, Zhou J, Cen K. Biodiesel from wet microalgae: extraction with hexane after

the microwave-assisted transesterification of lipids. *Bioresource Technology* 2014; **170**:69–75.
222. Ashokkumar V, Agila E, Sivakumar P, Salam Z, Rengasamy R, Ani FN. Optimization and characterization of biodiesel production from microalgae *Botryococcus* grown at semi-continuous system. *Energy Conversion and Management* 2014; **88**:936–946.
223. Malekzadeh M, Abedini Najafabadi H, Hakim M, Feilizadeh M, Vossoughi M, Rashtchian D. Experimental study and thermodynamic modeling for determining the effect of non-polar solvent (hexane)/polar solvent (methanol) ratio and moisture content on the lipid extraction efficiency from *Chlorella vulgaris*. *Bioresource Technology* 2016; **201**:304–311.
224. Nagle N, Lemke P. Production of methyl ester fuel from microalgae. *Applied Biochemistry and Biotechnology* 1990; **24–25**(1):355–361.
225. Schäfer K. Accelerated solvent extraction of lipids for determining the fatty acid composition of biological material. *Analytica Chimica Acta* 1998; **358**(1):69–77.
226. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 1959; **37**(8):911–917.
227. Soares RR, Simonetti DA, Dumesic JA. Glycerol as a source for fuels and chemicals by low-temperature catalytic processing. *Angewandte Chemie International Edition in English* 2006; **45**(24):3982–5.
228. Iverson SJ, Lang SL, Cooper MH. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* 2001; **36**(11):1283–1287.
229. de Boer K, Moheimani NR, Borowitzka MA, Bahri PA. Extraction and conversion pathways for microalgae to biodiesel: a review focused on energy consumption. *Journal of Applied Phycology* 2012; **24**(6):1681–1698.
230. Ranjan A, Patil C, Moholkar VS. Mechanistic assessment of microalgal lipid extraction. *Industrial and Engineering Chemistry Research* 2010; **49**(6):2979–2985.
231. Halim R, Danquah MK, Webley P a. Extraction of oil from microalgae for biodiesel production: a review. *Biotechnology Advances* 2012; **30**(3):709–732.
232. Ayala RS, De Castro MDL. Continuous subcritical water extraction as a useful tool for isolation of edible essential oils. *Food Chemistry* 2001; **75**(1):109–113.
233. Herrero M, Ibáñez E, Cifuentes A, Señoráns J. Pressurized liquid extracts from *Spirulina platensis* microalga. Determination of their antioxidant activity and preliminary analysis by micellar electrokinetic chromatography. *Journal of Chromatography A* 2004; **1047**(2):195–203.
234. Crampon C, Mouahid A, Toudji S-AA, Lépine O, Badens E. Influence of pretreatment on supercritical CO_2 extraction from *Nannochloropsis oculata*. *Journal of Supercritical Fluids* 2013; **79**:337–344.
235. Herrero M, Cifuentes A, Ibanez E. Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review. *Food Chemistry* 2006; **98**(1):136–148.
236. Hejazi MA, Wijffels RH. Milking of microalgae. *Trends in Biotechnology* 2004; **22**(4):189–194.
237. Kleinegris DMM, Janssen M, Brandenburg WA, Wijffels RH. The selectivity of milking of *Dunaliella salina*. *Marine Biotechnology New York NY* 2010; **12**(1):14–23.
238. Zuorro A, Miglietta S, Familiari G, Lavecchia R. Enhanced lipid recovery from *Nannochloropsis* microalgae by treatment with optimized cell wall degrading enzyme mixtures. *Bioresource Technology* 2016; **212**:35–41.
239. Steen EJ, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, Del Cardayre SB, Keasling JD. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. *Nature* 2010; **463**(7280):559–562.
240. Robles-Medina A, González-Moreno PA, Esteban-Cerdán L, Molina-Grima E. Biocatalysis: towards ever greener biodiesel production. *Biotechnology Advances* 2009; **27**(4):398–408.
241. Tian X, Dai L, Liu M, Liu D, Du W, Wu H. Lipase-catalyzed methanolysis of microalgae oil for biodiesel production and PUFAs concentration, 2016.
