Extraction and Conversion of Microalgal Lipids

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Abstract Efficient downstream processing is crucial for successful microalgal biodiesel production. Extraction of lipids and conversion of lipids are the main downstream steps in microalgal biodiesel production process. This chapter provides the overview of the conventional as well as novel extraction and conversion technologies for microalgal lipids. The extraction and conversion technologies have to be environmentally friendly and energy efficient for sustainable and economically viable microalgal biodiesel production.

Keywords Microalgae · Lipid · Biodiesel · Extraction · Transesterification

1 Introduction

Microalgal lipids can be converted to a renewable alternative biofuel, i.e., biodiesel. Fast growth rates, substantial lipid accumulation, suitable lipid profiles, minimal arable land requirement, utilization of wastewater as growth medium, CO_2 sequestration, and use of residual biomass make microalgae an excellent biodiesel feedstock. Various strategies can be applied such as nutrient stress and alteration of cultivation conditions to enhance the lipid accumulation capability of microalgae

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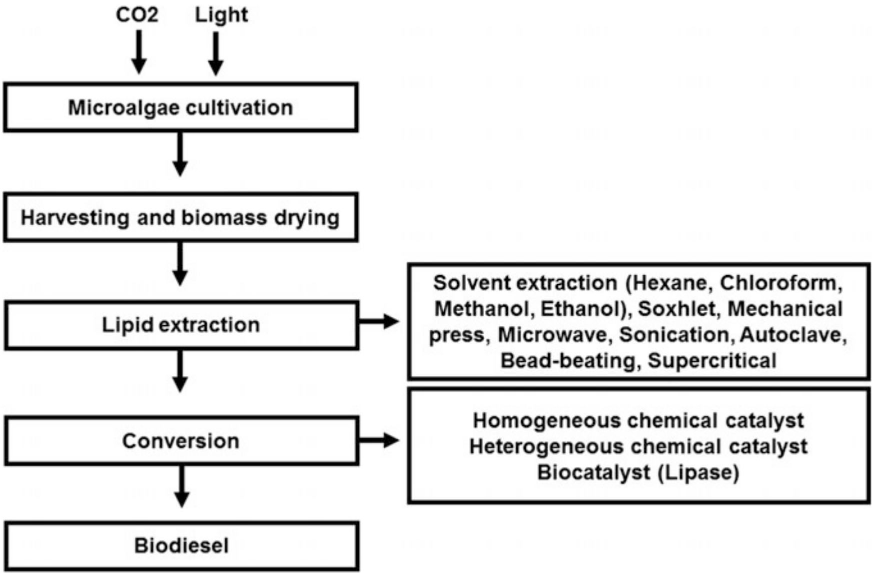

Fig. 1 Microalgal biodiesel synthesis process flow: different extraction and conversion techniques

(Rawat et al. 2013; Singh et al. 2015). Biodiesel synthesis from microalgae is a multistep process. The process involves cultivation of microalgae for biomass generation, harvesting of biomass, extraction of lipids, and conversion of lipids to biodiesel as major steps (Fig. 1). Much focus has been given to the upstream development such as cultivation of microalgae and enhancing the lipid yields. Downstream processes such as harvesting, extraction, and conversion are still, however, considered major bottlenecks for commercial-scale biodiesel production process.

Extraction of microalgal lipids and its conversion to biodiesel have proven to be challenging and are associated with environmental and economic concerns. There are no available technologies which are mature enough to be applied at commercial scale. Extraction of cellular lipids from microalgae faces challenges of cell wall disruption, high cost, and environmental concerns due to use of toxic solvents amongst others. Solvent extraction is widely used for lipid recovery from microalgae (Mubarak et al. 2015). Cell disruption techniques such as microwave, sonication, bead beating, autoclaving, etc. are generally coupled with solvent extraction to promote the process efficiency. Conversion of microalgal lipids to biodiesel can be achieved by transesterification. In transesterification, lipids are converted to fatty acid alkyl esters in the presence of a suitable acyl acceptor and catalyst. Glycerol is formed as a byproduct in this process. Transesterification can be accomplished using various homogeneous or heterogeneous chemical catalysts or lipase as a biocatalyst (Sharma et al. 2011). Efficient, environmentally friendly, and economical lipid extraction and conversion techniques are the foremost requisites for the sustainable biodiesel production from the microalgae.

