Current Approaches in Producing Oil and Biodiesel from Microalgal Biomass

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Abstract Alternative biofuel sources, such as biodiesel, produced from nontoxic, biodegradable, and renewable materials are currently attracting great interest given the growing global energy demand. Microalgae are an exceptional biofuel source due to their potential for generating significant quantities of biomass and oil and for combining their production with environmental technologies such as wastewater treatment. Technologies for producing biodiesel from microalgae have been widely studied, especially increasing the lipid content in the cell, strain selection, lipid extraction, and transesterification methods and innovations that use wet biomass for extraction, which reduces the environmental impact and production cost. These aspects are explored in this chapter to identify the different production methods and technologies under development for expanding biodiesel production from microalgae.

Keywords Microalgae • Oil • Biodiesel • Production technologies

1 Introduction

Fossil fuels currently meet 27 % of the world energy demand. However, the scarce reserves, high prices, and environmental problems associated with using these fuels have driven the development of alternative energy sources to ensure economic and environmental sustainability of the energy supply (Stephens et al. 2010; Lam and Lee 2012; Hong et al. 2014).

Biodiesel is a fuel composed of alkyl esters obtained from long-chain fatty acid transformation. It can be produced by animal or plant triglyceride transesterification with short-chain alcohols, such as methanol and ethanol (Elshahed 2010; Velasquez-Orta et al. 2012; Likozar and Levec 2014).

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Biodiesel is an exceptional renewable energy source because it releases less carbon dioxide, sulfur (SO_x), and carbon monoxide (CO) into the atmosphere. Moreover, it does not contain aromatic compounds and other chemical substances that are harmful to the environment and human health; it is also more biodegradable than diesel (Kirrolia et al. 2013). The important properties of biodiesel are that it is a clean energy source with adequate viscosity and its inherent lubricity, high flash point, and elevated cetane number for use as a fuel (Knothe and Steidley 2011; Hong et al. 2014; Huang and Su 2014).

Multiple raw material sources are used for biodiesel production, including microalgae. These microorganisms contain lipids and fatty acids as cell membrane components, reserve substances, metabolites, and energy sources. Certain species are excessively rich in oils and may have an oil content greater than 80 %, which is efficiently converted into methyl esters (biodiesel) (Demirbas 2010).

Lipids from microalgae are mostly neutral lipids and typically include a high level of saturation, which renders the microalgae even more attractive for biofuel research (Rawat et al. 2013). In this context, using mixed cultures is also important because it may be advantageous to use biomass generated from wastewater treatment lagoons in biodiesel production (Wahlen et al. 2011).

In addition to using microalgae with a high oil content and improving the culture conditions to maximize biomass and oil production, the limiting step in producing biodiesel from microalgae is extracting the lipid content from the cells. This step can be performed separately or concurrently with transesterification (in situ) in the wet or dry biomass.

The aspects that should be explored to facilitate biodiesel production from microalgae include culture conditions that yield greater lipid accumulation in cells, oil extraction techniques, combination methods to avoid certain production stages, and optimizing the transesterification process.

2 An Overview of Microalgae Production for Obtaining Oil

Microalgae are among the most promising sources of biofuels due to their ability to rapidly multiply, even in low-quality water; absorb large quantities of CO_2; and accumulate substantial quantities of reserve substances (Ashokkumar et al. 2014). Depending on the culture conditions and cultured species, large quantities of polysaccharides (sugars) and triglycerides (fats), which are raw materials for producing bioethanol and biodiesel, respectively, can be generated (Dębowski et al. 2013; Slade and Bauen 2013).

Maximizing lipid productivity is key for a successful biotechnological process using microalgae for biodiesel production, and one of the greatest challenges is scaling up from a laboratory to a commercial scale while maintaining high lipid productivity (Table 1) (Xu and Boeing 2014). Increased lipid extraction from microalgae depends on optimizing a series of nutritional conditions in the growth medium (Gupta et al. 2013; Huang and Su 2014).