242. Veillette M, Giroir-Fendler A, Faucheux N, Heitz M. Esterification of free fatty acids with methanol to biodiesel using heterogeneous catalysts: from model acid oil to microalgae lipids. *Chemical Engineering Journal* 2016; **308**:101–109.
243. Huang J, Xia J, Jiang W, Li Y, Li J. Biodiesel production from microalgae oil catalyzed by a recombinant lipase. *Bioresource Technology* 2015; **180**:47–53.
244. Fu X, Li D, Chen J, Zhang Y, Huang W, Zhu Y, Yang J, Zhang C. *A Microalgae Residue Based Carbon Solid Acid Catalyst for Biodiesel Production*. 2013.
245. Teo SH, Islam A, Yusaf T, Taufiq-Yap YH. Transesterification of *Nannochloropsis oculata* microalga's oil to biodiesel using calcium methoxide catalyst. *Energy* 2014; **78**:63–71.
246. Mathimani T, Uma L, Prabaharan D. Homogeneous acid catalysed transesterification of marine microalga *Chlorella* sp. BDUG 91771 lipid—an efficient biodiesel yield and its characterization. *Renewable Energy* 2015; **81**:523–533.

247. Wahlen BD, Willis RM, Seefeldt LC. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresource Technology* 2011; **102**(3):2724–30.
248. D'Oca MGM, Viêgas CV, Lemões JS, Miyasaki EK, Morón-Villarreyes JA, Primel EG, Abreu PC. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. *Biomass and Bioenergy* 2011; **35**(4):1533–1538.
249. Demirbas A. Biorefineries: for biomass upgrading facilities, 26. 2010.
250. Batista AP, Ambrosano L, Graça S, Sousa C, Marques PASS, Ribeiro B, Botrel EP, Castro Neto P, Gouveia L. Combining urban wastewater treatment with biohydrogen production—an integrated microalgae-based approach. *Bioresource Technology* 2015; **184**:230–235.
251. Goiris K, Van Colen W, Wilches I, León-Tamariz F, De Cooman L, Muylaert K. Impact of nutrient stress on antioxidant production in three species of microalgae. *Algal Research* 2015; **7**:51–57.
252. Wang H-MD, Chen C-C, Huynh P. Exploring the potential of using algae in cosmetics. *Bioresource Technology* 2015; **184**:355–362.
253. Manirafasha E, Ndikubwimana T, Zeng X, Lu Y, Jing K. Phycobiliprotein: potential microalgae derived pharmaceutical and biological reagent. *Biochemical Engineering Journal* 2016; **109**:282–296.
254. Chauton MS, Reitan KI, Norsker NH, Tveterås R, Kleivdal HT. A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: research challenges and possibilities. *Aquaculture* 2015; **436**:95–103.
255. Huang Y, Chen Y, Xie J, Liu H, Yin X, Wu C. Bio-oil production from hydrothermal liquefaction of high-protein high-ash microalgae including wild *Cyanobacteria* sp. and cultivated *Bacillariophyta* sp. *Fuel* 2016; **183**:9–19.
256. Yang C, Nie R, Fu J, Hou Z, Lu X. Production of aviation fuel via catalytic hydrothermal decarboxylation of fatty acids in microalgae oil. *Bioresource Technology* 2013; **146**:569–573.
257. Rodrigues DB, Menezes CR, Mercadante AZ, Jacob-Lopes E, Zepka LQ. Bioactive pigments from microalgae *Phormidium autumnale*. *Food Research International* 2015; **77**:273–279.
258. Wuang SC, Khin MC, Chua PQD, Luo YD. Use of *Spirulina* biomass produced from treatment of aquaculture wastewater as agricultural fertilizers. *Algal Research* 2016; **15**:59–64.
259. Ashokkumar V, Salam Z, Tiwari ON, Chinnasamy S, Mohammed S, Ani FN. An integrated approach for biodiesel and bioethanol production from *Scenedesmus bijugatus* cultivated in a vertical tubular photobioreactor. *Energy Conversion and Management* 2015; **101**:778–786.
260. Michels MHA, Vaskoska M, Vermuë MH, Wijffels RH. Growth of *Tetraselmis suecica* in a tubular photobioreactor on wastewater from a fish farm. *Water Research* 2014; **65**:290–296.
