Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 4, Issue, 12, pp. 2685-2693, December, 2014

Full Length Research Article

PRODUCTION OF BIODIESEL FROM THE ALGAE: STATE OF THE ART REVIEW

[1*]Amrik Singh, [1]Maji, S. and [2]Kumar Gaurav

[1]Mechanical Engineering Department Delhi Technological University, Delhi, India
[2]Department of Biotechnology, Delhi Technological University, Delhi, India

ARTICLE INFO

Article History:
Received 11th September, 2014
Received in revised form
30th October, 2014
Accepted 04th November, 2014
Published online 27th December, 2014

Key words:
Biodiesel,
Open pond,
Microalgae,
Photo bioreactor,
Lipid extraction

ABSTRACT

Production of biodiesel from microalgae is a rapidly growing field of research. The coming global oil crisis implies that alternatives to the diesel must be developed. Biodiesel can be produced from vegetable oils, plants like Jatropha and Sunflower etc. Studies shows that algae are the promising source of biodiesel production. Photosynthetic efficiency of microalgae is much higher than other plants and can be produced in non-arable land. Microalgae species have the potential to accumulate lipids at more than 50% of their biomass. This state of the art report presents the overview of the cultivation, harvesting and oil extraction techniques.

INTRODUCTION

Biodiesel is a renewable fuel which can displace crude oil based fuels. With increased demand of energy conventional fossil fuel reserves start to dwindle. Biodiesel have attracted the most attention during recent years as it has emerged as a potential alternative fuel. One of the biggest advantage of this fuel is that it can directly be used in existing diesel engines without any further modifications and can also be blended in a suitable ratio with petro diesel (Khan *et al*., 2009). The various sources include vegetable oils (Schuchardt *et al*., 1998 and Al-Widyan and Al-Shyoukh, 2002), animal fats (Mata *et al*., 2011) and algae (Radmer, 1994; Ranganathan *et al*., 2008 and Nayak *et al*., 2011). The production of biodiesel from oil crops in large quantities is not sustainable. So researchers have turned their focus on microalgae. Microalgae are sunlight driven cell factories that convert CO_2 to potential biofuels, foods, feeds and high value bioactive compounds (Chisti, 2007). Many microalgae are exceedingly rich in oil. Oil content of some microalgae exceeds 80% of the dry weight of algae biomass (Chisti, 2007 and Banerjee *et al*., 2002). This oil can be easily processed into biodiesel, jet fuel or other chemicals (Sudhakar and Premalatha, 2012).

Corresponding author: Amrik Singh
Mechanical Engineering Department Delhi Technological University, Delhi, India

Potential Sources of Biodiesel

There are more than 350 biodiesel feed-stocks (Atabani *et al*., 2012). The wide range of feedstock must full fill the main requirement: Large production scale and low production cost. The main feed stocks of biodiesel are edible oils, Non edible oils, animal fats and other sources as shown in table 1. The oil yield and land occupied are important parameters to consider any feedstock as biodiesel source. First generation bio-fuels are biofuels made from sugar, starch, and vegetable oils. Edible oil resources are considered as first generation of biodiesel. However edible oil resources such as rapeseed , soybeans etc use much of arable land for cultivation and give rise to food versus fuel crises and created many environmental problems such as deforestation and destruction of various vital soil resources. So non edible oil resources are gaining more attention as they are easily available. The main sources of non edible oil is shown in Table 1. The non edible oils and animal fats are regarded as second generation of biodiesel feedstock. But second generation feedstock are not enough to satisfy the energy demand and is reported the biodiesel produced from

Table 1. Feedstock of biodiesel (Atabani *et al*., 2012)

Edible oils Soybeans	Non-edible oils Jatropha curcas	Animal fats Pork lard	Other sources Bacteria
Rapeseed	Mahua	Beef tallow	Algae (Cyanobacteria)
Safflower	Pongamia	Poultry fat	Microalgae
Rice bran oil	Camelina	Fish oil	Tarpenes
Barley	Cotton seed	Chicken fat	Poplar
Sesame	Karanja		Switchgrass
Groundnut	Cumaru		Fungi
Sorghum	Neem		
Wheat	Tobacco seed		
Corn			
Coconut			
Canola			
Peanut			
Palm			
Sunflower			

vegetable oil has very poor performance in cold weather (Atabani *et al*., 2012). Recently, microalgae have received wide attention and emerged as third generation of biodiesel feedstock. Due to photosynthesis microorganism of algae convert sunlight, water and CO_2 to sugar more efficiently than other crops (Atabani *et al*., 2012). It is very popular because of high photosynthetic efficiency and high yield of oil compared to other feedstock Table 2. The oil produced by microalgae is 25 times higher than the palm oil and 250 times higher than the soybean (Atabani *et al*., 2012) shown in table 2. As compared to edible resources algae has shorter harvest time and they can double there mass every 24 hours.

