

# Lipid Extraction and Transesterification in Microalgae for Biodiesel Production: A Comprehensive Review

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**Abstract** - Microalgae have been receiving worldwide attention as promising feedstock for production of carbon neutral biodiesel. As an alternative fuel, biodiesel is very imperative for the sustainable development of mankind. Biodiesel production process is generally carried out through upstream and downstream processing to generate cellular biomass, extracted oil and finally transesterification reaction. Transesterification reaction is facilitated with suitable catalyst either heterogeneous or homogeneous. Selection of catalyst is highly dependent on amount of free fatty acids in oil. Heterogeneous catalysts offers high sensitivity, high activity as well as higher water tolerance properties which depends on strength and amount of active basic and acidic sites. Basic and acidic catalyst can be subdivided on type of metal oxides, their derivatives and active acidic sites. This review aims to collate and present different lipid extraction process from microalgal cells, transesterification and suitability of microalgal lipid compositions for biodiesel conversion.

**Index Terms** - Lipid extraction; Organic solvent; Microalgae biomass; Biodiesel; FAME; In situ transesterification; Acid/base catalysts.

## 1. INTRODUCTION

Biofuels are renewable energy resource which recently gain so much public attention as fossil fuel alternative (Demirbas and Demirbas, 2011). Biodiesel production from microalgae lipids is feasible approach since microalgae have higher lipid accumulation more than 50% of its dry cell weight (Xiong *et.al.* 2008). As microalgae grown in liquid media therefore lipid extraction usually classified as dry or wet extraction depending on whether the culture broth was dried or not before extraction. Currently the extraction process is carried out after drying the wet biomass for enhancing the efficiency of lipid extraction (Wahlen *et.al.* 2011; Rodríguez-Meizoso *et.al.* 2010). However, drying process consumed 59% total energy during the biodiesel production (Yanfen *et.al.* 2012). To make biodiesel production cost effective by eliminating drying process, wet extraction process should be improved by adding other operations such as ultrasonic, supercritical and microwave processes emerged as a very important issue (Xu *et. al.* 2011; Koberg *et.al.* 2011; Wahlen *et.al.* 2011; De Boer *et.al.* 2012). However, this review focuses on high yields of fatty acid methyl ester (FAME) from wet microalgae by combining the extraction and transesterification processes

into a single step named as in situ transesterification.

## 2. LIPID EXTRACTION

Depending on its pre-treatment pathway, microalgal biomass to be submitted to lipid extraction can assume one of the following physical states: concentrate or disrupted concentrate or dried powder. During lipid extraction, the microalgal biomass is exposed to an eluting extraction solvent which extracts the lipids out of the cellular matrices. Once the crude lipids are separated from the cell debris, the extraction solvent, and water (only when extraction is performed on concentrate or disrupted concentrate), their mass can be measured gravimetrically. Ideally, a lipid extraction technology for microalgal biodiesel production needs to display a high level of specificity towards lipids in order to minimize the co-extraction of non-lipid contaminants, such as protein and carbohydrates (Medina *et.al.* 1998). To reduce downstream fractionation/purification, the lipid extraction technology should also be more selective towards acylglycerols than other lipid fractions that are not as readily convertible to biodiesel, i.e. polar lipids and non-acylglycerol neutral lipids (free fatty acids, hydrocarbons, sterols, ketones, carotenes, and chlorophylls) (Medina *et.al.* 1998). Additionally, the selected

technology should be efficient (both in terms of time and energy), non-reactive with the lipids, relatively cheap (both in terms of capital cost and operating cost), and safe (Kates, 1986). Since dewatering the microalgal biomass beyond a paste consistency (200g dried microalgal biomass/L culture) is energy intensive, it will be economically beneficial if the selected lipid extraction technology is effective when directly applied to wet feedstock, i.e. concentrate or disrupted concentrate with concentrations between 10 and 200g dried microalgal biomass/L culture. Removing water, beyond 10-30 wt% dry biomasses, is energy intensive. Therefore, if a lipid extraction methodology can be applied to a wet feedstock, it can save a lot of energy (Halim *et.al.* 2011).

