eISSN: 2229 – 6905

Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Comprehensive review on lipid extraction methods for the production microalgae biofuel

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Abstract

Carbon dioxide emission has become a serious global issue and a lot of research is being carried out to look for environment-friendly and economically viable energy resources. The only alternative that appears to meet revolutionary needs is the utilization of renewable energy. Although various forms of renewable energy are being currently used, the prospects of producing carbon-neutral biofuels from microalgae appear bright because of its eco-friendly nature; non-toxic characteristics, biodegradability and lower net carbon cycle .They have some unique features such as high CO_2-sequestering capability, ability to grow in wastewater/seawater/brackish water and high-lipid productivity. Main benefits in micro algal biofuel technology are the cost-effectiveness and its efficiency in extraction of lipids .The objective of this article is to provide a comprehensive review on various methods of lipid extraction from microalgae available, to discuss the merits and demerits. The article contains about various methods of lipid extraction procedures including solvent extraction procedures, mechanical approaches, and solvent-free procedures apart from some of the latest extraction technologies. Further development is needed in this area for successful implementation of this technology at the large scale production.

Keywords: Lipid extraction, Algae, biofuel

Introduction

As the world's supplies of fossil fuels diminish and greenhouse gas emissions grow to be a pressing issue, nations across the globe are working to provide sources of alternative energy The importance of investigating new options offered by algae cultivation is motivated by the fact that algae are very efficient at converting light, water and carbon dioxide (CO_2) into biomass in a system that does not necessarily require agricultural land. Depending on the concept, the water can be salty and the nutrients can come from waste streams. Depending on the species and cultivation conditions, algae can contain extremely high percentages of lipids or carbohydrates that are easily converted into a whole range of biofuels including biodiesel or bioethanol. Algae- based products can serve as an alternative to a wide range of products that are currently produced from fossil resources or land-based agriculture, but without requiring high quality land and in some cases without requiring fresh water3 , with CO_2 as the only carbon input.

1. Biochemical conversion and thermo chemical conversion

Biofuels which can be derived from oil crops, animal fats, kitchen used oils and algal biomass through biochemical conversion and thermochemical conversion (Kumar *et al*., 2015). From amongst the various biological resources, environmental and economic sustainability algae biomass considered to be rocketed in renewable fuels source of near future. The microalgae are rich in nutrients in that nitrogen, phosphorus and potassium (N, P & K) are the most important macronutrients for their growth and stored as secondary metabolites especially carbohydrates and proteins for the production of high-energy biofuels through fermentation technology. Table-1 represents carbohydrates and proteins

The potentially viable algae strains that can be used to produce copious amount of carbon-neutral important green- bioenergies such as bio-diesel, bio-methane, bioethanol, bio-hydrogen and bio-butanol due to the presence of macromolecules carbohydrates, protein and lipids in there biomass as well as it has most similar properties to that of fossil fuel. The main advantages of microalgal contain macromolecules (lipids and carbohydrates) derived biofuels: (i) atmospheric CO_2 fixation via the process of photosynthesis, (ii) marginal environmental pollution when compared to fossil fuels (iii) produces less harmful gas emissions such as sulfur oxide, (iv) biodiesel is biodegradable and (vi) can be used without modifying existing engines (Rawat *et al*., 2013).The recent methods employed for extraction of fuels from microalgae biomass by biochemical conversion and thermochemical conversion (Farias *et al*., 2007; Larson 2008; Nigam and Singh 2011and Kumar *et al*., 2015).

Table 1. Microalgae metabolites protein and carbohydrates amounts from various microalgae on a dry matter basis (%).

S.NO	Name of the Organism/ group	Protein (% dwt)	Carbohydrate (% dwt)
1	*Anabaena cylindrical* / Cyanophyceae	43-56	25-30
2	*Chlamydomonas rheinhardii* / Chlorophyceae	48	17
3	*Chlorella pyrenoidosa* / Chlorophyceae	57	26
4	*Chlorella vulgaris* / Chlorophyceae	51-58	12-17
5	*Dunaliella bioculata* / Chlorophyceae	49	4
6	*Dunaliella salina* / Chlorophyceae	57	32
7	*Euglena gracilis* /Euglenaceae	39-61	14-18
8	*Porphyridium cruentum* /Porphyridiaceae	28-45	40-57
9	*Prymnesium parvum* / Prymnesiaceae	28-45	25-33
10	*Scenedesmus obliquus* / Chlorophyceae	50–56	10–17
11	*Scenedesmus quadricauda* / Chlorophyceae	47	-
12	*Scenedesmus dimorphus*/ Chlorophyceae	8 -18	21-52
13	*Scenedesmus* sp. / Chlorophyceae	51.14	23.19
14	*Spirogyra* sp. / Chlorophyceae	6-20	33-64
15	*Spirulina maxima* / Cyanophyceae	28-39	13-16
16	*Spirulina platensis* 52 8–14 / Cyanophyceae	52	8-14
17	*Synechoccus* sp. / Cyanophyceae	46-63	15
18	*Tetraselmis maculate* / Cyanophyceae	52	15