Table 2. Percentage of yield and oil content in different Feedstocks (Atabani *et al*., 2012)

Feedstocks	Oil content (%)	Oil yield (L/ha/year)
Microalgae (high oil content)	70	136,900
Palm oil	30–60	5950
Calophyllum inophyllum L.	65	4680
Coconut	63–65	2689
Euphorbia lathyris L.	48	1500–2500[a]
Pongamia pinnata (karanja)	27–39	225–2250[a]
Jatropha	50-60	1982
Jojoba	45–50	1818
Castor	53	1413
Olive oil	45–70	1212
Rapeseed	38–46	1190
Peanut oil	45–55	1059
Sunflower	25–35	952
Tung	16–18	940
Rice bran	15–23	828
Sesame	–	696
Soybean	15–20	446
Cottonseed	18–25	325
Corn (Germ)	48	172

[a] (kg oil/ha).

Cultivation of Microalgae

Microalgae with high oil content are required for producing biodiesel. Microalgal biodiesel can be produced commercially. Adequate amount of sunlight, CO_2, H_2O and inorganic salts are required to grow microalgae. Essential elements for its growth are N, P and Fe. Nutritional requirement of microalgae can be estimated using approximate molecular formula of microalgal biomass; ($CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$) which is based on data presented by Grobbelaar (Sudhakar and Premalatha, 2012). Generally continuous culture during daylight is used for large scale production of microalgae. In this method of operation, fresh culture medium is fed at a constant rate and the same quantity of microalgal broth is withdrawn continuously (Grima Molina *et al*., 1999). Feeding ceases during the night, but the mixing of broth must continue to prevent settling of biomass (Grima Molina *et al*., 1999). Figure 1 showing the culture harvesting, and biodiesel production process. Practically the methods used for large scale microalgae production are Raceway ponds (Grima Molina *et al*., 1999 and Terry Kenneth and Raymond Lawrence, 1985) and tubular photobioreactors (Grima Molina *et al*., 1999 and Tredici, 1999).

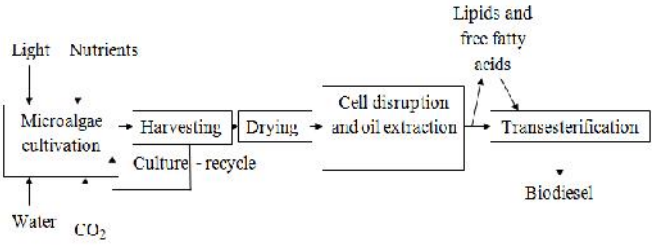

Fig. 1. Process showing cultivation, harvesting and biodiesel production from microalgae

Open Pond System

Open pond system is used widely nowadays because it is easier to construct and has low economic as well as operational cost. As algae growth depends upon sunlight that is freely available in open pond system. Open system comprises lakes, ponds and raceway ponds. Open pond system is widely used but still they depend upon prevailing climatic conditions (Cloudy days, Change of season and Rainfall). Furthermore in open pond system predators attack effect the production.

Raceway Ponds

Raceway ponds are made in concrete and have closed loop recirculation channel that is about 0.3m deep. Algae broth and nutrient mixing is produced by paddlewheel. Behind paddlewheel on completion of the loop algae broth is harvested. To prevent sedimentation paddlewheel operates all the time as shown in Figure 2.

Photobioreactor: Some species of algae do not grow in open system, so closed system which is free from contaminants such as heavy metals and microorganisms. The basic designs are Tubular photobioreactors, Flat bioreactor and Column

photobioreactor. Tubular photobioreactor is generally made of transparent plastic or glass. Photobioreactor can be vertical, horizontal, inclined or conical shaped. Pump is used to circulate algae through the tube (Fig.3). Solar collector is used to collect the sunlight. The diameter of solar collector tube is less than or equal to 0.1m to ensure light productivity. Diameter is limited because light do not penetrate too deeply in the dense culture. Microalgae is circulated from degassing column to solar collector and back to degassing column. Tubes are arranged horizontally or in the form of helix and are oriented from north to south. The ground is covered with white sheet of plastic (Tredici, 1999) to increase reflectance. To prevent sedimentation in tubes the flow is maintained as highly turbulent. The mixing is done by mechanical pump or airlift pump. Mechanical pumps can damage the biomass (Chisti, 1999a; García Camacho *et al*., 201 and García Camacho *et al*., 2007), but are easy to operate and handle.