## 2.1. Selection of organic solvents

In addition to satisfying the previously mentioned criteria for an ideal lipid extraction technology, the selected organic solvents should preferably be volatile for low-energy distillation from the crude lipids (Kates, 1986; Medina *et.al.* 1998).

Chloroform/methanol (1/2 v/v) is the most frequently used organic solvent mixture for lipid extraction from any living tissue. Using this organic solvent system, residual endogenous water in the microalgal cells acts as a ternary component that enables the complete extraction of both neutral and polar lipids. It is noted that this method does not require the complete drying of microalgal biomass. Once the cell debris is removed, more chloroform and water are added to induce biphasic partitioning. The lower organic phase (chloroform with some methanol) contains most of the lipids (both neutral and polar) while the upper aqueous phase (water with some methanol) constitutes most of the non-lipids (proteins and carbohydrates) (Medina *et.al.* 1998).

Extraction using chloroform/methanol (1/2 v/v) is fast and quantitative. Chloroform, however, is highly toxic and its usage is undesirable. The method was originally developed by Folch *et.al.* (1951) for the isolation of total lipids from brain tissues. For this reason, its efficacy in extracting lipids from microalgal biomass still needs further assessment. In a study by Lee *et.al.* (1998), the performance of five different organic solvent mixtures in extracting lipids from bead-beaten *Botryococcus braunii* cells was compared. Chloroform/methanol obtained the highest final total lipid yield at 0.29 g/g dried microalgal

biomass. On the other hand, dichloroethane-based organic solvent mixtures (dichloroethane/methanol and dichloroethane/ethanol), previously recommended for lipid extraction from the green algae *Cladofora*, were found to have limited efficacies when applied to *B. braunii* (Lee *et.al.* 1998).

Hexane/isopropanol (3/2 v/v) mixture has been suggested as a low-toxicity substitute to chloroform/methanol system (Halim *et.al.* 2011). The mixture works in a similar fashion with chloroform/ methanol system. Upon biphasic separation, the upper organic phase (hexane with some isopropanol) contains most of the lipids (both neutral and polar) while the lower aqueous phase (water with some isopropanol) contains most of the non-lipids (proteins and carbohydrates). When evaluated for microalgal lipid extraction, hexane/isopropanol mixture was found to be more selective towards neutral lipids compared to chloroform/methanol system (Guckert *et.al.* 1988). Guckert *et.al.* (1988) attributed the neutral lipid selectivity of hexane/isopropanol mixture to its inability to extract the polar lipid constituents of microalgal membranes (chloroplast membranes contain glycolipids and cell membranes contain phospholipids). The hexane/isopropanol system, however, yielded a surprisingly low total lipid recovery when applied to *B. braunii* (Lee *et.al.* 1998).

The highest values of the lipid extracts i.e. those above 10% has highest efficiencies for the extraction with chloroform, ethanol, and hexane from the biomass, it may be concluded that a range of lipids varying from polar to non-polar were present in the algal biomass. It was also significant to note that acetone was the solvent with the lowest efficiency, extracting an average of 2.32% of lipids, when compared to chloroform which extracted the highest quantity of lipids, with an average value of 10.78% lipids. This also serves to confirm chloroform as the solvent of choice in reports by Bligh and Dyer and Folch and Christie, for the extraction of lipids (D'Oca *et.al.* 2011). A comparative study was conducted using the optimized chloroform:ethanol (1:1) and a modified Bligh and Dyer method using chloroform and methanol mixture (1:1) (Manirakiza, 2001). The results compared favorably with the Bligh and Dyer method which produced a slightly higher extract. A comparison of the various ratios of the three binary mixtures indicates that the 1:1 chloroform:hexane mixture extracted the least amount of lipids at 0.98%

whereas the 1:1 chloroform:ethanol mixture recorded the highest quantity of lipids at 11.76%. The various ratios of chloroform:hexane show extraction levels below 2% hence offering lower efficiency than the chloroform:ethanol mixtures which varied from 2.5% to approximately 12% (Ramluckan *et.al.* 2014).