Microalgae biomass can be used for production of bio-oil (Li *et al*., 2008), methane (Minowa and Sawayama, 1999), methanol (Hirano *et al*., 1998) and hydrogen (Turner *et al*., 2008). Technologies to derive biofuels from algal biomass can be segregate in to three main divisions such as thermochemical, bio-chemical and physico-chemical. The thermochemical technologies can be subcategorizes into three elementary types of mechanism pyrolysis, gasification and combustion (Huang *et al*., 2011). Thermochemical conversion technologies (TCCTs) is used for conversion of the biomass into energy fuels in the form of solid or liquid or gases in the absence of oxygen at temperatures of 300–650°C (Manara and Zabaniotou 2012) as given in Fig.1.

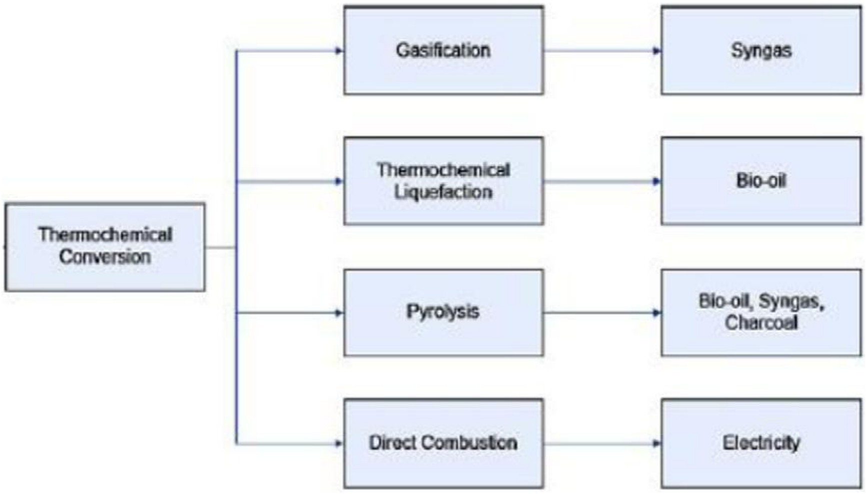

Fig.1 Potential energy products derived by thermal decomposition of microalgal biomass (Rawat *et al*., 2010).

Among the various TCCTs, pyrolysis and gasification are mostly employed for production of fuels from biomass. A primary research goal in biomass thermochemical conversion is directed towards the optimization of these processes to reduce the amount of unwanted byproducts. The thermo-analytical methods provide valuable information on pyrolysis kinetics, but they cannot provide information about the nature of the evolving volatile components (Bahng *et al*., 2009). Thermogravimetric analysis (TGA) is used to study the thermal events of the solid biomass under pyrolysis conditions and reaction kinetics. It presents a computation of weight loss as an objective of time and temperature (Silva *et al*., 2012). The kinetics of these thermal events has been determined by the application of the Arrhenius equation reciprocal to the separate slopes of constant mass degradation. With reference to TGA experimental data, thermal behaviour and kinetic parameters like reaction order, activation energy and pre-exponential factor of the biomass were resolved. These information are necessary for developing the biomass based pyrolytic conversion systems. Many researchers have found and described about the thermal behaviors and pyrolysis kinetics of different species of microalgae such as *S.platensis* and *C. protothecoides* (Peng *et al*., 2001), *D. tertiolecta* (Shuping *et al*., 2010), *C. vulgaris* (Bhola *et al*., 2011) and *Scenedesmus* sp. (Kumar *et al*., 2015). Information available on thermal behaviour and kinetic parameters viz., reaction order, activation energy and pre-exponential factor of different microalgae are very handy in construction of pyrolytic processing system for production of energy.