2 Microalgal Lipids for Biodiesel Production

A steady supply of lipid feedstock is pertinent for the commercial-scale biodiesel production. The quantity and quality of the lipid feedstock is of vital importance for the ease of production and final quality of biodiesel. Microalgal lipids have shown both the substantial quantitative yields as well as suitable composition, to make it an excellent feedstock for biodiesel production. Various microalgal strains have been studied to assess their potential as a biodiesel feedstock and have yielded varying lipid content and profiles. The reported lipid contents of microalgae vary greatly and are strongly dependent on the strain and cultivation conditions. Widely studied microalgal genera such as *Chlorella, Dunaliella, Nannochloropsis, Scenedesmus, Neochloris, Nitzschia, Porphyridium, Phaeodactylum,* and *Isochrysis* yield lipid content in the range of 20–50 % (Amaro et al. 2011). High lipid productivity is important to ensure the economic viability of microalgal biodiesel production. Various stress conditions like nitrogen limitation, light, CO_2, etc. are known to enhance the lipid accumulation in microalgae, and however may negatively affect biomass production and thus lipid productivity (Singh et al. 2015).

The major constituents of microalgal oils are neutral lipids, polar lipids, and some amount of hydrocarbons, sterols, waxes, and pigments (Sharma et al. 2012). The neutral lipids are the best suited for the biodiesel synthesis due to their ease of conversion to fatty acid alkyl esters (FAAE). Neutral lipids function as the energy storage components of microalgal cells and composed mainly of triglycerides (TAG) and some amount of free fatty acids (FFA). The polar lipids are responsible for structural functions (phospholipids in cell membrane) and physiological roles such as cell signaling (sphingolipids) (Sharma et al. 2012). Triglycerides are the suitable for conversion to biodiesel via base catalyzed transesterification, whilst free fatty acids can be converted to biodiesel via esterification prior to transesterification or acid/lipase catalysis. Microalgal lipid accumulation can be enhanced by altering the nutrient composition in the media or cultivation conditions.

Fatty acid composition of lipids is an important criterion for selection of suitable microalgal strains for biodiesel synthesis. Fuel properties of the biodiesel are primarily influenced by the carbon chain length, degree of unsaturation, and percentage composition of saturated and unsaturated fatty acid in microalgal lipids. Microalgal lipids are composed of saturated, monounsaturated, and polyunsaturated fatty acids. Many of the lipid-accumulating microalgae have been shown to comprise C14:0, C16:0, C18:1, C18:2, and C18:3 as major contributing fatty acids of their lipids, which are considered to be suitable for good quality biodiesel (Song et al. 2013). Table 1 shows fatty acid profile and lipid content of various microalgal strains studied for biodiesel production.

Table 1 Fatty acid profile and lipid content of different microalgae

Microalgal strain	Lipid content (%)	Fatty acid composition (%)									Reference
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	SFA	PUFA	
Scenedesmus sp.	16	–	15.62	4.06	2.97	15.23	7.00	22.99	18.59	56.86	Talebi et al. (2013)
Chlorella vulgaris	17.3	–	14.55	1.183	10.51	23.62	13.80	32.10	25.06	70.7	Talebi et al. (2013)
Chlorella vulgaris	20.82	0.78	36.97	5.10	0.5	4.96	4.38	8.42	–	–	Song et al. (2013)
Staurastrum sp.	10	4.97	40	16.50	–	–	–	–	–	–	Song et al. (2013)
Chlorella sp.	30	0.30	0.41	2.41	25.94	3.82	0.47	0.99	–	–	Praveenkumar et al. (2012)
Monoraphidium sp. KMN5	20.84	–	41.03	–	17.67	10.16	3.03	1.53	58.7	16.26	Tale et al. (2014)
Scenedesmus sp. KMN4	28.63	–	41.27	–	20.53	9.21	1.91	0.57	61.82	12.77	Tale et al. (2014)
Nannochloropsis gaditana.	–	3	16	17.1	–	3.9	8	9	19.4	68.3	Hita Peña et al. (2015)
Scenedesmus sp. ISTGA1	20	–	14.6	12.1	21.2	24.9	8.8	4.2	35.8	54.3	Tripathi et al. (2015)
Ankistrodesmus falcatus KJ671624	23.3	–	–	21.15	–	28.18	19.25	14.33	–	–	Singh et al. (2015)
Neochloris oleoabundans	29	0.43	19.35	1.85	0.98	20.29	12.99	17.43	20.76	64.6	Gouveia et al. (2009)

3 Lipid Extraction Techniques

Lipid extraction from microalgae is a crucial step in biodiesel synthesis. The techniques of lipid extraction from microalgae studied by researchers include various physical and chemical methods. Solvent extraction is a most widely used method and can be coupled with cell disruption techniques to intensify the process for improved yields. Various cell disruption methods investigated include autoclaving, osmotic shock, microwave-assisted extraction, and sonication-assisted extraction (Halim et al. 2012; Mubarak et al. 2015). Novel methods of lipid extraction such as supercritical fluid (SCF) extraction, electrochemical extraction, high pressure extraction, etc. are more recent developments. Ideally, lipid extraction should be high yielding, energy efficient, time saving, and environmentally friendly with usage of nontoxic chemicals and easy handling. The other important aspect of the extraction technique is that it should not have deteriorating effects on the quality of lipids. The efficiency of lipid extraction techniques depends on the number of factors such as microalgal strain, cell wall structure, their lipid content, quality of lipids, etc. Table 2 depicts the various techniques and solvents used for extraction of lipids from the microalgae.