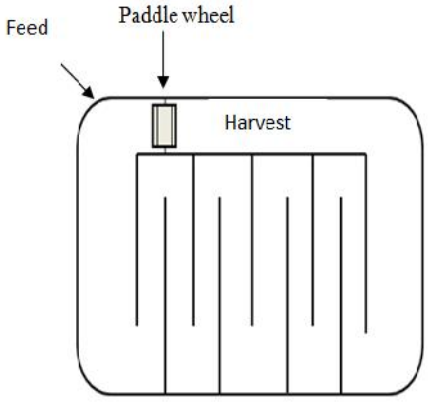

Fig. 2. View of a raceway pond

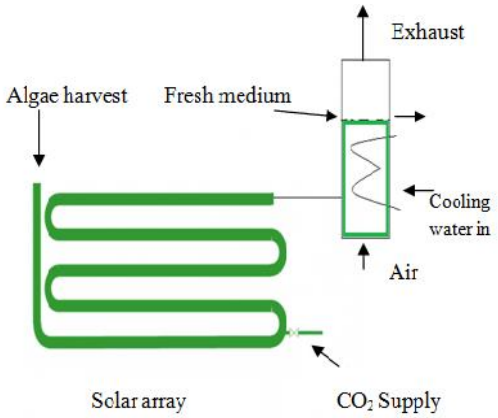

Fig.3. Tubular photobioreactor

Harvesting

The cost of biomass recovery from the broth can make upto 20 – 30% of the total cost of producing the biomass. Microalgal biomass can be harvested physically, chemically or biologically. Various combination of these methods are also common (Bernhardt and Clasen, 1991; Danquah *et al*., 2009 and Kumar *et al*., 1981). There is no proven single best method of harvesting microalgae (Shelf *et al*., 1984).

Centrifugation

Separation of algal cells from medium is a difficult task because of smallest size of the algal cell. Mixtures are separated by spinning in a centrifuge. Centrifugation equipment are either fixed wall devices (hydroclone) or rotating wall devices (sedimenting centrifuges) (Shelf *et al*., 1984). The general types of centrifuges used are multi chamber centrifuge, Disc – Stack Centrifuge and Decanters. Nozzle type of centrifuges are easily sterilized and cleaned but have high operating cost (Shelf *et al*., 1984). In multi chamber centrifuge the feed is passed through vertical concentric cylinders rotating at high speed. Larger particles deposit at the inner chamber and finer particles at the outer chamber (Mohn, 1980b). Disc stack centrifuge consist of disc stack plate, liquid is collected at the top and solids are removed under gravitation (Shelf *et al*., 1984 and Oswald, 1988b). Decanter centrifuge have screw conveyor (rotating at slow speed) fixed with solid cylindrical bowl (rotating at high speed).

Filtration

Filtration is method of harvesting suitable only for large size microalgae. The different type of filtration process available are Tangential Flow filtration, Vaccum filtration, Dead end filtration, Ultrafiltration, Microfiltration and Pressure filtration. Generally broth is passed through the filters. It allows the medium to pass and accumulate the algae. Small size microalgae is also harvested by using diaphragm filter (Borowitzka Michael and Borowitzka Lesley, 1988). Microalgae having coarse particles are harvested by vaccum band filters. Sand filters cannot operate continuously because after some interval of time they have to be rinsed off. Sand filters that are modified can be cleaned continuously by mammoth pump (Borowitzka Michael and Borowitzka Lesley, 1988). Sedimentation is the tendency of the particle to settle down the fluid under the gravity. Benefits of sedimentation are low manpower requirement, low design cost, low power requirement.

Floatation

In floatation method algae is made to float on the surface of the medium and removed as scum. The floatation methods that are generally used are Froth floatation, Dissolved air floatation, Bio floatation. In froth floatation method air is bubbled through column to create froth, so that algae is accumulated above the media. Smaller the air bubble, higher is the floatation efficiency. Because of lower buoyancy in froth floatation bubble size is large, making it less efficient. The stability of air microalgae mixture is governed by the pH of the culture. The pH required depends on different species of algae. This is not suitable because of high cost and lower floatation efficiency. In dissolved air floatation algae is harvested by passing stream of air bubbles. Adhesion of particles to bubbles is required, so chemical flocculants are used. The process is efficient if micro fine bubbles are introduced but this increases the cost. Use of floatation method is limited due to limited evidence of economic and technical viability (Brennan and Owende, 2010).