## **2.2. Modifications to organic solvent extraction**

A majority of the laboratory-scale organic solvent extractions reported in the literature have been performed as a batch process. Even though batch extraction is limited by lipid mass transfer equilibrium, a continuous organic solvent extraction able to overcome this limitation requires a large amount of organic solvent and becomes too expensive. Through its ingenious cycles of solvent evaporation and condensation, the Soxhlet apparatus continuously replenishes cells with fresh organic solvent (hence evading equilibrium limitation) while simultaneously minimizing solvent consumption (de Castro and Garcia-Ayuso, 1998; Wang and Weller, 2006). The apparatus has 3 compartments: a continuously heated round bottom flask to store the extracting organic solvent, the Soxhlet extractor to hold the microalgal biomass (existing as concentrate or disrupted concentrate or dried powder), and the continuously cooled condenser. Organic solvent from the heated round-bottom flask enters the condenser and is immediately channeled into the Soxhlet extractor. The organic solvent comes in contact with the microalgal biomass and performs lipid extraction. The thimble in the extractor prevents the microalgal biomass from being carried away by the organic solvent flow and, as such, serves as a filter to remove cell debris. Once the organic solvent in the extractor reaches the overflow level, a siphon unloads the organic solvent-lipids mixture from the extractor back into the round-bottom flask. The organic solvent is heated and evaporates again while the extracted crude lipids remain in the round-bottom flask. This cycle is repeated until no more crude lipids are extracted in the Soxhlet extractor. Despite its advantageous design in avoiding equilibrium limitation, the Soxhlet apparatus suffers from high energy requirement for continuous distillation (Wang and Weller, 2006).

Despite its improved total lipid recovery, Soxhlet extraction potentially suffered from lipid degradation resulting from the use of elevated

temperature throughout the process. Guckert *et.al.* (1988) noted that the crude lipids recovered using a Soxhlet system contained less PUFAs than those obtained by batch extractions and ascribed this observation to potential thermal degradation due to the harshness of the Soxhlet method. A couple of modifications to organic solvent extraction have also been introduced: microwave-assisted organic solvent extraction and accelerated or subcritical organic solvent extraction. Each modification utilizes an auxiliary process that enhances the kinetics of lipid extraction by the organic solvent through speedy disruption of the cellular structures (Wang and Weller, 2006).

Microwave-assisted organic solvent extraction uses the aid of electromagnetic radiation within a specific frequency range to deliver large amount of thermal energy to the microalgal cells (Balasubramanian *et.al.* 2011). When the cells receive this energy, local internal superheating occurs leading to instantaneous temperature rise within the matrices and rapid pressure effects on the cell wall/membrane structure. Cell structures are immediately ruptured forcing cell constituents to spill out. This effective expulsion of cell materials facilitates a more rapid diffusion of microalgal lipids into the extracting organic solvent. Microwave-assisted heating is substantially more rapid than conventional heating as heat is delivered via radiation rather than convection and conduction. Balasubramanian *et.al.* (2011) examined the use of microwave-assisted hexane extraction to recover lipids from *S. obliquus*. Microwave-assisted hexane extractions were found to result in higher oil yields compared to conventionally water heated hexane extraction control methods at all extraction temperatures and times.

### *2.2.1. Bligh and Dyer's method*

Lam and Lee found Bligh and Dyer method to have highest lipid extraction efficiency (Lam *et.al.* 2012). The Bligh and Dyer method of lipid extraction, yields  $\geq 95\%$  of total lipid and further to it, this method can be used for any tissue containing water up to 80%. Therefore, for lipid extraction, the Bligh and Dyer method has been considered for both dry and wet route (Iverson *et.al.* 2001).

The critical ratios of methanol, chloroform and water should be 2:1:1.8 and that of solvent to tissue should be 3:1. After the solvent and culture are mixed, in the given ratio, they are

homogenized to form a monophasic system and then re-homogenized with another similar quantity of chloroform. Therefore, the overall ratio of methanol, chloroform and water should be 2:2:1.8 and that of solvent to tissue is [(3:1):1] (Iverson *et.al.* 2001). Considering the critical ratios, for dry route, since water content is insignificant in comparison to biomass, solvent to tissue ratio of [(3:1):1] should be considered, while for wet route because of high water content, methanol, chloroform and water ratio of 2:2:1.8 should be considered. The homogenization by centrifuge, separates the biphasic layer (lipid dissolved in chloroform and methanol dissolved in water) formed in the process. Thereafter, the lipid is separated from chloroform and methanol from water by fractional distillation (Iverson *et.al.* 2001).