3.1 Conventional Lipid Extraction Techniques

Conventional lipid extraction methods such as solvent extraction, soxhlet, and mechanical press extraction are in practice, since the biodiesel started gaining the interest of researchers and industries. These methods are widely used at industrial scale for the extraction of oil from edible and nonedible plant seeds. Due to the smaller cell size and cell wall composition of microalgae, physical methods have been found to be inefficient. The conventional methods are low yielding and time consuming. These drawbacks negatively affect the economics of microalgal biodiesel.

3.1.1 Soxhlet Extraction

The soxhlet technique is the most widely employed organic solvent extraction strategy for the extraction of oil from different plants and microalgal strains. In the soxhlet technique, oil and fat from the biomass matrix are concentrated by repeated washings with an organic solvent, typically *n*-hexane or petroleum ether, under reflux in a specialized apparatus called soxhlet extractor. This method is used extensively by oil industry (Kirrolia et al. 2013). This method is temperature dependent as variations in extraction temperature affect the lipid yield. Temperatures ranging from 30 to 60 °C enhance the yield of lipids (Fajardo et al. 2007). However, temperatures above 70 °C decrease lipid yield due to loss of thermolabile ingredients by oxidative degradation. Soxhlet extraction is simple and nonlabor-intensive process which is economical and scalable. Soxhlet extraction is,

Table 2 Extraction of lipids from microalgae using different cell disruption techniques and solvents

Microalgae	Cell disruption	Solvents (ratio)	Lipid yields (%)	Reference
Scenedesmus sp.	Microwave	Chloroform: ethanol (1:1)	29.65	Guldhe et al. (2014)
Botryococcus sp.	Microwave	Chloroform: methanol (1:1)	28.6	Lee et al. (2010)
Chlorella sp.	Microwave	Chloroform: methanol (2:1)	38	Prabakaran and Ravindran (2011)
Nannochloropsis	Microwave	Chloroform: methanol (2:1)	32.8	Koberg et al. (2011)
Chlorella sp.	Sonication	Chloroform: methanol (2:1)	40	Prabakaran and Ravindran (2011)
Scenedesmus sp.	Sonication	Chloroform: ethanol (1:1)	19.85	Guldhe et al. (2014)
Botryococcus sp.	Sonication	Chloroform: methanol (1:1)	8.8	Lee et al. (2010)
Chlorella sp.	Autoclave	Chloroform: methanol (2:1)	24	Prabakaran and Ravindran (2011)
C. vulgaris	Autoclave	Chloroform: methanol (1:1)	10	Lee et al. (2010)
Botryococcus sp.	Bead beating	Chloroform: methanol (1:1)	28.1	Lee et al. (2010)
Scenedesmus sp.	Osmotic shock	Chloroform: methanol (1:1)	7.9	Lee et al. (2010)
Schizochytrium limacinum	Soxhlet	*n*-Hexane	45	Tang et al. (2011)
Scenedesmus caucus	–	*n*-Hexane	4	Shin et al. (2014)
*Nannochloropsi*s *oculata*	–	Dichloromethane	9	Liau et al. (2010)
*Nannochloropsi*s *oculata*	–	*n*-Hexane	5.79	Liau et al. (2010)
Nannochloropsis oculata	–	Petroleum ether	8.2	Converti et al. (2009)

however, a time-consuming method and generally needs large amounts of solvents. The lipid yields from soxhlet extraction are low, which could be because of poor extraction of polar lipids.

3.1.2 Mechanical Press

Mechanical press is a physical extraction technique which utilizes mechanical pressure to break and compress out oil from the dry biomass. This method is common for extraction of oils from the plant seeds. For its efficient performance this technique needs large amount of biomass (Harun et al. 2010). The small cell size of some algae renders the process ineffective for microalgal lipid extraction. This technique is slow and tedious compared to other methods of oil extraction and does not recovery the total lipid content as in organic solvent extraction. The benefit of mechanical pressing is that the technique maintains the chemical composition of lipids as no chemicals and/or extra heat is applied.