Table 1 Lipid concentrations extracted from the biomass of certain microalgal species

Espécie	Teor de lipídios (%)	Referência
Haematococcus pluvialis	24.3	Wu et al. (2013)
Chlorella vulgaris	27.6	Wu et al. (2013)
Chlorella vulgaris	27.38	Heredia-Arroyo et al. (2011)
Chlorella pyrenoidosa	17.4	Wu et al. (2013)
Chlorella sorokiniana	32	Wu et al. (2013)
Chlorella sp.	26.43–27.96	Ahmad et al. (2014)
Scenedesmus obliquus	12–14	Dragone et al. (2010)
Scenedesmus dimorphus	16–40	Dragone et al. (2010)
Chlorella protothecoides	14.6–57.8	Maity et al. (2014)
Dunaliella tertiolecta	16.7–71	Maity et al. (2014)
Prymnesium parvum	22–38	Demirbas (2011)
Euglena gracilis	14–20	Demirbas (2011)
Chlamydomonas sp. TAI-2	18.4	Wu et al. (2012)
Chlorella emersonii	25–63	Mata et al. (2010)
Nannochloropsis sp.	12–53	Mata et al. (2010)
Neochloris oleoabundans	29–65	Mata et al. (2010)
Botryococcus braunii	25–75	Mata et al. (2010)
Synechocystis aquatilis	15.85	Kaiwan-Arporn et al. (2012)

Lipid accumulation in microalgae may result from a lack of nutrients, especially nitrogen, and excess organic carbon (Chandra et al. 2014). To abundantly produce valuable bioproducts from microalgae, particularly biofuels, it is important that the conditions offered to the microorganisms transition them toward a heterotrophic metabolism, where organic carbons, such as in sugars and organic acids, act as carbon and energy sources (Liang 2013).

Temperature, luminosity, pH, salinity, and salt minerals are other variables that, when controlled and applied as stress, can improve growth performance and energy reserve accumulation in microalgae (Venkata Mohan and Devi 2014).

Nitrogen, sulfur, and phosphorus are important components of algal cells and DNA and are essential for cell growth; these elements become limiting factors at low concentrations. Information on nitrogen deficiency in the algal metabolism is scarce, but certain reports indicate that a nitrogen deficit can lead to oxidative stress (Hockin et al. 2012). Zhang et al. (2013) observed substantial neutral lipid accumulation by *Chlorella sorokiniana C*3 in response to oxidative stress induced by a hydrogen peroxide treatment.

Nutrient limitation, particularly nitrogen, increases neutral lipid production and accumulation in microalgae. However, this limitation yields severe losses in biomass productivity. To reduce the negative effect of subjecting microalgae cultures to nutrient-limiting conditions, biomass production must first be maximized, and later, a neutral lipid accumulation-stimulating factor must be introduced (Bertozzini et al. 2014).

To maximize algal biomass production using *Chlorella vulgaris* and *Scenedesmus* sp., thereby increasing lipid accumulation, different concentrations of calcium, magnesium, and sodium chloride were examined. The results showed that a short-term reduction in the magnesium concentration induced lipid production (Chen et al. 2014).

Among the micronutrients, iron, potassium, and inorganic salts are the most important metabolic activators and are used in algal culture media formulations (Zeng et al. 2011; Cai et al. 2013).

Yang et al. (2014) applied a response surface method to define the optimal conditions for promoting greater lipid accumulation in *Scenedesmus* sp. The results showed that adding $NaHCO_3$, i.e., a source of inorganic carbon, as well as NaH_2PO_4, $2H_2O$ and $NaNO_3$ significantly enhanced lipid accumulation in microalgae without influencing the fatty acid composition of *Scenedesmus* sp.

To synthesize cyanocobalamin (vitamin B12), algae require cobalt (Co), which should be used at low concentrations. According to Li et al. (2007), such care is necessary due to both the potential for toxicity to the algae and the researcher in addition to introducing this toxic element into the food chain.

Another important factor that merits study is supplementing the culture medium with glycerol, which is a by-product of biodiesel production that increases lipid production in microalgae. In studies conducted by Cerón-Garcıa et al. (2005), glycerol, fructose, glucose, mannose, and lactose were used as carbon sources to stimulate growth and fatty acid accumulation in *Phaeodactylum tricornutum*, which enabled effective gains in biomass yield and intracellular oil accumulation.

High glycerol concentrations in microalgal cultures subjected to mixotrophic growth conditions tend to change the thylakoids in chloroplasts that favor glycerol assimilation, which can be considered an adaptive response to stress imposed by mixotrophic conditions (Cerón-García et al. 2013).