### 2.2.2. Ionic Liquids

Ionic liquids (ILs) are salts that consist of relatively large asymmetric organic cations coupled with smaller inorganic or organic anions. The cations generally consist of nitrogen containing ring structure (e.g., imidazolium or pyridine) with a broad range of functional side groups, which decide the polarity of the ILs. The anions vary from single ions like chloride, to larger complex ions like  $[N(SO_2CF_3)_2]^-$  (Young *et.al.* 2010). They are also known as green solvents and their characters like non-volatile nature and thermal stability; make them an attractive alternative to volatile organic solvents (Kim *et.al.* 2012).

Kim *et.al.* (2012) used mixture of ionic liquid [Bmim]  $[CF_3SO_3]$  and methanol in volume ratio of 1:1, ionic liquid [Emim]  $[MeSO_4]$  and methanol in volume ratio of 1:1. Methanol was used to decrease the high viscosity of ionic liquids. The two mixtures of ionic liquids and methanol dissolved algal biomass leaving lipids insoluble. Undissolved lipids, being lighter than the ionic liquids and methanol mixture, floated during the dissolution process after which the lipid phase was separated by centrifugation. On comparison with Bligh and Dyer's extraction, it was found that [Bmim]  $[CF_3SO_3]$  and [Emim]  $[MeSO_4]$  extracted 12.5% and 11.9% of the lipids, respectively, while only 10.6% of lipid was extracted by the Bligh and Dyer's method (Kim *et.al.* 2012).

The extraction efficiency of lipids is highly dependent on the anion structure of ILs. Generally, hydrophobic and water immiscible ILs

such as [Bmim] $[PF_6]$  and [Bmim]  $[Tf_2N]$  showed a low extraction efficiency, while hydrophilic and water miscible ILs such as [Bmim]  $[CF_3SO_3]$ , [Bmim]  $[MeSO_4]$ , and [Emim]  $[MeSO_4]$  showed a high extraction efficiency, with the exception of [Bmim]  $[Cl]$  and [Emim]  $[Ac]$ . These results can be partially attributed to the solubility of lipids in the ILs. Higher solubility of hydrophobic ILs for lipids can induce the partitioning of lipids to the methanol and IL mixture phase (Kim *et.al.* 2012).

### 3. OIL EXTRACTION AND BIODIESEL PRODUCTION

Harvesting is followed by oil extraction. The extracted lipid is then converted into biodiesel (Rawat *et.al.* 2011). Direct transesterification of dried biomass has also been reported in some microalgal and fungal species (Maceiras *et.al.* 2011). In this section, the technically viable conversion options for algal biomass and end-use of derived energy or energy carriers (liquid or gaseous fuels) are considered. The conversion of algal biomass-to-energy encompasses the different processes ordinarily used for terrestrial biomass and which depend, to a large extent, on the types and sources of biomass, conservation options and enduses (McKendry, 2002). The conversion technologies for utilizing microalgae biomass can be separated into two basic categories of thermochemical and biochemical conversion. Factors that influence choice of conversion process include: the type and quantity of biomass feedstock; the desired form of the energy; economic consideration; project specific; and the desired end form of the product (McKendry, 2002).

### 4. EXTRACTION AND PURIFICATION FOR ALGAL METABOLITES

Cell disruption is often required for recovering intracellular products from microalgae. Cell walls can strongly modulate any extraction process by reducing the cell biodegradability (Sialve *et.al.* 2009). Most cell disruption methods applicable to microalgae have been adapted from applications on intracellular non-photosynthetic bioproducts (Middelberg, 1994). Cell disruption methods that have been used successfully (Mendes-Pinto *et.al.* 2001) include high-pressure homogenisers, autoclaving, and addition of hydrochloric acid, sodium hydroxide, or alkaline lysis. Solvents are widely used to extract metabolites such as astaxanthin, b-carotene and fatty acids from algal biomass (Molina Grima *et.al.* 2003). The process entails cell uptake of solvent molecules on

exposure to a solvent, which causes alterations to the cell membrane to enhance the movement of globules toward the outside of the cell. Properties of the cell membrane play an important part in solvent extraction process (Hejazi & Wijffels, 2004).