3.1.3 Solvents for Lipid Extraction

Use of the organic solvents to extract lipids from microalgae is the most employed technique. Lipids are soluble in organic solvents, and thus they are employed for lipid extraction process. Organic solvents such as *n*-hexane, methanol, ethanol, and mixed polar/nonpolar chemical solvents (e.g., methanol/chloroform and hexane/isopropanol) are effective for extraction of the microalgal lipids, but yield factor depends on microalgal strain and its lipid content (Halim et al. 2012). Microalgal lipids are of two types viz., neutral lipids where the carboxylic group end of the fatty acid molecule bonded to an uncharged head group (e.g., Glycerol) and polar lipids where fatty acid carboxylic group head is attached to a charged head group (e.g., Phosphate complex). Polar lipids are divided into two parts that are phospholipids and glycolipids. Neutral lipids (storage lipids) take the function of energy storage, while the polar lipids (membrane lipids) are involved in structural components of cell such as cell membrane. The basic concept of lipid extraction by solvent is like dissolving like. The solvents used for lipid extraction are polar and nonpolar or mixture of both in particular ratios. The polar solvents extract the polar lipids, while the nonpolar solvents extract the neutral lipids. The mixture of polar and nonpolar solvents can extract both neutral lipids and polar lipids (Halim et al. 2012).

Nonpolar organic solvents (viz., hexane, chloroform) penetrate the cell membrane and interact with neutral lipid present in the cytoplasm (Halim et al. 2012). The solvent–lipid interaction is reported to be due to the van der Waal's forces that lead to the formation of organic solvent and neutral lipid complex. The concentration gradient drives this complex to diffuse across the cell membrane. The neutral lipids get extracted from the cell to dissolve in the organic solvent that is of nonpolar nature. However, some amounts of neutral lipids found in cytoplasm are present as complex with polar lipids (i.e., phospholipids, glycolipids, sterols, carotenoids). The complex is said to be strongly linked by hydrogen bonds with proteins in the cell membrane which can be broken using polar organic solvents (e.g., methanol, ethanol). Hence, to break the membrane-based lipid–protein association, it is necessary to use both the nonpolar and polar organic solvents. Thus, the use of mixture of polar and nonpolar solvents like chloroform and methanol is advocated by many researchers for efficient lipid extraction (Guldhe et al. 2014; Lee et al. 2010). When *n*-hexane was used by Keris-Sen et al. (2014) for lipid extraction from a mixed culture (i.e., *Scenedesmus* sp., *Chlorococcum* sp.) the yield was 24 %. While Guldhe et al. (2014) applied mixture of chloroform and ethanol (1:1 v/v) for lipid extraction from *Scenedesmus obliquus* and Lee et al. (2010) applied mixture of chloroform and methanol (1:1 v/v) for lipid extraction from *Botryococcus* sp., the yields were 29.65 and 28.1 %, respectively.

The major drawback of using solvents is their hazardous nature and adverse effects on the environment. Some of the organic solvents like chloroform and methanol are highly toxic in nature, and could pose a serious threat while handling. Researchers are focusing on the use of greener solvents and minimizing the amount of solvents needed for extraction. The solvent extraction method can be linked with efficient cell disruption technique to improve the lipid extraction yields.

3.2 Process Intensification by Cell Disruption

To alleviate the drawbacks of conventional lipid extraction techniques, various cell disruption methods are coupled with solvent extraction to intensify the extraction process. These techniques facilitate the easy contact of the solvents and lipids in the microalgal cells. These techniques are associated with the advantages of high lipid yields, minimum solvent requirement, and reduced time of extraction. Microwave, sonication, autoclaving, bead beating, etc. are the cell disruption techniques commonly used for microalgal lipid extraction.

3.2.1 Microwave

Microwave is highly efficient cell disruption technique with reported high lipid yields from the microalgae (Koberg et al. 2011). Microwave energy generated in this technique causes rotation of the molecular dipole and results in disrupting the weak hydrogen bonds. This phenomenon increases the movement of dissolved ions that facilitate the diffusion of solvent resulting in effective lipid extraction. In the microwave technique the heating is efficient as the electromagnetic waves interact with cell matrix at the molecular level. Also in this technique both the mass and heat transfer take place from inside the cell toward the outside solvent. These reasons make the microwave-assisted lipid extraction from microalgae a highly efficient process. Prabakaran and Ravindran (2011) used microwave technique for extraction of lipids from *Chlorella* sp. with a lipid yield of 38 %. Koberg et al. (2011) obtained 32.8 % lipid yield when microwave technique is applied for extraction of *Nannochloropsis* sp. The advantages of microwave-assisted cell disruption techniques are rapid, minimal solvent consumption, high lipid yield, and easily scalable. However, this technique is energy intensive as the electromagnetic waves are generated for cell disruption.