Sun et al. (2014) studied the effects of culture media with different glycerol concentrations (1, 5, 10, 15, and 30 g L^{-1}) and glucose (10 g L^{-1}) as supplementary carbon sources on growth and lipid accumulation in *Chlorella vulgaris*. The presence of glycerol in the culture medium enhanced lipid production and accumulation, whereas the presence of glucose favored cell growth. The positive effect of glycerol supplementation on lipid accumulation in microalgae is limited over a given concentration (10 g L^{-1}), after which inhibitory effects on biomass growth and lipid production were observed.

Biofuel production using current algal cultivation technology is not economical (Pittman et al. 2011). A more attractive option for reducing greenhouse gas emissions and the demand for freshwater resources and fertilizers is algal growth using wastewater. The high productivity of microalgal biomass grown in sewage and wastewater suggests that this culture method is a viable means for generating biofuels and will likely be one of the main technologies used for producing sustainable and renewable energy in the future.

Wastewaters contain organic carbon, nitrogen, phosphorus, and other compounds (Liang 2013; Sahu et al. 2013), which are used for microalgal growth and form a readily available substrate for biomass production that is easily assimilated

and is inexpensive (Rawat et al. 2011; Ummalyma and Sukumaran 2014; Zhu et al. 2014).

Culturing microalgal using wastewater is influenced by multiple factors, and growth efficiency depends on controlling the variables considered critical, such as pH, temperature, light availability, CO_2, O_2, and especially nutrient concentrations, which can impede algal biomass development. Municipal wastewater typically contains approximately 350 mg L^{-1} chemical oxygen demand (COD), 50 mg L^{-1} of N—$NH4^+$, and 10 mg L^{-1} of P—PO_4^{-3} as well as a considerable organic load and high nutrient concentrations (nitrogen and phosphorus) (Boelee et al. 2014).

Treated wastewater effluents also contain trace elements (K, Ca, Mg, Fe, Cu, and Mn) that are essential for microalgal metabolism and growth. Therefore, secondary and tertiary effluents may be broadly applied to microalgal culture media (Schneider et al. 2012; Aravantinou et al. 2013). However, the levels of cadmium, mercury, toxic organic compounds, and biotic contaminants, such as pathogenic bacteria and predators (zooplankton), in the culture medium should be considered because they can inhibit microalgal growth (Pittman et al. 2011; Van Den Hende et al. 2014).

Luminosity is a critical factor for microalgal growth when using urban wastewater as a culture medium due to the levels of suspended solids and turbidity in this type of effluent (Zhou et al. 2014). Microalgal cultures may overcome light limitations by consuming high levels of total organic carbon (TOC) as acetic acid, propionic acid, butyric acid, or ethanol from municipal wastewater for rapid growth under photoheterotrophic or mixotrophic conditions (Ji et al. 2014).

Likely one of the main requirements for large-scale biofuel production from microalgae is using microalgal strains adapted to local environmental conditions. Thus, there is a need for effective and rapid isolation of microalgal strains with a high potential for growth and biomass production as well as sufficiently high lipid and/or polysaccharide content for economic exploitation (Abdelaziz et al. 2014).

Microorganism communities overcome the limitations inherent to isolation through symbiosis. These interactions involve subtle signals and horizontal gene transfer, and they create competitive or cooperative scenarios where microorganisms can compete or provide resources. Cultures with multiple microbial species contain a broader range of genes and better metabolic capacity compared with monocultures (Hays et al. 2015).

The symbiotic relationship between microalgae and bacteria has been well characterized with regard to the oxygen supplied by microalgae for the aerobic bacteria to biodegrade organic pollutants and, in turn, consume the CO_2 released during bacterial respiration. However, the interactions between these two microbial groups are not limited to CO_2/O_2 exchange. When properly characterized, the microalgae-bacteria consortium benefits both species; however, this association should be carefully evaluated because it can also produce antagonistic relationships (Oswald 2003; Schumacher et al. 2003).

Microbial consortia generally perform more complex tasks and can perform functions that are difficult or even impossible for individual species or strains (Brenner et al. 2008). Bacteria can increase the microalgal cell growth capacity

by releasing certain growth factors, and microalgae assist bacteria by releasing biosurfactants and extracellular compounds in the medium, which improves bacteria-pollutant degradation activity (Tang et al. 2010).