## 5. TRANSESTERIFICATION

Transesterification is an equilibrium derived process, through which monohydric-alcohol and triglyceride ester reacts in the ratio of 1:3 to acquire an equivalent quantity of mono-alkyl esters (biodiesel) and a mole of glycerol (glycerine) as byproduct (Galadima & Muraza 2014). In this reaction, the excess supply of alcohol maintained equilibrium shift to the product and improve reaction rate (Singh & Singh, 2010). The viscosity of crude biodiesel is relatively high, thus requiring conversion of lower molecular constituents in the form of fatty acid alkyl esters. Transesterification convert triacylglycerols/free fatty acids in raw microalgal lipid into non-toxic, renewable and biodegradable biodiesel for direct consumption (Rawat *et.al.* 2011). While comparing the emission of biodiesel with fossil fuel, shows clear reduction in emission through combustion engines as follows: 48% CO, 100% SO<sub>2</sub>, 67% CO<sub>2</sub>, 47% particulate matter (Lotero *et.al.* 2005). Hence, biodiesel leads to no sulfur, zero net CO<sub>2</sub> and significantly reduced permanent gas emissions (Antolin *et.al.* 2002).

### 5.1. Catalyst

Catalysts that are used in the transesterification reaction are bases, acids or enzymes. Kaieda *et.al.* (1999) have described the kinetics of triglyceride transesterification with methanol, i.e. methanolysis, catalyzed by *Ryzopus oryzae* lipase seems to be in accord with successive reaction mechanism. Transesterification through lipases at

low temperatures yields more biodiesel as compare to other transesterification methods. However its amount affects the reaction rate; 75% immobilized lipase with 10% water gives a 98.15% conversion in 12h (Table 1). Unfortunately, lipases are deactivated by impurities and are very expensive for commercial biodiesel production (Fjerbaek *et.al.* 2009; Akoh *et.al.* 2007; Du *et.al.* 2008). In enzyme catalyzed methanolysis free fatty acids could be easily converted to methyl esters. Free fatty acids content in the region of 20-50% is responsible for saponification during base catalyzed transesterification (Fukuda *et.al.* 2001).

#### 5.1.1. Base catalysis

Homogeneous base catalysts like sodium hydroxide, sodium methoxide or potassium hydroxide are used in the production of biodiesel primarily due to their low cost and high reaction rates. They can be used at low operating temperatures typically between 45°C and 55°C. The major drawback of these catalysts is their difficult recovery and highly corrosive nature (Leung and Guo 2006). Homogenously catalyzed processes are the conventional technologies. However, their large-scale applicability is compromised due to their characteristic challenges. Batch processes and continuous processes are used for industrial purposes with typical capacity of 7.26-7.5 Gg y<sup>-1</sup> and 8-125 Gg y<sup>-1</sup> respectively, and heterogeneous catalysis may be sustainable for the continuous processes (Table 1). Heterogeneous catalysts from renewable sources may be both environmentally and economically viable (Aransiola *et.al.* 2014). Teo & Idris 2014, investigated that maximum biodiesel production efficiency of 90.71% was attained at 1:12 lipid to methanol ratio during the application of simultaneous cooling and microwave heating (SCMH) under alkali transesterification as shown in Table 1.