3.2.2 Sonication

In sonication, the cavitation impact generated due to sound waves causes the cell disruption of microalgae. At the point when ultrasound is transmitted to the media cavitation bubbles are formed. These microbubbles moving in the media collapse with each other and implode generating chemical and mechanical energies in the form of heat, free radicals, stun waves, etc. This energy generated from cavitation bubbles disrupts the cell envelope, facilitating the solvent and cellular lipid interaction and easy mass transfer (Guldhe et al. 2014). Sonication technique provides the high lipid yield and greater penetration of solvents into the microalgal cells. When lipids from *Chlorella* sp. were extracted using sonication-assisted solvent extraction the lipid yield obtained was approximately 40 % (Prabakaran and Ravindran 2011). This technique does not involve the input of external heating source and thus operates at moderate temperatures. Generating the ultrasound waves is energy intensive and this technique requires sophisticated instrumentation which could be cost intensive.

3.2.3 Autoclaving

Autoclaving has also been investigated for the extraction of lipids from microalgae (Mendes-Pinto et al. 2001). The lipid yields from extraction by autoclave technique are lower than the microwave or sonication techniques. In microwave technique the cell disruption is caused by the heating at molecular level inside the cell, while in autoclaving the heat is diffused from the surroundings into the cell, which makes the cell disruption inefficient. Lee et al. (2010) in their comparative study of different cell disruption techniques found 10 % lipid yield while using autoclave as a cell disruption technique for extraction from *Chlorella vulgaris*. Autoclaving is also an energy-intensive process.

3.2.4 Bead Beating

Bead beating or milling causes cell disruption by the grinding of beads against the microalgal cells inside the vessel. The bead mills are of two types, viz., shaking vessels and agitating beads. In the first type cell disruption is caused by shaking the whole vessel filled with microalgal cells and beads. In the second type the vessel is fixed but provided with the rotary agitator, which facilitates the grinding by beads. The efficiency of bead beating depends upon number of factors such as size and structure of the beads, the velocity and configuration of the agitator, and time (Halim et al. 2012). The bead mills can be designed in horizontal or vertical orientation and can be easily scaled up from laboratory scale to industrial scale. The only constraint with this method is that it is economically viable with the high concentrations of microalgal biomass (100–200 g l^{-1}) (Show et al. 2014).

Table 3 Extraction of lipids from microalgae by supercritical fluid extraction method

Microalgae	Solvent	Conditions (Temp, pressure, time)	Lipid yields (%)	Reference
Schizochytrium limacinum	CO_2 and ethanol	40 °C, 35 MPa, 30 min	33.9	Tang et al. (2011)
Pavlova sp.	CO_2	60 °C, 306 bar, 6 h	34	Cheng et al. (2011)
Nannochloropsis sp.	SC–CO_2	55 °C, 70 MPa, 6 h	25	Andrich et al. (2005)
Chlorococcum sp.	SC–CO_2	60 and 80 °C, 30 MPa, 80 min	81.7	Halim et al. (2011)

3.3 Supercritical Lipid Extraction

Supercritical liquid extraction is the recent technology that can possibly replace the conventional organic solvent extraction. In this technique, specific pressure and temperature are maintained above the critical temperature and pressure of the liquid to attain the supercritical solvent properties. The microalgal biomass is exposed to SCF under specific temperature and pressure conditions, which causes dissolution of lipids from sample into the SCF. SCFs can be tuned by adjusting the temperature and pressure conditions, to preferentially extract the neutral lipids from microalgal biomass (Halim et al. 2012). Supercritical properties of liquid facilitate its rapid penetration in the cell matrix and efficient extraction of lipids. Thus, this lipid extraction technique is rapid and highly efficient. Supercritical properties of solvent are the function of density, which can also be adjusted by controlling the temperature and pressure. The lipid obtained from the supercritical extraction does not need to undergo solvent recovery step as they are free from solvents. Supercritical CO_2 ($SCCO_2$) is the commonly used solvent for extraction of microalgal lipids. $SCCO_2$ has critical pressure (72.9 atm) and moderate temperature (31.1 °C), which makes it a suitable solvent for lipid extraction without thermally degrading the lipid components (Mubarak et al. 2015). Advanced instrumentation is required for controlling the temperature and pressure at critical levels which could be energy and cost intensive. Table 3 depicts the extraction of lipids from microalgae using SCF.

3.4 Effect of Preceding Processing Steps on the Lipid Extraction

The steps in the biodiesel production process preceding lipid extraction such as harvesting also have an impact on the lipid extraction of microalgae. Some of the recent approaches to the harvesting of microalgae have been shown the influence on the lipid yields during the extraction. Harvesting processes like ozoflotation, electrolyte-assisted electrochemical harvesting, etc. aid in weakening of the cell wall, and thus facilitate the easy cell disruption and higher lipid yields. Komolafe et al. (2014) have reported that the treatment of microalgae, *Desmodesmus* sp. with ozone during harvesting (ozone flotation), led to the formation of higher content of saturated fatty acids in the oil. The ozoflotation of microalgae has been reported to aid in disruption of the microalgal cells and improves the lipid extraction process. Misra et al. (2014) investigated the electrochemical harvesting process for the *Chlorella sorokiniana*. In their study they found that when electrolyte (NaCl) is applied to intensify the electrochemical harvesting process, the lipid yields were also increased. The electrolyte aided in the weakening of the cell wall by osmotic shock and thus resulted in the higher lipid recovery yields. The right combination of preceding step with efficient extraction technique or merging this technique could make the biodiesel production process sustainable and economical.