Increased cell growth in the microalgae *Chlorella minutissima* was observed when it was cocultured with *Escherichia coli* under mixotrophic conditions, in which both light and organic compounds are used for energy and carbon production. This association demonstrates the potential for increasing microalgal biomass production with 1 % substrate consumption compared with isolated *Chlorella minutissima* cultures (Higgins et al. 2015).

Microalgae and bacteria co-selection in a consortium system is important when the objective is to increase biomass production, and the effects of these interactions may affect the system stability; thus, the system should be thoroughly investigated (Muñoz and Guieysse 2006).

Microalgal biomass can be converted into solid, liquid, or gaseous biofuels and may supply 30 % of the global fuel demand without affecting food production (López Barreiro et al. 2013) via various processes, including thermochemical (liquefaction, pyrolysis, and gasification) (Amin 2009) and biochemical processes (fermentation, transesterification, and anaerobic digestion) (Demirbas 2011).

When processed via chemical or biological reactions, in addition to biodiesel and bioethanol, microalgal biomass can provide different types of renewable biofuels, such as bio-hydrogen, bio-methane, and butanol, as well as light hydrocarbons, such as ethane (C_2H_6) and ethylene (C_2H_4), for direct use as energy sources or to generate electricity (Mussgnug et al. 2010; Efremenko et al. 2012; Nasir Uddin et al. 2013; Lakaniemi et al. 2013; Nayak et al. 2014). For microalgae-based fuels, the main focus is biodiesel production, whereas bioethanol and biomethane are considered a part of integrated processes (Oncel 2013) that generate residual biomass during oil extraction, which can be aerobically fermented into ethanol and anaerobically fermented into biomethane (Chinnasamy et al. 2010). The relevant factors for microalgal production and their potential applications in biofuel production are illustrated in Fig. 1.

3 Biodiesel Production

3.1 Oil Extraction

Several methods can be used to extract lipids from microalgae, including supercritical extraction, ultrasonic extraction, microwave extraction, high-pressure homogenizer extraction, hydrothermal liquefaction, and solvent extraction (Bligh and Dyer, 1959; Friedrich and Pryde 1984; Chao et al. 1993; Converti et al. 2009; Bucy et al. 2012; Iqbal and Theegala 2013; Toor et al. 2013; Reddy et al. 2014). However, high quantities of solvent are necessary, which may lead to

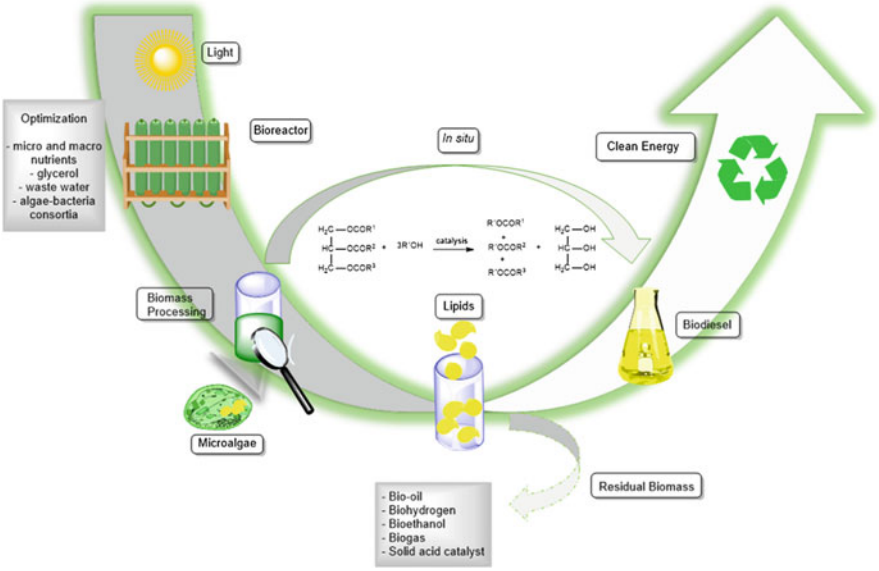

Fig. 1 Diagram illustrating the relevant factors for microalgal production and their potential applications in biofuel production

environmental pollution and increase costs as well as energy consumption during the process (Xu et al. 2006).

Since the introduction of the Folch method, researchers have investigated various techniques to effectively extract lipids using different ratios of chloroform and methanol. These techniques include the Bligh-Dyer method, which is considered a conventional lipid extraction method that successfully uses a mixture of chloroform and methanol and is currently adjusted to the conditions of several laboratories (Bligh and Dyer 1959).