2. Microalgae for bioethanol production

Bioethanol does not have negative environmental impacts and also has lower carbon dioxide emission that fossil fuel hence it would be an ideal substitute fuel source. Bioethanol has been receiving abundant interest worldwide (Demirbas, 2008). There are two methods commonly involved to produce ethanol are fermentation (Biochemical) and gasification (Thermo chemical). Ethanol has been used for incipient industry of internal combustion engines since 1894 by Germany and France. Ethanol has been utilized by Brazil since 1925. Ethanol produced from microalgal biomass blended (10%) with petrol can be used in vehicles directly without further modification of engine or the requirement for fuel purification. Ethanol as a fuel product has high energy molecules, which lead to theoretical efficiency advantages over gasoline viz: 1) ethanol octane number has higher (108), 2) flammability limits, flammable speed are broader and higher 3) higher compression ratio, 4) higher heats of vaporization than gasoline and 5) shorter burning time and leaner burning engine (Demirbas, 2008).

Bioethanol can produced from various forms of biomass such as corn, sugar cane, sorghum etc. these have many common problems; food and feed security, requirement of large quantity of arable land, seasonal harvesting and high cost of ethanol production etc. A reduction in the cost of ethanol production compared to conventional fermentation can be achieved using low-cost raw material such as non-edible microalgae by a simple and quick fermentation method patented by (Ueda *et al*., 1996).

Nguyen *et al*., (2012) reported that the certain microalgae are ideal candidate for bioethanol production as carbohydrates from microalgae can be extracted to produce fermentable sugars. It has been estimated that approximately 5000–15,000 gal of ethanol/acre/year (46,760–140,290 L/ha) can be produced from microalgae. This yield is several orders of magnitude larger than yields obtained for other feedstock Table.2. Currently extensive work is being focused with great interest on exploring alternative energy sources from microalgae. This has the potential of solving the problems with direct and indirect land use and microalgae biomass potentially sequesters more carbon than alternative fuels (Richardson *et al*., 2010). Goh & Lee, 2010 have reported that microalgae bioethanol represents the world's third alternative in bioethanol generation. Carbohydrates and proteins form major components of microalgae depending on the species. Different microalgae species blended with bacteria and yeast or fungi are able to ferment under anaerobic condition to produce ethanol.

Table. 2 Ethanol yield from different sources (Nguyen *et al*., 2012).

S.No	**Source Ethanol**	**Ethanol yield(gal/acre)**	**Ethanol yield (L/ha)**
1	Corn stover	112–150	1,050–1,400
2	Wheat	277	2,590
3	Cassava	354	3,310
4	Sweet sorghum	326-435	3,050-4,070
5	Corn	370-430	3,460-4,020
6	Sugar beet	536-714	5,010-6,680
7	Switch grass	1,150	10,760
8	Microalgae	5,000-15,000	46,760-140,290

Besides ethanol, carbon dioxide and water are also formed as byproducts. The process of substantial bioethanol production through effective fermentation by the addition of yeast *Saccharomyces uvarum* / *Saccharomyces saravesei* to algae biomass broth was patented (Bush & Hall, 2006) Clostridium sp is also involved in ethanol production. Microalgae have a very good platform for the low-cost, feasible approach of amenable production of bioethanol. During bioethanol purification, CO_2 and water are waste products that can be recycled to microalgae cultivation ponds as nutrients for microalgae growth and thus reduce green house gases emission. In general, according to simplified reaction equation below, theoretical maximum yield is 0.51 kg ethanol and 0.49 kg CO_2 per kg of carbon sugar, glucose (Harun *et al*., 2010).

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2$$

(Hirano *et al*., 1997) confirmed the microalgae *C.vulgaris* are good sources for ethanol production because the biomass contains a huge amount starch (ca. 37% dry wt.), that could be increase the efficiency of conversion to bioethanol and has been recorded up to 60 %. (Ueno *et al*., 1998) found that microalgae *Chlorococcum littorale* achieved maximum ethanol production 450 µmol g_1 dry wt. at 30 ºC via dark fermentation process. Microalgae contain biochemicals as well as carbohydrates. These mixed with volatile fatty acids and yeast / *Zymomonas mobilis* released very low amounts of CO_2 and provides a higher carbon yield of ethanol (Chang *et al*., 2008).It could provide a new platform with versatile applications via an economical process (Chang *et al*., 2010). However, bioethanol production from microalgae is still under investigation due to the technology still being in its early stage and thus has not been commercialized for wide spread uses.