4 Novel Lipid Extraction Techniques and Recent Advances

The quest for efficient, scalable, energy, and cost-effective lipid extraction technology is still ongoing. Researchers are investigating several novel aspects of production such as novel extraction techniques, cell disruption methods, wet biomass extraction, and use of green solvents. Steriti et al. (2014) applied hydrogen peroxide (H_2O_2) and ferrous sulfate ($FeSO_4$) to disrupt the cell wall of *Chlorella vulgaris* and found increased lipid yield of 17.4 % compared to 6.9 % without any cell disruption. In the electrochemical lipid extraction process, the electrical field develops a potential difference across the membrane. This potential difference causes the breakdown of membrane and makes the cell wall permeable for lipid extraction (Daghrir et al. 2014). Pulse electric field is an attractive lipid extraction technique where microalgal cell disruption is caused by the electroporation method. The main advantage of this process is that it can be applied directly to the microalgal culture, and as a result the harvesting and drying of biomass can be skipped (Flisar et al. 2014). Taher et al. (2014) investigated the extraction of lipids from wet biomass of microalgae *Scenedesmus* sp. using enzymes lysozyme and cellulose. Ionic liquids are considered as the green solvents because of their non-volatile characteristic, synthetic flexibility, and thermal stability. Kim et al. (2012) extract lipid from *Chlorella vulgaris* using mixture of ionic liquids and methanol, which enhance the overall yield of lipid from 11.1 to 19 % compared to original lipid extraction techniques of Bligh and Dyer method. These novel techniques have shown promising potential in the quest for sustainable, economical, and efficient lipid extraction method.

5 Microalgal Lipids Conversion to Biodiesel

Conversion of microalgal lipids to biodiesel is commonly accomplished by transesterification or alcoholysis of triglycerides with acyl acceptor employing a suitable catalyst that yields fatty acid alkyl esters (FAAE) and glycerol. Commonly employed acyl acceptors are short-chain alcohols like methanol and ethanol, while the catalysts used are either homogeneous and heterogeneous chemical catalysts or enzyme biocatalysts (lipase) (Table 4). This three-step process first converts triglycerides to diglycerides, then diglycerides to monoglycerides, and finally monoglycerides to glycerol with yield of a monoalkyl ester of fatty acid in each of the three steps. Stoichiometrically, three moles of alcohol are required for converting one mole of triglyceride into biodiesel. Normally, more than three moles of alcohol are added to shift the equilibrium reaction in the forward direction.

5.1 *Chemical Catalysis*

Catalysts play an important role in the synthesis of biodiesel. For the completion of the transesterification in acceptable timeframes, either homogeneous or heterogeneous catalyst is employed. The types of catalyst used are acidic, alkaline, or enzyme. Commonly employed acid homogeneous catalysts include sulfuric acid. The commonly employed alkaline homogeneous catalysts are potassium hydroxide, sodium hydroxide, and sodium methoxide. The heterogeneous catalyst includes a wide range of compounds. These include Brønsted or Lewis catalysts. The catalyst used in transesterification more often depends on the free fatty acid (FFA) content of the feedstock. For alkaline transesterification, the oil must contain low amounts of FFA. It is reported that the FFA should be lower than 4 mg $KOHg^{-1}$ for efficient alkaline transesterification (Sharma et al. 2008). Microalgal lipids have been found to generally contain higher amounts of free fatty acids. Thus, either acid catalyst or two-step method of acid esterification followed by transesterification by alkaline catalyst are the suitable strategies for microalgal lipid conversion (Singh et al. 2014). Heterogeneous catalysts have the advantage of fast reaction rates and reuse over the homogeneous catalysts. Miao and Wu (2006) studied conversion of *Chlorella protothecoides* lipids using sulfuric acid catalyst and the biodiesel yield obtained was 60 %. When heterogeneous Al_2O_3-supported CaO catalyst was employed for the conversion of lipids extracted from *Nannochloropsis oculata* biodiesel, yield of 97.5 % was observed (Umdu et al. 2009).