Halim et al. (2012) compared lipid extraction in different solvents. Although chloroform can dissolve the lipid content of cells, including acilglicerois e ácidos graxos livres as well as polar lipids, fosfolipídios e glicolipídios, researchers are reluctant to use chloroform in lipid extraction due to the toxicity of chlorinated solvents. Hexane is often suggested as a hydrophobic alternative because it selectively extracts neutral lipids and is less toxic. Another option is mixing hexane/isopropanol (3:2 v/v), which features good lipid extraction yield.

Ethanol is an inexpensive solvent and has a strong affinity for the lipid complex; thus, lipids can be efficiently extracted. Moreover, ethanol is a safe and eco-friendly solvent, which suggests that the residual biomass can be used in the food industry (Reddy et al. 2014). Several authors (Ibáñez González et al. 1998; Fajardo et al. 2007) have efficiently used ethanol to extract lipids from microalgae, such as *Phaeodactylum tricornutum* and *P. tricornutum* UTEX 640. However, these studies use dehydration or high temperatures for extraction.

Dimethyl carbonate (DMC)/methanol can also be used as an extraction solvent and generates a 38.9 % yield. In fact, the DMC can be subsequently used for transesterification (Lee et al. 2013). According to Su et al. (2009), short-chain dialkyl carbonates can be acyl group acceptors with carbon dioxide formation and favor methyl ester formation.

Because the microalgal biomass generated features a high water content, high levels of energy are consumed before extraction for drying and efficient extraction of the lipid content. Jiménez Callejón et al. (2014) studied extraction with wet biomass, and the results indicated that 69.1 % of the unsaponifiable lipids were extracted. The authors used the high-pressure homogenization technique and added hexane when using wet biomass for lipid extraction.

Therefore, lipids are typically extracted with solvents and dry biomass; other processes for using wet biomass will be discussed in the section on in situ transesterification.

3.2 Transesterification

Several processes can be used for microalgal lipid content transesterification, which are combined through transesterification of extracted oil or biomass directly using in situ methods. During optimization, researchers consider the necessary physical treatments associated with whether or not to use catalysts or biocatalysts. Certain biodiesel production initiatives are presented in Table 2 and are the most investigated methods for assessing potential biodiesel production from microalgae.

Catalysts should also be considered an important aspect for converting microalgal lipids into biodiesel. Two catalyst groups are considered, chemical and biocatalysts (enzyme catalysts), with their specific modes of action.

3.3 Chemical Catalysts

Methods that involve chemical catalysts during transesterification are the most attractive and widely used due to high yield and increased reaction speed. Because this reaction is reversible, excess alcohol is typically used to shift the equilibrium toward product formation (Arias-Peñaranda et al. 2013; Kiran et al. 2014).

Homogeneous alkaline catalysis has been the most widely used pathway for biodiesel production because the reaction is catalyzed at a low temperature and atmospheric pressure, and it yields high levels of conversion in less time. The most frequently used alkaline catalysts are homogeneous catalysts, such as potassium hydroxide, sodium hydroxide, sodium methoxide, and potassium methoxide (Puna et al. 2010; Macario and Giordano 2013).

Often, an alkaline catalyst is not effective for converting microalgal lipids through transesterification due to the high free fatty acid content. Soaps may form

Table 2 Conditions for biodiesel production from microalgae using different methods and microalgal strains