3. Microalgae for biodiesel production

Generally, the primary metabolites such as carbohydrates, proteins and lipids produced as high amount in a short time by microalgae are accumulated within their cells and it is necessary to breakdown the microalgae cells in order to obtain the particular macromolecules for biodiesel production. The chemical reaction of transesterification of triglycerides with alcohol in presence of catalyst is showed in Fig.2. The Free Fatty Acids (FFA) of oil recovered from dried biomass must be studied before choosing the biodiesel production method.

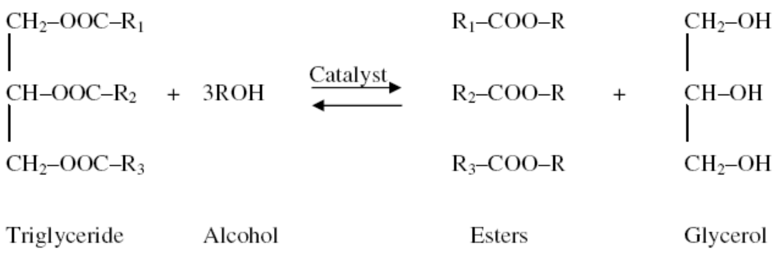

Fig.2. Transesterification of triglycerides (biodiesel) with alcohol

Generally, the microalgal lipids contain higher FFA content, which needs to be neutralized. The FFA amount in the feedstock is main factor to affects the methyl conversion rate, reaction time and biodiesel yield. It is also directly related to cost of the biodiesel production due to pre-treatment of the high FFA raw oil. The conversion efficiency of the traditional methods for biodiesel production (homogeneous acid or base-catalyzed transesterification of oil) is highly dependent on the FFA and water content in the lipids feed stocks (Lopez *et al*., 2005; Freedman *et al*., 1984; Kusdiana and Saka, 2001). High FFA feedstock on base-catalyzed transesterification reaction is leads to saponification results in end product like viscous gel/soap formation. The soaps support the formation of stable emulsions, which prevents the separation of biodiesel from crude glycerin. This reduces ester conversion rate and increases the products separation costs. The oil selected for biodiesel production should not contain more than 1% FFA for the base catalyzed method (Freedman *et al*., 1984). The use of supercritical method for microalgae biodiesel production is also restricted due to process economics and safety concerns related to the reaction conditions (Ehimen *et al*., 2010). Generally, microalgae oil contains higher amount of FFA content (Rodolfi *et al*., 2009). Such type of feedstock needs additional FFA purification or stepwise conversion process for biodiesel production, which may leads to increase in biodiesel price. Developing a technology for continuous production may be the possible solution for reduction of the production cost and hence reduce the overall cost of biodiesel may lead to the price of biodiesel competitive with respect to conventional diesel fuel (Knothe 2005 and Van Gerpen, 2005).

The Mcgyan process is a recently developed technology for continuous biodiesel production from various feedstocks. In this process, transesterification and etherification reactions simultaneously occurred in a continuous fixed-bed reactor filled with a sulfated metal oxide catalyst. The combination of alcohol and lipids are sent into reactor at elevated temperature and pressure to perform these reactions. The reactants are distilled for excess alcohol recovery and residual free fatty acids in biodiesel is removed and returned to the reactor (Um and Kim, 2009). The added advantages of this process are insensitivity to free fatty acid/water content of the feed-stocks and no side reactions for free fatty acids. The Mcgyan process is well suited for conversion of microalgae oil into biodiesel due to its tolerance to elevated FFA levels and its flexibility towards varying feedstock composition (Brian 2012).

Cost for the biodiesel production is combination of two main components viz., feedstock cost and processing cost (Huang G *et al*., 2010). Generally, cost of the feedstock is ranged from 60 to 85% of total biodiesel cost (Haas *et al*., 2007). An estimation made in 2003 indicates that the cost of biofuel production is 2.3 times higher than fossil fuels (Kondili and Kaldellis 2007). Estimated the cost of microalgae Oil should be lower than $ 0.48/L (Chisti 2007). The cost of microalgae biodiesel processing is depending on feedstock cost, process technology used, chemicals, quality of by products and etc. The biodiesel is still too expensive compared to diesel fuel due to feedstock production, processing costs and under-utilization of byproducts. The continuous biodiesel production process is only solution to reduce the overall cost of biodiesel production. An alternate solution to reduce the biodiesel cost is tax discount incentives offered by the government to support the biodiesel producing industries and environment.