Flocculation

Harvesting of the algae depends upon the effective particle size. Chemical flocculants are added to increase the particle size so that another method like floatation and filtration are used to harvest the algae. In suspension microalgae have

negative charge that prevents them from self aggregation. Addition of flocculants reduces or neutralizes the negative surface charge. To flocculate the cells electrolytes and synthetic polymers are added (Bernhardt and Clasen, 1991). For charge neutralization ferric cations, aluminium sulphate are often used because they have +3 charge. Natural polymer and cationic starch are also used as flocculants (Pal *et al*., 2005). Adding cationic polymers and multivalent cations as flocculants, surface charge can be reduced or neutralized (Grima Molina *et al*., 2003). Flocculants should be non toxic and inexpensive.

Auto Flocculation

The pH of water increases due to photosynthesis in microalgae. Phosphate, calcium and magnesium ions are precipitated with increase in pH of water (Becker, 1994). These salts have positive charge so can neutralize the negative charge on the microalgae and flocculate the algae. For auto flocculation pH should be above 10.

Method for extraction of lipids

Microalgae generally include polar lipids and neutral lipids. These lipids must be extracted from the cell and collected. Table 4 represents biomass composition of microalgae on a dry matter basis. The cell disruption can be achieved by chemical and mechanical disruption, sonication, super critical fluid extraction, solvent extraction and combination of these techniques (Lee *et al*., 2010; DOE, 2010 and Mercer and Armenta, 2010).

Mechanical cell disruption method

Mechanical disruption is utilized at large processing, homogenization and bead milling (Mercer and Armenta, 2010 and Greenwell *et al*., 2009). Mechanical disruption maintains the chemical integrity of the substance and contamination from the external sources is minimized. In mechanical pressing high pressure on the microalgae is applied which rupture cell wall and oil is extracted. Mechanical pressing is used to extract oil from plant seeds but this method can be applied to microalgae (Mercer and Armenta, 2010).

Table 3. Biomass composition of microalgae on a dry matter basis

Strain	Lipid
Scenedesmus dimorphus	16-40
Prymnesium parvum	22-39
Botryococcus braunii	33
Chlorella vulgaris	10-22
Spirogyra sp.	11-21
Chlamydomonas rheinhardii	21
Euglena gracilis	14-20
Scenedesmus obliquus	12-14
Porphyridium cruentum	9-14
Dunaliella tertiolecta	11
Synechoccus sp.	11
Spirulina platensis	4-11
Dunaliella bioculata	8
Spirulina maxima	6-7
Anabaena cylindrica	4–7
Dunaliella salina	6
Tetraselmis maculata	3
Chlorella pyrenoidosa	2
Scenedesmus quadricauda	1.9

In homogenization cell wall is ruptured by forcing the biomass to pass through small orifice at high pressure. At the opening of the orifice large pressure drop and liquid shear forces rupture the cells allowing the lipids to be extracted (Greenwell *et al*., 2009). Bead milling is packed with very small beads which agitates the algal biomass resulting in cell disruption. This technique is used for size reduction and for the disruption of cells (Doucha and Livansky, 2008). Mechanical force of the beads pulverizes the algal cells and helps in extracting the lipids (DOE, 2010). Degree of disruption depends upon the contact between beads and biomass, size, shape and strength of microalgal cell walls. For the extraction of lipids most effective and simplest method is use of microwave by (Lee *et al*., 2010) but this technique is very difficult to use for industrial scale (DOE, 2010).

Solvent extraction method

This technique is very effective for lipid extraction from vegetable seed as well as from microalgae (Russin *et al*., 2010). This is because of high solubility of lipids in organic solvents such as hexane, chloroform, petroleum ether and benzene (Ahmad *et al*., 2011). To extract lipids from microalgae, five step mechanism is proposed by (Halim *et al*., 2011) shown in Fig. 4. 1: The solvent enters the cytoplasm by penetrating the cell membrane 2: The non polar lipids interact with the non polar solvent, as both are non polar there exist vander wall forces.3: Non polar solvent and lipid form a complex. 4: This complex diffuses across the cell membrane. 5: Organic solvent lipid complex diffuses through the static film into the bulk solvent