Microalgae	Conditions	Yield (%)	References
Chlorella pyrenoidosa	120 °C/180 min/H_2SO_4/hexane/methanol Acid catalyst and cosolvent	> 90	Cao et al. (2013)
	60 °C/40 s/H_2SO_4/chloroform/methanol Microwave	10.5[a]	Cheng et al. (2013)
Chlamydomonas sp.	45 °C/15 min/ NaOH/hexane/methanol In situ and cosolvent	>97	Chen et al. (2015)
Chlorella vulgaris	40–45 °C/48 h/immobilized *Burkholderia* lipase/methanol/hexane/ in situ/from ultrasonic pretreated wet biomass	>90	Tran et al. (2013b)
	60 °C/75 min/NaOH/methanol/preheated in the oven at 100 °C for 1 h alkali (NaOH)	77.6	Velasquez-Orta et al. (2012)
	45 °C/48 h/enzyme/methanol/10 min sonication Enzymatic catalysis after sonication	> 95	Tran et al. (2013a)
Mixed cultures	60 °C/100 min/H_2SO_4/metanol/chloroform In situ microwave	74	Wahlen et al. (2011)
	65 °C/7 h/H_2SO_4/methanol Cosolvent chloroform and hexane	82.1 and 55.3	Soydemir et al. (2015)
Schizochytrium limacinum	211.6 °C/120 min/methanol Subcritical and supercritical methanol	37.5	Bi et al. (2015)
Nannochloropsis sp.	255 °C/25 min/methanol Supercritical methanol	>85	Patil et al. (2011)
Nannochloropsis salina	105 °C/30 min/H_2SO_4/methanol or ethanol In situ/wet biomass	>90	Kim et al. (2015a, b)
Nannochloropsis oceanica	95 °C/90 min/H_2SO_4/methanol/chloroform Acid catalyst in situ	> 90	Im et al. (2014)
Scenedesmus obliquus	35 °C/36 h/*Aspergillus niger* whole cell/methanol Enzymatic catalysis	53.7	Guldhe et al. (2016)

[a]Relative to dry biomass

under this condition, and they will consume the catalyst and impede separation of the esters and glycerol formed (Gog et al. 2012).

More reports describe biodiesel production from microalgae via acid catalysis than alkali metals because acid catalysts esterify free fatty acids and transesterify triglycerides (Suwannakarn et al. 2009; Leung et al. 2010; Nigam and Singh 2011; Lee et al. 2014). Acid catalysts are typically employed for lipids with free fatty acid content greater than 2 % (Velasquez-Orta et al. 2012).

The most commonly used acids for homogeneous catalysts in biodiesel production are H_2SO_4 and sulfonic acid; HCl, BF_3, and H_3PO_4 are also used (Bharathiraja et al. 2014). The use of this type of catalyst requires a greater response time and

higher temperatures than alkaline catalysts (Hidalgo et al. 2013; Vonortas and Papayannakos 2014).

To overcome the problems with using homogeneous catalysts, certain authors report using both in the same transesterification process, which consists of two reaction steps. An acid catalyst may initially be used to convert free fatty acids to methyl esters by esterification such that the free fatty acid content in the oils is reduced to less than 1 %; thereafter, an alkaline catalyst is used (Park et al. 2015).

Reactions using homogeneous catalysts can generate many environmental and corrosion problems. Therefore, transesterification reactions with heterogeneous catalysts have emerged as an alternative for biodiesel production using different raw materials. Thus, researchers expect that heterogeneous catalysts will be easier to recover and reuse. Umdu et al. (2009) reported transesterification of oils from *Nannochloropsis oculata* using CaO and MgO supported on alumina with a 97.5 % yield. Using metal oxides composed of ZrO, TiO, and Al_2O_3, lipids from the microalgae *Dunaliella tertiolecta* and *Nannochloropsis oculata* were converted (85 %) into biodiesel with free fatty acid esterification and triglyceride transesterification simultaneously under supercritical conditions (Krohn et al. 2011).

3.4 Biocatalysts

Lipases (EC 3.1.1.3) are tools that catalyze various synthetic reactions, such as hydrolysis, esterification, transesterification, and aminolysis. Lipases are used as biocatalysts for transforming oil/lipids into biodiesel due to low operating costs and, particularly, high product purity under mild conditions (20–50 °C) (Gog et al. 2012; Tran et al. 2012; Christopher et al. 2014; Huang et al. 2015).

Furthermore, comparing the enzyme and chemical catalyst technologies, researchers have observed that enzymes catalyze free fatty acid (FFA) and triglyceride (TG) esterification in a reaction step without the need for a wash step and prevent by-product formation, which ensures an easily recovered product (Kulkarni and Dalai 2006; Robles-Medina et al. 2009).

Certain lipases synthesized by *Mucor miehei*, *Rhizopus oryzae, Pseudomonas cepacia, Candida antarctica* (Taher et al. 2011), *Candida rugosa*, *Candida cylindracea*, *Pseudomonas fluorescens*, *Rhizomucor miehei* (Teo et al. 2014), and *Burkholderia* sp. (Tran et al. 2012) are used as enzyme biocatalysts for biofuel production.