4. Microalgae for biohydrogen production

Miandad *et al*., (2016) reviewed that H_2 is the most ideal energy carrier which are abundantly available elements on the earth with highest energy content per unit weight (142 KJ/g) and efficiency of producing electricity. It can be stored in liquid and gas forms and can be converted into different forms of energy. This makes H_2 a promising alternative fuel and future energy carrier (Table.3).

Table.3 Characteristics of H_2 (Miandad *et al*., 2016)

Characteristics	Unit	Values
Boiling point	k	20.3
Liquid density	kg/ m^3	71
Gas density	kg/m	30.08
Heat of vaporization	kJ/kg	444
Lower heating value (mass)	MJ/kg	120
Lower heating value (liquid, volume)	MJ/m^3	8960
Diffusivity in air	cm^2 /s	0.63
Lower flammability	limit vol. % (in air)	4
Upper flammability	limit vol. % (in air)	75
Ignition	temperature (in air)°C	585
Ignition energy	MJ	0.02
Flame velocity	Cm/s	270

Hydrogen can be produce both renewable and non renewable sources however, the past one decade hydrogen productions from renewable sources like microalgal biomass have gained significant attention because hydrogen production as a clean energy from microalgal biomass and that can be achieved through the implementation of various processing technologies including anaerobic digestion, pyrolysis, gasification, catalytic cracking, and enzymatic or chemical transesterification (Rawat *et al*., 2013). Hydrogen production by a unique process has been developed using renewable energy and is one of the popular scenarios from microalgae biomass with consortium of bacteria. This must be subjected to anaerobic fermentation to produce hydrogen and carbon dioxide (Demirbas 2010). Miandad *et al*., (2016) has reported that the mechanism of biological H_2 production was first discovered by Hans Gaffron in the early 1940s, when he found that green algae can either consume H_2as an electron donor in carbon dioxide (CO_2) fixation process or produce H_2 under anaerobic conditions in both dark and light. The photosynthetic cell-factories microalgae have higher capacity to absorb freely abundant solar energy through photosynthetic system I & II and use it to spilt water to produce molecular oxygen of photo-biological production as well as evolved H+ and e- that are combined to produce biosolar hydrogen (Rawat *et al*., 2013and Kruse & Hankamer, 2010). Generally photosynthetic bio-hydrogen production involved into two stage process they are i) an aerobic and ii) an anaerobic stage. These stages was explained by Melis & Happe (2001), the first stage involves microalgae grown photosynthetically (accumulation of carbohydrates) under normal conditions, the second stage under involves fermentation of microalgae (carbohydrates) that are deprived of sulfur and physiological reactions take place after 60 h of fermentation for consistent hydrogen production. Theoretical maximum yields of hydrogen by bio-prospecting green microalgae could be about 198 kg H_2 ha^{-1} day^{-1} in two stage hydrogen production process. During hydrogen production, this process does not produce any toxic or harmful products but can provide value added byproducts from the biomass (Rawat *et al*., 2013 and

Melis & Happe, 2001). Demirbas (2010a) denoted that anaerobic hydrogen production potentially proceeds photofermentatively as well as without the presence of light. Anaerobic bacteria use organic substances as the sole source of electrons and energy, converting them into hydrogen. The reactions involved in hydrogen production (Eqs. (1) and (2)) are rapid and these processes do not require solar radiation.

$$\text{Glucose} + 2H2O \longrightarrow 2\text{Acetate} + 2CO2 + 4H2 \qquad (1)$$