5.2 *Biocatalysis*

Biocatalytic conversion is greener approach as it is associated with the advantages of less wastewater generation and low energy input. Lipases are the enzymes which

Table 4 Conversion of microalgal lipids to biodiesel by different catalytic methods

Microalgae	Catalyst	Catalyst loading (% wt/oil wt)	Molar ratio Alcohol: oil	Reaction conditions [temperature (°C), time (h), stirring (RPM)]	Biodiesel yield (Y)/conversion (C) %	Reference
Chlorella protothecoides	Sulfuric acid	100	56:1	30, 4, 160	$Y \approx 60$	Miao and Wu (2006)
Oedogonium sp.	Sodium hydroxide	–	–	–, 3, 300	$Y > 90$	Hossain et al. (2008)
Spirogyra sp.	Sodium hydroxide	–	–	–, 3, 300	$Y > 90$	Hossain et al. (2008)
Nannochloropsis oculata	Al_2O_3 supported CaO	2	30:1	50, 4, 1100	$Y = 97.5$	Umdu et al. (2009)
Chlorella protothecoides	*Candida* sp. 99–125 sp. lipase	30	3:1	38, 12, 180	$C = 98.15$	Xiong et al. (2008)
Chlorella pyrenoidosa	*Penicillium expansum* lipase (PEL)	20	3:1	50, 48	$Y = 90.7$	Lai et al. (2012)

can be employed as the catalyst for transesterification of microalgal lipids. The enzyme-catalyzed reactions are less energy intensive than the chemical-catalyzed reactions as they can be carried out at moderate temperatures. The lipase-catalyzed conversion yields high-quality products, i.e., biodiesel and glycerol. Lipases are capable of catalyzing both the transesterification and esterification reactions which make them advantageous over most of the chemical catalysts as they can be used for feedstocks with high free fatty acid content (Guldhe et al. 2015a). Lipases can be used in two ways, i.e., extracellular and intracellular (whole cell catalyst) (Guldhe et al. 2015b). The main drawback associated with the enzyme catalysts is their high cost. However, immobilization of enzyme makes its reuse possible and thus improves the overall economics. Separation and purification of product is easier in the enzyme catalysis compared to chemical catalysis. The short-chain alcohols used in reaction and glycerol produced have a negative impact on the activity of lipase used for transesterification. Greener enzyme catalysis and sustainable microalgal feedstocks hold promising potential for environmentally friendly biodiesel production. This area is scarcely studied and needs further investigation to alleviate the constraints of lipase-catalyzed biodiesel conversion. Xiong et al. (2008) applied the *Candida* sp. 99–125 sp. lipase for the conversion of lipids extracted from *Chlorella protothecoides*, and observed 98.15 % biodiesel conversion.

5.3 Solvents Used in Conversion

The widely used acyl acceptors in transesterification reactions are short-chain alcohols like methanol and ethanol. The alcohol and oil in the reaction mixture form a two-phase system. Low dissolution rate of oil in the alcohol causes slower reaction rates. To increase the reaction rate solvents are added to the transesterification reaction mixture. A solvent in the reaction mixture increases the solubility of the reactants and also aids in the proper mass transfer and thus increases the reaction rate. Tetrahydrofuran (THF), diethyl ether, hexane, and tert-butanol are the solvents commonly employed in the transesterification reaction (Lam et al. 2010). The ideal solvent should be inert and nontoxic, and should be easily recovered after the completion of reaction. For high quality of the biodiesel, the solvent should be completely removed from it. The solvents can be used for transesterification catalyzed by either chemical catalyst or biocatalyst.

SCFs can also be applied as a solvent for transesterification reaction. SCFs form a single-phase system which drives the reaction at faster rates. With supercritical methanol a noncatalytic method of biodiesel production can be developed. In this method supercritical methanol and oil form a single phase and reaction is completed in short duration of time. In other mode SCFs can be used as solvents for transesterification reaction in the presence of catalyst. Taher et al. (2011) have advocated the enzymatic production of biodiesel from microalgal in supercritical CO_2. The supercritical condition leads to faster completion of reaction. Another advantage with this method is the ease in separation of products. However, the high temperature and pressure requirements to maintain the supercritical conditions make this process an energy-intensive route. The supercritical method also leads to simultaneous extraction of lipid from algal biomass and transesterification of the lipids to biodiesel. The optimum yield of FAAE was reported to have obtained at 265 °C, and at pressure of ca. 80 bar using microwave reactor. Supercritical method has been reported to be suitable for noncatalytic transesterification of wet microalgae by Kim et al. (2013). At a high temperature when supercritical methanol is used, the water–methanol mixture in wet microalgae exhibits both hydrophobic and hydrophilic characteristics which will reduce the reaction time as well as product separation. It has, however, been reported that in situ transesterification will not be feasible when the water content is in excess of 31.7 % (Kim et al. 2013). The reason attributed for this is hydrolysis during transesterification.