Table 1. Transesterification through different catalysts

Catalyst	Conversion to FAME	Advantages	Disadvantages	Reference
Lipase Enzyme	98.15% conversion in 12h	Low operating temperature yield more biodiesel as compare to other tranesterification methods	Deactivated by impurities and are very expensive for commercial biodiesel production	Akoh <i>et.al.</i> 2007; Du <i>et.al.</i> 2008; Fjerbaek <i>et.al.</i> 2009
Homogeneous base catalysts	88.8%-93.5% conversion	Low operating temperatures, low cost and high	Difficult recovery, highly corrosive nature,	Leung & Guo 2006; Dias et al., 2008

(NaOH, CH <sub>3</sub> NaO, KOH)	ion in 15min at 70°C	reaction rates	Saponification	
Heterogeneous Base catalysts	90.71% biodiesel production efficiency	Production cost of these catalysts is low and they show a very high performance	Energy intensive, recovery of glycerol is difficult, the catalyst has to be removed from the product, alkaline waste water requires treatment, free fatty acid and water interfere with the reaction, and low selectivity leads to undesirable side reactions	Fukuda et al., 2001; Noureddini et al., 2005; Maçaira et al., 2011; Aransiola <i>et.al.</i> 2014; Teo & Idris 2014
Homogeneous acidic catalyst (Sulphuric, hydrochloric, sulfonic and phosphoric acids)	89.5%-99% conversion efficiency	Higher tolerance to FFA, less capital intensive, No soap formation, Low energy requirement, preferred for low grade oil	High alcohol to oil molar ratio, longer reaction time and recycling is problematic	Canakci & Van Gerpen, 1999; Demirbas 2007; Dias et al., 2008; Sharma & Singh 2008; Liu et al., 2008; Yan et al., 2010
Heterogeneous acid catalysts (sulphated zirconia, zeolites, ion exchange resins and mixed metal oxides)	96.22% yield under optimal conditions of 60°C, a reaction time of 10 min	Less corrosive, less toxic, and generates fewer environmental problems	Higher reaction temperature, High cost, Low micro porosity, Leaching and low surface areas	Demirbas 2007; Park et al., 2008; Zhang et al., 2010; Yan et al., 2010
Heterogeneous catalysts from natural sources (Eggshell, limestone calcite, cuttlebone, dolomite and hydroxyapatite)	86.13%-98% conversion efficiency	Recyclable, economically and environment friendly	Lengthy process of synthesis and may be complicated in some cases, requiring great expertise	Demirbas 2007; Kotwal et al., 2009; Sharma et al., 2010;

### 5.1.2. Acid catalysis

Acid catalysts convert FFAs to esters or biodiesel through esterification and simultaneously transesterification at a relatively higher temperature (100°C) (Lotero *et.al.* 2005). Experimentally, base-catalyzed reactions are approx. 4000 times faster than acid-catalyzed reaction. The optimization of base catalyzed

reactions are is done at 60°C for 90 min under atmospheric pressure. At higher temperatures and pressures, the reaction proceeds faster but is more expensive (Hossain & de lasa 2008). The optimum conditions for acidic transesterification are 100% catalyst (e.g. sulfuric acid), a methanol-to-oil ratio of 56:1, and a temperature of 30°C (Table 1). The specific gravity of oil reduces from 0.912 to 0.8637 in 4h (Demirbas, 2007).

Mixed oxides of transition elements like Ca, Ce, Zr, Fe and La have also been evaluated for transesterification and recorded 95% biodiesel yield. For instance, CaTiO<sub>3</sub> produce 79% biodiesel during 10h reaction time, whereas CaZrO<sub>3</sub> and CaCeO<sub>3</sub> yields between 95% and 70% of biodiesel in 10 h by using oil to methanol ratio (1:6) at 60°C (Galadima & Muraza 2014). The transesterification of *Nannochloropsis oculata* was done in the presence of Al<sub>2</sub>O<sub>3</sub> and CaO catalysts at 50°C to give 97.5% oil conversion

(Umdu *et.al.* 2009). In another experiment, transesterification of algae carried in the presence of titania, alumina, and zirconia catalysts at 350-400°C and 2500 psi (17.23 MPa), giving 90.2% oil conversion (McNeff *et.al.* 2008). *C. protothecoides* was transesterified in the presence of 75% lipase (from *Candida* sp.) and methanol at 38°C, with an oil conversion of 98.15% after 12 h (Cheng *et.al.* 2009) shown in Table 2.