The lipases may exhibit regiospecificity, specificity for fatty acids or the nature of the alcohol, and stereospecificity, which includes *sn*-1,3 specific (hydrolyzable ester linkage at position sR1 or R3 of the triglyceride), *sn*-2 specific (hydrolyzable ester linkage in the R2 position of the TAG), and nonspecific (no distinction between the ester positions) (Kapoor and Gupta 2012).

In general, enzymatic transesterification for oils features certain disadvantages, including the reaction time and the potential enzyme inactivation by methanol

(Persson et al. 2002; Adamczak and Bednarski 2004; Jung et al. 2006). These disadvantages limit the industrial applications of biodiesel production processes due to the high cost of enzymes associated with this process (Bajaj et al. 2010). Using the whole cell or immobilized enzyme yields higher conversion and less activity loss but allows for enzyme reuse (Teo et al. 2014; Huang et al. 2015).

The lipids are more viscous compared with the organic solvents, and dissolving lipids in this class of solvents may improve reactant diffusion in the liquid phase and hence transesterification performance (Lam and Lee 2013).

Studies on biocatalysis for transesterification are only performed in media with the alcohol used for the conversion or with cosolvents, especially hexane, which solubilizes oil and biodiesel well and is less aggressive toward enzymes (Tran et al. 2013a). Researchers have also examined using ionic liquids for biodiesel production using oil from three different microalgae (*Botryococcus braunii*, *Chlorella vulgaris*, and *Chlorella pyrenoidosa*). Researchers have observed high levels of methyl ester conversion using *Penicillium expansum* lipase (PEL) and *Candida antarctica* lipase B (Novozyme 435) (Lai et al. 2012).

According to Amoah et al. (2016), researchers must also consider the phospholipids in microalgae at concentrations reaching 30 % of the total lipid content, which negatively affect enzyme catalysis because they can form water-in-oil phospholipid-based reverse micelles. The water required for enzyme activity is trapped by the micelle.

To produce biodiesel from microalgae, Huang et al. (2015) used a recombinant *Rhizomucor miehei* lipase to convert oil from *Chlorella vulgaris* with more than 90 % conversion to methyl esters and ethyl esters.

Notably, enzymatic transesterification can be advantageous for in situ biodiesel production from alternative raw materials, such as microalgae.

3.5 In Situ Transesterification

In situ transesterification is an efficient means to directly convert lipids from biomass to biodiesel. Because it is a simple process, various studies have used in situ transesterification with different raw materials, including oilseed plants (Hincapié et al. 2011; Martínez Arias et al. 2012) and microalgae (Hidalgo et al. 2013). This method is an alternative to the conventional transesterification process that may reduce biodiesel production costs where the lipid/oil extraction step features a high environmental or economic impact.

In this process, fatty acids are converted into alkyl esters directly inside the biomass, which eliminates the extraction step and postharvest biomass drying (Amaro et al. 2011). Microalgae lipid content can be transformed to biodiesel by enzymatic transesterification in situ, which uses methanol as raw material for the process and as solvent to assist microalgae cell wall rupture (Steriti et al. 2014), as shown in Fig. 2.

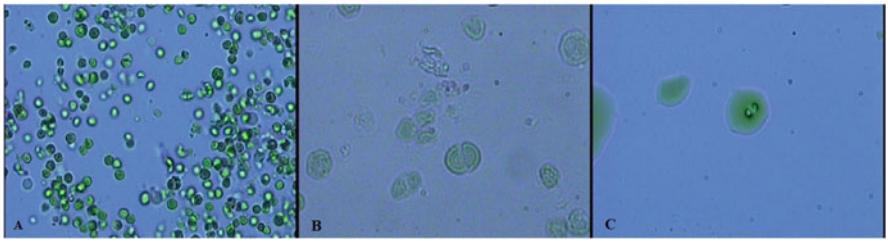

Fig. 2 Optical microscopy images of *Desmodesmus* sp. cells at different times of transesterification in situ: (**a**) intact cells at the beginning of the process (400x); (**b**) cell wall rupture after 48 h of transesterification (1000x); (**c**) the lipid content extracted from cells after transesterification in situ and evaporation of the solvent (400x)

This method may be particularly advantageous for microalgae because lipids are typically extracted using solvents, not through physical methods for oil extraction from conventional crops, as previously presented. Alcoholysis of the TG in the biomass produces better biodiesel yields compared with the conventional transesterification method with the additional advantage of reducing waste generation, power consumption, and consequently the environmental impact from these methods (Ehimen et al. 2010).