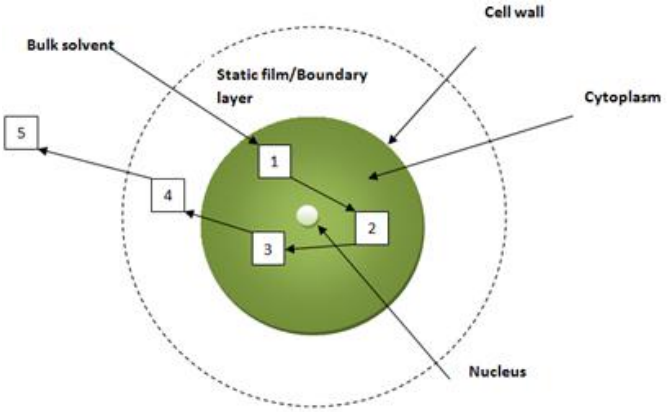

Fig. 4. Schemetic diagram shows mechanism for lipid extraction

Number of techniques exists for extraction such as Bligh and Dyer, Folch extraction and Gold Fisch (Bligh and Dyer, 1959; Folch *et al*., 1957 and Gloria *et al*., 1985). The Bligh and Dyer technique uses chloroform - methanol solvent to extract lipids from fish tissue. Bligh and Dyer method is modified for the extraction of lipid from the algae and found to recover 70% of total lipids (Enssani, 1987). To obtain optimum result biomass should be dried. If the water is not removed it interfere by shielding lipids from solvent (DOE, 2010; Halim *et al*., 201 Converti *et al*., 2009 and Young *et al*., 2010). But drying of biomass requires large energy (Lardon *et al*., 2009). In contrast ideal extraction method should be safe, intoxic, inexpensive and have low energy requirement. In industries, alkanes and short chain alcohol are commonly used for lipid extraction. Hexane an ideal solvent to extract edible oil, is an

alkane hydrocarbon with chemical formula C_6H_{14}. It is non reactive and non polar. Hexane solvent extract a fraction of lipids available in algae depending on the lipids present in biomass (Halim *et al*., 2011). Research efforts are being made to determine effective solvent capable of removing maximum lipids from algae (Lewis *et al*., 2000 and Dufreche *et al*., 2007).

Supercritical fluid extraction method

Now-a-days study is focused on supercritical extraction of lipids because of shortcoming associated with organic solvent based lipid extraction method (Carrapiso and Garcia, 2000). This method typically uses carbon dioxide. When CO_2 heated beyond critical pressure and temperature, to its supercritical state it exhibits the characteristic of both liquid and gas, making it more diffusive with low viscosity (Herrero *et al*., 2006 and Gong and Jiang, 2011). Because of this the CO_2 penetrate solids and extract the target molecules. CO_2 is used as supercritical fluid because of relatively low critical temperature (31.1^0C) and pressure (72.9 atm) (Herrero *et al*., 2006 and Mercer and Armenta, 2010). Extraction efficiency of supercritical CO_2 is affected by four main factors i.e. Temperature, pressure, extraction time and CO_2 flow rate. Use of modifiers (ethanol as a co - solvent) can increase the polarity of the extracting solvent, subsequently viscosity of fluid is altered. Use of CO_2 for the extraction of algae lipid have numerous advantages. Lipid extraction by solvent requires large volume of toxic and hazardous compounds and organic solvents are not required (Carrapiso and Garcia, 2000). Supercritical extraction method uses only CO_2 as extracting solvent (Carrapiso and Garcia, 2000 and Halim *et al*., 2011). CO_2 can be easily removed as it act as a gas at ambient condition leaving behind a pure extract (Mercer and Armenta, 2010).

Ultrasonic extraction

In many species of algae, cells are resistant to solvent extraction, requiring pre treatment to degrade the cells to remove lipids (Grima Molina *et al*., 2003; Lee *et al*., 2010; Gouveia and Oliveira, 2008 and Singh and Gu, 2010). After cell degradation lipid yield increases (Lee *et al*., 2010; Converti *et al*., 2009 and Widjaja *et al*., 2009). So ultrasonic cell disruption method is used. Ultrasonic waves are applied with high frequency and energy intensity to a solution containing cell culture. These waves creates cavitation bubbles in a solvent material, when these bubbles collapse it creates small regions of extremely high pressure which shear cell walls apart (Chaplin, 2004). It is an effective method of destroying cell walls at laboratory scale (U.S. Department of Energy, 2009). This method requires a large amount of energy per unit volume. So it is not practically possible for industrial scale method of cell distruption (Chisti and Moo-Young, 1986).