$$\text{Glucose} \longrightarrow \text{Butyrate} + 2CO2 + 2H2 \qquad (2)$$

Renewable, efficient, eco-friendly carbon neutral, alternative energy biohydrogen production has been studied extensively in many microalgae species; the green microalgae *Chlamydomonas reinhardtii* has the remarkable ability to produce hydrogen via hydrolysis of water during illumination (Kruse *et al*., 2010; Melis and Happe 2001), *Chlamydomonas reinhardtii* has the ability to produce biosolar hydrogen (H_2) under anaerobic conditions (Momirlan and Veziroglu 2005; Kruse ., 2005; Melis *et al*., 2000). Some of the cyanobacteria are a good source of hydrogen production. (Block & Melody, 1992) reported that cost of photobiologically produced hydrogen is much less (US$25 m−3) than photovoltaic splitting of water (US$170 m−3). *Scenedesmus obliquus* (Das and Veziroglu 2008), *Chlorococcum littorale* (Hallenbeck and Benemann 2002 ; Hallenbeck *et al*. 2009), *Chlorella fusca* (Gaffron and Rubin 1942), *Anabaena* sp., *Oscillatoria* sp., *Calothrix* sp., *Synechococcus* sp., *Gloeobacter* sp. (Melis and Happe 2001), *Anabaena cylindrical* (Schulz 1996 ; Melis and Happe 2001), *Anabaena variabilis* (Benemann 1997 and Ni *et al*. 2006), etc.Microalgae feedstocks production of hydrogen is one of the alternative promising renewable fuels for transportation and domestic application. However with improving novel discoveries, future work should be perceived on potentials and the possibility of achieving economic production of highlighted microalgae hydrogen gas. Therefore bio hydrogen fuel would be deemed as low-cost fuel for the future whilst being less polluting than the fossil fuel based economy.

5. Microalgae for bio methane production

Biomethane production using fermentation technology has been carried out for more than 50 years (Mussgnug *et al.*, 2010). The astonishing biodiversity of microbial world has rendered some biomass the capacity to produce biomethane action primarily and smaller quantities of carbon dioxide from biogas productions (Pöschl *et al.*, 2010). For an extended period of time, many projects have been carried out extensively on selection of promising microalgal species for biomethane production there are; microalgae sludge (*Chlorella–Scenedesmus*) (Golueke *et al*., 1957), *Spirulina maxima* (Samson and Leduy,1982 and 1986), *Chlorella vulgaris* (Sanchez and Travieso, 1993), *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorella kessleri*, *Dunaliella salina* and *Euglena gracilis* (Mussgnug *et al*.,2010). The microalgae have the potential for superior quality methane production by anaerobic digestion because the cell wall is fats degrading protein with little cellulose and almost no lignin (Mussgnug *et al*.,2010) . Therefore, a the process of anaerobic digestion is stable and efficient (Vergaraf Fernandez *et al.*, 2008). Methane production has received much attention recently as there is potential to recover high volume energy from the microalgal biomass post lipid extraction (Vergaraf Fernandez *et al.*, 2008). The biogas produced from microalgal biomass consists of two major gases:- methane (55-75 %) and -carbon dioxide (25 – 45 %), with trace amounts of other gases such as hydrogen sulphide very low (below the standard limit) quantities (Sialve *et al*., 2009). Table 4. represents % of methane produced from different microalgae. For utilization in the biomass production process and supplied to the grid Methane yield can be increased by long solid retention times and high organic loading in reactors (Chynoweth, 2005). However, economical methane production needs few more research in order elucidate the affects of organic loading ,retention time, pH, temperature and necessary characteristics for purification of of the biogas before feasible biomethane production can be considered at large scale.

Table 4 **Theoretical methane productions from various microalgae species of the total biomass (Singh *et al*., 2011).**

S.No	Microalgal species	CH_4 (L g-1 VS)
1	*Euglena gracilis*	0.53–0.8
2	*Chlamydomonas Reinhardtii*	0.69
3	*Chlorella Pyrenoidosa*	0.8
4	*Chlorella vulgaris*	0.63–0.79
5	*Dunaliella salina*	0.68
6	*Spirulina maxima*	0.63–0.74
7	*Spirulina platensis*	0.47–0.69
8	*Scenedesmus obliquus*	0.59–0.69

6. Hydrothermal liquefaction (HTL), Pyrolysis, Gasification

There are many methods used for biofuel production such as chemical, biochemical and thermochemical from microalgal biomass. However, thermochemical conversion technologies getting a promising route for biofuel production. Fig.3 represents the various thermochemical conversion technologies for biofuel production from microalgae, classifying them according to the desired primary product and the water content of the feedstock (Barreiro *et al*., 2013). Thermochemical processes for wet biomass, such as hydrothermal gasification, hydrothermal liquefaction or hydrothermal carbonization appear to be more suitable for microalgae feedstock.