Table 2. Transition Elements as catalysts

Mix Oxides	Biodiesel Yield	References
CaTiO <sub>3</sub>	79% during 10h reaction time	Galadima & Muraza 2014
CaZrO <sub>3</sub>	95% in 10h by using oil to methanol ratio (1:6) at 60°C	Galadima & Muraza 2014
CaCeO <sub>3</sub>	70% in 10h by using oil to methanol ratio (1:6) at 60°C	Galadima & Muraza 2014
Al <sub>2</sub> O <sub>3</sub> and CaO	97.5% oil conversion at 50°C	Umdu <i>et.al.</i> 2009
Titania, alumina, and zirconia	90.2% oil conversion at 350-400°C and 2500 psi (17.23 MPa)	McNeff <i>et.al.</i> 2008

### 11.2. In Situ Algal Biomass Transesterification

In situ transesterification is an emerging technique with potential for the reduction of costs and processing units for fuel conversion process. It increases biodiesel production up to 20% when compare with conventional method (Ehimen *et.al.* 2010). In situ transesterification is carried out by addition of methanol/potassium hydroxide solution in dried microalgae samples and then ultrasonicated the mixture. Centrifugation method could be used to separate reacted biomass sample from cell debris, glycerin and excess of methanol. In situ transesterification have higher comparative biodiesel yield and low cost than the conventional route biodiesel production (Harrington & Arcy-Evans, 1985). This method controls process wastes and pollution (Haas *et.al.* 2007).

### 11.3. Simultaneous extraction and transesterification of microalgal lipids

Recent studies investigating biodiesel production from microalgae have focused their efforts on the

development of an alternative downstream processing step termed simultaneous extraction and transesterification (Wahlen *et.al.* 2011). A wet lipid extraction procedure was developed that was capable of extracting 79% of transesterifiable lipids from wet algal biomass (84% moisture) via acid and base hydrolysis (90°C and ambient pressures) (Table 3), and 76% of those extracted lipids were isolated, and converted to FAMES (Sathish, & Sims, 2012). The yield of FAME gained through hexane after microwave-assisted transesterification (EHMT) of lipids in wet microalgae increased 6-fold when compare FAME extracted through chloroform of dried algae in direct transesterification. FAME content attained through EHMT is 86.74% is higher when compare with chloroform-based process is 75.93% (Cheng *et.al.* 2014). Former studies (Wahlen *et.al.* 2011; Patil *et.al.* 2012, 2013) employing microwaves or supercritical fluids for in situ transesterification reveal a conversion yield of 84% with 50 wt. % moisture. Whereas 90.6% yield of biodiesel attained by chloroform with the microalgae containing 65 wt.% moisture which shows solvent with high polarity achieved higher conversion yield as well as have lower production cost of biodiesel from microalgae (Im *et.al.* 2014).

Table 3. Transesterification of wet microalgae for biodiesel production

Method of Transesterification	Organic Solvent	Reaction time	% of water	Assisted	Reactant/Catalyst	Biodiesel yield (%)	References
Direct transesterification	Hexane	90°C for 30 min	84%	Acid and base hydrolysis	Methanol/Sulfuric acid	76%	Sathish & Sims, 2012
Hexane after microwave-assisted transesterification (EHMT)	Hexane	90°C for 30 min	77%	Microwave	Methanol/Sulfuric acid	86.74%	Cheng et al., 2014
Simultaneous Hydrolysis-esterification	Hexane	140°C for 6 h	80%	Microwave	Methanol/Sulfuric acid	90%	Takisawa et al., 2013a
In situ transesterification	n-Hexane	65-95°C for 30-120 min	65%	Immersing the tube in a waterbath	Methanol/Sulfuric acid	91%	Im et al., 2014

## 6. CONCLUSION

Microalgae have several advantages such as high growth rate, high oil content and used as potential candidate for renewable biofuel. However, extraction of lipid from algae feedstock has faced challenge while considering large scale application as well as it is also necessary to make this process cost effective. The potential storage of lipid is hindered by presence of polyunsaturated fatty acid (PUFA) and high moisture content in biomass. After lipid extraction, oil is transesterified into FAME which is then used as biodiesel. In situ transesterification is an emerging technology for cost reduction at large scale but it still needs optimization so that biodiesel quality meets the existing global standards.

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