261. Bergmann P, Trösch W. Repeated fed-batch cultivation of *Thermosynechococcus elongatus* BP-1 in flat-panel airlift photobioreactors with static mixers for improved light utilization: influence of nitrate, carbon supply and photobioreactor design. *Algal Research* 2016; **17**:79–86.
262. He Q, Yang H, Hu C. Culture modes and financial evaluation of two oleaginous microalgae for biodiesel production in desert area with open raceway pond. *Bioresource Technology* 2016; **218**:571–579.
263. Osundeko O, Pittman JK. Implications of sludge liquor addition for wastewater-based open pond cultivation of microalgae for biofuel generation and pollutant remediation. *Bioresource Technology* 2014; **152**:355–363.
264. Zhang C-D, Li W, Shi Y-H, Li Y-G, Huang J-K, Li H-X. A new technology of CO_2 supplementary for microalgae cultivation on large scale—a spraying absorption tower coupled with an outdoor open runway pond. *Bioresource Technology* 2016; **209**:351–359.
265. Reyes JF, Labra C. Biomass harvesting and concentration of microalgae *Scenedesmus* sp. cultivated in a pilot phobioreactor. *Biomass and Bioenergy* 2016; **87**:78–83.
266. Wang T, Yang W-L, Hong Y, Hou Y-L. Magnetic nanoparticles grafted with amino-riched dendrimer as magnetic flocculant for efficient harvesting of oleaginous microalgae. *Chemical Engineering Journal* 2016; **297**:304–314.
267. Lananan F, Mohd Yunos FH, Mohdm Nasir N, Abu Bakar NS, Lam SS, Jusoh A. Optimization of biomass harvesting of microalgae, *Chlorella* sp. utilizing auto-flocculating microalgae, *Ankistrodesmus* sp. as bio-flocculant. *International Biodeterioration and Biodegradation* 2016; **113**:391–396.
268. Kim K, Shin H, Moon M, Ryu B-G, Han J-I, Yang J-W, Chang YK. Evaluation of various harvesting methods for high-density microalgae, *Aurantiochytrium* sp. KRS101. *Bioresource Technology* 2015; **198**:828–835.
269. Wang S, Chen J, Li Z, Wang Y, Fu B, Han X, Zheng L. Cultivation of the benthic microalga *Prorocentrum*

lima for the production of diarrhetic shellfish poisoning toxins in a vertical flat photobioreactor. *Bioresource Technology* 2015; **179**:243–248.
270. Gutiérrez R, Ferrer I, Uggetti E, Arnabat C, Salvadó H, García J. Settling velocity distribution of microalgal biomass from urban wastewater treatment high rate algal ponds. *Algal Research* 2016; **16**:409–417.
271. Chatsungnoen T, Chisti Y. Continuous flocculation–sedimentation for harvesting *Nannochloropsis salina* biomass. *Journal of Biotechnology* 2016; **222**:94–103.
272. Che R, Huang L, Yu X. Enhanced biomass production, lipid yield and sedimentation efficiency by iron ion, 2015.
273. Bilad MR, Discart V, Vandamme D, Foubert I, Muylaert K, Vankelecom IFJ. Harvesting microalgal biomass using a magnetically induced membrane vibration (MMV) system: filtration performance and energy consumption. *Bioresource Technology* 2013; **138**:329–338.
274. Barrut B, Blancheton J-P, Muller-Feuga A, René F, Narváez C, Champagne J-Y, Grasmick A. Separation efficiency of a vacuum gas lift for microalgae harvesting. *Bioresource Technology* 2013; **128**:235–240.
275. Bui TT, Han M. Removal of *Phormidium* sp. by positively charged bubble flotation. *Minerals Engineering* 2015; **72**:108–114.
276. Shuman TR, Mason G, Marsolek MD, Lin Y, Reeve D, Schacht A. An ultra-low energy method for rapidly pre-concentrating microalgae. *Bioresource Technology* 2014; **158**:217–224.
277. Hidalgo P, Ciudad G, Navia R. Evaluation of different solvent mixtures in esterifiable lipids extraction from microalgae *Botryococcus braunii* for biodiesel production, 2016.
278. Seo YH, Sung M, Oh Y-K, Han J-I. Lipid extraction from microalgae cell using persulfate-based oxidation, 2016.
279. Batista G, Surek GAS, Benincá C, Corazza ML, Zanoelo EF. Cyclic pressurization assisted extraction of lipids from microalgae for biodiesel production: non-equilibrium and equilibrium data. *Fuel* 2016; **163**:133–138.