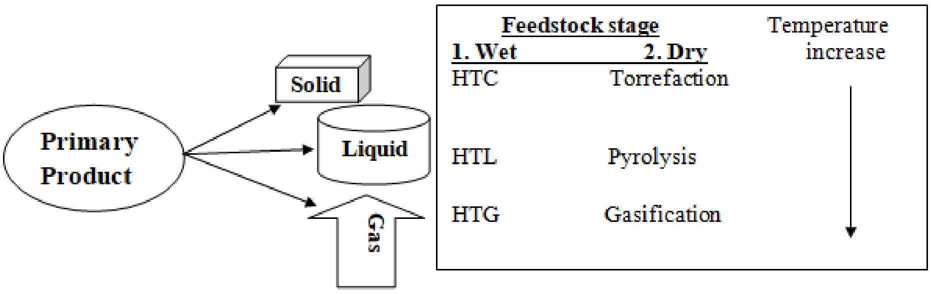

Fig. 3 Thermochemical conversion processes for microalgal feedstock source adapted form (Barreiro et al., 2013). HTC (hydrothermal carbonization); HTL (hydrothermal liquefaction); HTG (hydrothermal gasification).

Conversion of biofuels from microalgae using hydrothermal liquefaction (HTL) is not new method which has been using since 1994 (Dote *et al*., 1994). HTL also called hydrous pyrolysis, is a process for the reduction of complex organic materials such as bio - waste or biomass into crude oil and other chemicals and also it is an approach that requires no drying because the whole microalgae biomass is decomposed and converted in hot compressed water. Biocrude oil is obtained as the main product, next to gaseous, aqueous and solid by-products. Microalgal biomass used to produce bio-fuels. Bio-oil or bio-crude, bio-char, water extractives, and bio-gas are the major products from algal liquefaction process, usually can be achieved from 200–400° C within 10–60 min (Miao *et al*., 2012). As conducted many experiment and mentioned in publications, reaction temperature (250-350°C), pressure (5-15 Mpa), residence time, biomass/ water ratio play a significant role in the quantity and quality of bio-oil. Bio-oil liquefied. Due to existence of micro algae complex biomolecules break down and resolve into mixture of compounds that divides into self separating bio oil phase when condition return into medium temperature and pressure.

HTL has been examined with a broad range of microalgal species like *Dunaliella tertiolecta* (Zou *et al*., 2010), *Chaetomorpha linum* (Aresta *et al*., 2005), *Spirulina* (Jena et *al*., 2011; Vardon *et al*., 2011), *Chlorella* (Biller *et al*., 2013), *Nannochloropsis* (Brown *et al*., 2010), Detailed multi-method characterization demonstrates that feedstock organic content and nutritional composition greatly affect HTL biocrude oil yields and chemistry, despite having similar bulk elemental distributions. The feedstock nutritional profile was reflected in the heteroatom content, type of compounds, and functionality observed in the resulting HTL biocrude oils. However, the tie between feedstock nutritional profile and HTL biocrude molecular makeup emphasizes the need for feedstock characterization and selection based on the intended downstream application. The molecular-level information gained from complementary methods can also help researchers design functional group-specific chemical strategies and processes to further reduce heteroatom content and improve HTL biocrude properties.

7. Microalgae cultivation and condition

Large scale microalgal production has a number of challenges that need to be overcome in order to make it commercially viable. These include strain selection, maintaining culture integrity, nutrient supply, photosynthetic activity, and gaseous exchange (Brennan & Owende, 2010). Open ponds are able to culture only certain species of microalgae (Richmond, 2004). Microalgae that grow under extreme conditions (high pH, nutrient level, etc.) provide a competitive advantage thus limiting contamination by other microalgae. Contamination is however inevitable and requires constant propagation of seed culture in order to maintain the culture of choice as the dominant culture (Day *et al.*, 2012). Contamination by non-target microalgae is only regarded as problematic should the contaminating species have a trait that is not desired , have a negative impact on the culture or be capable of outgrowing the culture of choice. Increased control of the growth environment is can effectively reduce contamination but increases the cost. Biofouling becomes a possibility if the microalgae adhere to the walls of the bioreactor. This effectively increases shading thereby reducing productivity. Biofouling can also impede culture flow, requiring more energy and thus increasing the cost of production (Day *et al.*, 2012). Supply of photosynthetically active radiation (PAR) becomes a limiting factor in dense cultures above specific concentrations in both open and closed systems thus reducing productivity. Supply of carbon dioxide is essential for the prevention of carbon limitation. Despite ambient air containing CO_2 that is sufficient for normal microalgae growth, CO_2 needs to be dissolved in the culture medium for uptake. Less than 10 % of the CO_2 resources in nature are available to the microalgae for uptake. Bubbling of air is not an effective delivery system for open ponds due to short residence time (Mata *et al.*, 2010). Optimization of bubbling technology for dissolving of CO_2 remains an engineering challenge. Removal of oxygen is imperative for the prevention photo-oxidative stress in photobioreactors. Oxygen above atmospheric concentration inhibits photosynthesis. This problem is usually remedied by sparging of air through the reactor or a section of the reactor in order to strip away excess oxygen. This increases energy consumption and thus cost (Christenson & Sims, 2011).