6 Influence of Microalgal Lipids on Biodiesel Properties

The lipid profile has an important role to play in the characteristics of biodiesel. Fatty acid composition of lipids in microalgae has an influence over fuel properties such oxidative stability, cetane number, viscosity, iodine value, cold flow properties, etc. Long chain length and low degree of unsaturation are considered good for oxidative stability and high cetane number (Shekh et al. 2013). The feedstock which is rich in monounsaturated fatty acids is considered suitable for synthesis of biodiesel as it improves the cold flow property of the fuel. A substantial amount of saturated fatty acid content in the feedstock is equally important to impart oxidation stability in the fuel. As there is an inverse relationship between the cold flow property and the oxidation stability of the fuel, it has been reported that a high content of oleic acid (a monounsaturated fatty acid) could lead to strike a balance between these two properties (Tale et al. 2014). Tale et al. (2014) reported a high content of oleic acid for the five microalgae studied. The unsaturated fatty acid (comprising monounsaturated and polyunsaturated fatty acid) content in three strains of *Chlorella* species (isolate: KMN 1, KMN 2, KMN 3) ranged from 31.62, 31.26, and 35.01 %, respectively. The saturated fatty acids in the species were 40, 43.92, and 47.48 %, respectively. The species *Scenedesmus* sp. and *Monoraphidium* sp. possessed comparatively lesser content of unsaturated fatty acids of 12.77 and 16.26 %, respectively, and a much higher content of saturated fatty acids (61.82 and 58.7 %, respectively). It has been reported that the appropriate balance of UFA and SFA will be suitable for the production of biodiesel.

Cheng et al. (2014) reported that polyunsaturated fatty acids in microalgal lipids are prone to degradation and autoxidation when subjected to high temperature during either extraction of lipid or transesterification. This could further lead to loss of FAAE yield. Cheng et al. (2014) have thus suggested comparatively lower temperature (at 90 °C) of transesterification of lipids in wet microalgae by microwave instead of high temperature operation (at 250 °C) using supercritical alcohol.

Table 5 Fuel properties of biodiesel produced from different microalgae

Biodiesel characteristics	Units	ASTM 6751	EN 14214	*Chlorella protothecoides*	*Scenedesmus* sp.	*Nannochloropsis* sp.	*Dinoflagellate*
Cetane number	–	Min 47	Min 51	–	–	–	–
Calorific value	MJ kg^{-1}	–	–	41	–		–
Density	kg m^{-3}	860–900	860–900	0.864	0.852	0.854	0.878
Methyl ester content	%	–	Min 96.5	–	91.0	92.2	96.6
Linolenic acid methyl ester content	%	–	Max 12	–	8.26	–	–
Acid value	mgKOH g^{-1}	Max 0.8	Max 0.5	0.374	0.52	0.46	0.44
Iodine number	g100 g^{-1}	–	Max 120	–	–	–	–
Cold filter plugging point (CFPP)	°C	–	–	–11	–	–	–
Oxidative stability	h	Min 3	Min 6	–	5.42	1.93	1.02
Sulfur	wt%	Max 0.05	–	–	0.02	0.06	0.04
Reference	–	–	–	Miao and Wu (2006)	Chen et al. (2012)	Chen et al. (2012)	Chen et al. (2012)

Cheng et al. (2014) have also suggested that a high concentration of sulfuric acid as catalyst could promote polymerization of double bonds in the unsaturated fatty acids. Patil et al. (2013) have reported microwave-assisted transesterification of dried biomass of microalgal using supercritical ethanol. Table 5 depicts the fuel properties of biodiesel produced from different microalgal strains.

7 Challenges in Lipid Extraction and Conversion

The major challenge for lipid extraction and biodiesel conversion techniques is their economical viability. Biodiesel is a bulk commodity which is considered as a low-cost product compared to pigments and other therapeutic proteins from the microalgae. Thus for its successful industrialization, the crucial steps like extraction of lipids and conversion to microalgal biodiesel have to be economical. The other bottlenecks are the environmental implications and the scalability of the techniques. The commonly used solvents applied for the lipid extraction and conversion process are toxic and volatile in nature, which could be hazardous as well as threat to the environment. The wastewater generated during the conversion process contains acidic or basic catalyst, solvent, glycerol, etc. The techniques like microwave and sonication for extraction have shown excellent results in terms of yields. But yet these techniques have not been investigated at the industrial-scale microalgal biodiesel production plant. Applications of the heterogeneous chemical and biocatalysis are considered as the environmental friendly and efficient conversion methods. The fuel properties of microalgal biodiesel are still a concern. These fuel properties have to comply with the specification set by the international standards. However, the recent investigations and technical advances in the microalgal lipid extraction and biodiesel conversion processes have shown potential to alleviate these challenges toward the sustainable microalgal biodiesel production.

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