280. Seo YH, Sung M, Oh Y-K, Han J-I. Lipid extraction from microalgae cell using UV–Fenton-like reaction, 2015.
281. Cho H-S, Oh Y-K, Park S-C, Lee J-W, Park J-Y. Effects of enzymatic hydrolysis on lipid extraction from *Chlorella vulgaris*. *Renewable Energy* 2013; **54**:156–160.
282. Taher H, Al-Zuhair S, Al-Marzouqi AH, Haik Y, Farid M, Tariq S. Supercritical carbon dioxide extraction of microalgae lipid: process optimization and laboratory scale-up. *Journal of Supercritical Fluids* 2014; **86**:57–66.
283. Araujo GS, Matos LJBL, Fernandes JO, Cartaxo SJM, Gonçalves LRB, Fernandes FAN, Farias WRL. Extraction of lipids from microalgae by ultrasound application: prospection of the optimal extraction method. *Ultrasonics Sonochemistry* 2013; **20**(1):95–98.
284. Flisar K, Meglic SH, Morelj J, Golob J, Miklavcic D. Testing a prototype pulse generator for a continuous flow system and its use for *E. coli* inactivation and microalgae lipid extraction. *Bioelectrochemistry* 2014; **100**:44–51.
285. Shen X-F, Liu J-J, Chauhan AS, Hu H, Ma L-L, Lam PKS, Zeng RJ. Combining nitrogen starvation with sufficient phosphorus supply for enhanced biodiesel productivity of *Chlorella vulgaris* fed on acetate. *Algal Research* 2016; **17**:261–267.
286. Park W-K, Moon M, Kwak M-S, Jeon S, Choi G-G, Yang J-W, Lee B. Use of orange peel extract for mixotrophic cultivation of *Chlorella vulgaris*: increased production of biomass and FAMEs. *Bioresource Technology* 2014; **171**:343–349.
287. Daroch M, Shao C, Liu Y, Geng S, Cheng JJ. Induction of lipids and resultant FAME profiles of microalgae from coastal waters of Pearl River Delta. *Bioresource Technology* 2013; **146**:192–199.
288. Shen X-F, Liu J-J, Chu F-F, Lam PKS, Zeng RJ. Enhancement of FAME productivity of *Scenedesmus obliquus* by combining nitrogen deficiency with sufficient phosphorus supply in heterotrophic cultivation. *Applied Energy* 2015; **158**:348–354.
289. Lu S, Wang J, Niu Y, Yang J, Zhou J, Yuan Y. Metabolic profiling reveals growth related FAME productivity and quality of *Chlorella sorokiniana* with different inoculum sizes. *Biotechnology and Bioengineering* 2012; **109**(7):1651–1662.
290. Li Z, Yuan H, Yang J, Li B. Optimization of the biomass production of oil algae *Chlorella minutissima* UTEX2341. *Bioresource Technology* 2011; **102**(19):9128–9134.
291. Lin Q, Gu N, Lin J. Effect of ferric ion on nitrogen consumption, biomass and oil accumulation of a *Scenedesmus rubescens*-like microalga. *Bioresource Technology* 2012; **112**:242–247.
292. Kang NK, Jeon S, Kwon S, Koh HG, Shin S-E, Lee B, Choi G-G, Yang J-W, Jeong B, Chang YK. Effects of overexpression of a bHLH transcription factor on biomass and lipid production in *Nannochloropsis salina*. *Biotechnology for Biofuels* 2015; **8**(1):200.
293. Zhu L, Wang Z, Shu Q, Takala J, Hiltunen E, Feng P, Yuan Z. Nutrient removal and biodiesel production

by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research* 2013; **47**(13):4294–4302.

294. Shen X-F, Chu F-F, Lam PKS, Zeng RJ. Biosynthesis of high yield fatty acids from *Chlorella vulgaris* NIES-227 under nitrogen starvation stress during heterotrophic cultivation. *Water Research* 2015; **81**:294–300.
295. Chu F-F, Chu P-N, Shen X-F, Lam PKS, Zeng RJ. Effect of phosphorus on biodiesel production from *Scenedesmus obliquus* under nitrogen-deficiency stress. *Bioresource Technology* 2014; **152**:241–246.

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