Commercial operations have produced monocultures of *Spirulina* and *Dunaliella*, with little or no inoculum production being required, due to the use of selective growth media (alkaline or saline). Such extreme environments do not allow high productivity and suggest that “extremophiles” are not a good target for future research (Lundquist *et al*., 2010).

New methods can be implemented for production for substantial mass cultivation of micro algae at large scale. Grobbelaar (2009) reported that to produce micro algal biofuels in economical manner, the micro algal culture‘s growth will have to be uninterrupted for continuous period and without the demand for re- inoculation. In context to that many open ponds are utilised for mass micro algal culture which yield huge monetary products (eg. Nutraceuticals and pharmaceuticals products) and uses of water treatment , which make feasible growth medium for microalgae, to produce many by-products especially automobile fuels has unrealized potential .the factors which will be affected the liner type , mixing methods, flux gas, water and nutrients, harvesting methods and processing techniques ,wastewater treatment and mixing et, are some other factors involved. In context to such issues it is better to understand in beginning stage how these economical factors can curb the arrangement and engineering of microalgae oil production plant and its component systems. Apart from the cost, the energy inputs processes (embedded energy in the system and required operational energy for the process) need to studied and the total green house emission correlated with algal fuel production.

Supplementary factors that should be taken are: Localisation, with respect to climatic change, water resources, availability of waste water treatment (which act as sources for nutrients and water), sources of CO_2 and issues dealing with land and biodiversity factors involved. The prospective of by-products (Nutraceuticals, pharmaceutical, pigments etc) to produce microalgal biofuel production technology that bring economically viable transport oil. To develop a proper system that requires structured engineering design and economically modelled system and also need a proper infrastructure for detailed analysis and experimentation with component technologies for oil extraction. The possibility for integration with other industries should take into consideration, and treasured by-products from the system, process should be investigated in order to enhance the overall beneficial aspects of the plants. With respect to the cost, two other important factors should be seen in the engineering design; energy balance and GHG emissions.

Research has been conducted around the world to find the best strains of microalgae for different climates/seasons, to develop the best growth medium, to develop the best pond structure, and to test harvesting and separation technologies (Sheehan *et al*., 1998). Recently, Chisti (2007) discussed the production potential of micro microalgae and the area necessary to replace 50% of all US transportation fuels. Chisti (2007) showed how minute those areas are when compared to current sources of biodiesel. He also addressed various oil-content levels based on different strains of microalgae and the variations within each strain. Huntley and Redalje (2007) estimated a cost of $84/barrel of oil, based on raceway costs of $74,782 per hectare and photobioreactor costs of $197,000 per hectare. This assumed that 80% of the production facility area was for raceways while the remaining 20% was for photobioreactors. Huntley and Redalje (2007) concluded that a 75% reduction in photobioreactor costs would put costs at $51/barrel. They also reported updates on the growth patterns of the microalgae depending on climatic conditions.

8. Conclusion

There are various aspects of micro-algae production which influence the future feasibility of algal fuel production: the energy and carbon balance, environmental factors and cost of production. Every aspects of micro-algae production have been presented. A positive energy balance is required for implementation of technological advances and highly optimized production systems. The remission of environmental impacts, and in particular water management, gives both challenges and opportunities, many issues can be resolved. Proper planning is needed which require an empirical data on performance of system which has been designed for production of biofuels. The algal diversity is so vast that it can be useful for the discovery of new products through new applications. With advancements in cultivation techniques and increasing in algal production it would be found that biofuels have a role to play. It is quite possible that many of the challenges identified are being addressed, but that the information about how this is being achieved is yet to make it into the public domain

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