The reactions are simple and include adding alcohols, catalysts, biomass, and, occasionally, cosolvents (Rodrigues Da Silva Baumgartner et al. 2013; Atadashi et al. 2013).

For microalgae, this method has been applied using *Nannochloropsis salina*, *Chlorella* sp., *Scenedesmus* sp., *Nannochloropsis gaditana*, *Chlorella pyrenoidosa*, and *Schizochytrium limacinum*, among others (Carvalho et al. 2011; Cao et al. 2013; Jin et al. 2014; Sathish et al. 2014; Kim et al. 2015a, b; Ma et al. 2015). Tran et al. (2013b) used immobilized *Burkholderia* lipase for direct transesterification (in situ) to convert lipids into methyl esters after ultrasonically pretreating the biomass. The results showed that the ideal hexane/methanol ratio was 1.65, and different proportions of methanol relative to the lipid content yielded more than 90 % conversion to esters in the 48-h reaction period.

Biodiesel can also be produced from microalgae through in situ non-catalytic supercritical methanol transesterification, reaching 45.6 % biodiesel using the microalgae *Chlorella* sp. (native) (Jazzar et al. 2015) or *Schizochytrium limacinum* with supercritical CO_2 and adding methanol, which generates a 37.5 % yield (Bi et al. 2015). Notably, supercritical fluids are promising for in situ transesterification and should be further explored in the near future (Zeng et al. 2014).

3.6 *Prospects for Industrial Production*

The viability of biodiesel production using microalgae at the industrial level faces several challenges involving reduction of energy loss. The process includes a pretreatment step for free fatty acid esterification with methanol and acid catalysis followed by alkaline-catalyzed transesterification, and the cost of purifying biodiesel and glycerol through distillation was recently calculated as $0.592/L of biodiesel. This value does not consider lipid extraction from the biomass, which also impacts the production cost and may be investigated to save energy (Song et al. 2015).

In the first phase of biodiesel production from microalgae, volatile solvents are used to separate the lipids and for subsequent transesterification, which are ineffective for microalgae with high water content according to Cooney et al. (2009). Thus, the microalgae must be dried. Next, the organic solvents must be removed through reducing the pressure. The energy cost associated with the drying, distillation, and solvent recovery processes must also be considered. Modifications to the process that decrease energy consumption and optimize the use of solvents and other inputs may be the factors responsible that render biodiesel production from microalgae feasible. Peralta-Ruiz et al. (2013) performed an energy analysis for the microalgal lipid extraction pathways, and hexane was the most suitable substance for large-scale production with a 51 % maximum energy efficiency.

Lipid extraction technologies that generate a high yield are developed on a laboratory scale and are not easily converted to larger scales, such as the industrial scale. Successful industrial production is directly linked to using innovative technologies, such as in situ transesterification.

Countries such as Japan, Israel, India, and the United States have successfully ventured into this area, but the potential for using microalgae on an industrial level may be much greater when it is associated with, for example, wastewater phytoremediation. The potential for biofuel production from microalgae has driven studies and the transfer of technology to industry (http://www.algaeindustrymagazine.com).

The environmental and economic impacts of biomass production, lipid extraction, and biodiesel production have been studied in recent years (Monari et al. 2016; Delrue et al. 2012; Dassey and Theegala 2013; Collet et al. 2014; Mata et al. 2014; Sawaengsak et al. 2014; Yu et al. 2015). However, different production systems must be studied with a focus on environmental issues and the real potential of microalgae in biodiesel production to determine the actual environmental and economic gains from industrial biodiesel production using microalgae, as shown by Yu et al. (2015) in Singapore.

4 Conclusion

Biodiesel production from microalgae depends on the species, biomass production conditions, and technologies used for lipid separation and transesterification, and it is becoming feasible or is already a reality in certain countries. Several initiatives in the field have sought to use techniques that consume less energy and produce a better final biodiesel yield. Among these technologies, recent microalgal culture studies have sought greater lipid accumulation in the cells through adding different sources of carbon, metals, and vitamins and have sought to avoid using extraction solvents, such as through in situ transesterification. Technological advances from research have facilitated efficient biodiesel production from microalgae, but large-scale feasibility has not been analyzed to maximize production potential. The environmental gains from these processes should also be highlighted to render biodiesel production from microalgal biomass even more relevant in the medium and long term.

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