Soxhlet extraction

Soxhlet extraction is oldest approach widely used for extraction of solid samples. It consist of a simple distillation process repeated a number of times. In this approach fresh solvent is always in contact with the sample phase which enhances the displacement of target compound from the matrix. The extraction procedure is divided in to three stages boiling, refluxing and recovery. The advantage of soxhlet extaction method is that the compounds are not discomposed due to moderate extraction condition (Lee *et al*., 2010).

Biodiesel production

Once the lipids are extracted they must be converted to biodiesel. Biodiesel production from any parent oil is performed via a chemical conversion process known as transesterification (esterification for FFA). The oils which are originally in the form of Triglycerides, complex lipids or FFA are converted to alkyl esters, which has physical and chemical resemblance with petroleum base diesel (Meher *et al*., 2006 and Vyas *et al*., 2010). Transesterification can be performed by reacting lipids with alcohol with or without the presence of catalyst (Demirbas, 2006 and Huang *et al*., 2010). The various steps followed in transesterification are: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol (Chisti, 2007). R is a mixture of fatty acid chain. Alcohal generally used for transesterification is methanol as it is cheap. Ethanol and Iso-Propanol may also be used, results in biodiesel with better fuel properties.

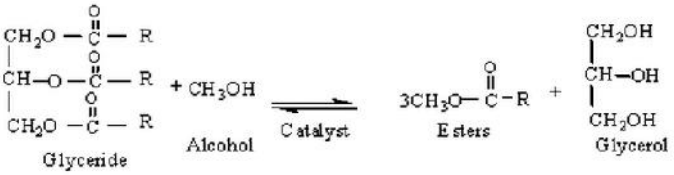

Fig. 5. Transesterification of oil to Esters

The Catalyst used for transesterification process can be alkali catalysts, acid Catalyst, enzyme and heterogeneous catalyst. The basic catalyst commonly used include sodium hydroxide (NaOH), potassium hydroxide (KOH), Potassium methoxide ($KOCH_3$), and sodium methoxide ($NaOCH_3$) (Singh *ET AL*., 2006). The acid catalyst commonly used are hydrochloric acid, sulphuric acid and sulphonic acid. The catalyst whether they are alkaline or acidic, they all are homogeneous catalyst which mean the reactants and catalyst are in the same phase. In general, acid catalyzed transesterification is performed at low to moderate temperature and pressure. Acid catalyzed reaction require high acid catalyst concentration to obtain the beast product yields with reasonable reaction time. Acid catalyzed transesterification for sunflower oil is carried at low temperature and pressure, 200:1 molar ratio under these conditions reaction takes 4h to complete (Marinkovic Siler and Tomasevic, 1998). Acid catalyst is generally used because of its insensitiveness to free fatty acid in the feed stock.

But they are largely ignored because of the relatively slow reaction rate. Concentration of free fatty acid can change according to type and quality of lipid feedstock used (Meher *et al*., 2006 and Canakc and Van Gerpen, 2003). Fatty acids react differently depending on acid or base is used as catalyst (Marchetti *et al*., 2007). In alkali-catalyzed transesterification alkalis such as potassium and sodium hydroxide are the two types of most commonly used catalyst at a concentration from 0.4 to 2% w/w of oil (Freedman *et al*., 1984). The main drawback of base catalyzed transesterification is that the oil containing free fatty acids cannot be converted into biodiesel. The free fatty acid reacts with base catalyst to produce soaps that inhibits the separation and production of biodiesel (Georgogianni *et al*., 2008).

Table 4. Properties of biodiesel produced from different feedstocks in comparison with diesel (Atabani *et al*., 2012; Balat and Balat, 2010; Meher *et al*., 2006; Moser and Vaughn, 2010 and Demirbas, 2005)

Fuel properties	Diesel fuel	Biodiesel	Test method		
	ASTM D975	ASTM D6751	DIN 14214	ASTM	DIN (Deutsches Institut fur Normung) German
Density 15°C (kg/m3)	850	880	860-900	D1298	EN ISO 3675/12185
Viscosity at 40°C (cSt)	2.6	1.9-6.0	3.5-5.0	D-445	EN ISO 3104
Cetane number	40-55	Min-47	Min-51	D-613	EN ISO 5165
Calorific value (MJ/kg)	42–46	–	35	–	EN 14214
Pour point (◦C)	−35	−15 to −16	–	D-97	–
Flash point (◦C)	60–80	Min. 100–170 >120 D-93 ISO DIS 3679	>120	D-93	ISO DIS 3679
Cloud point (◦C)	−20	−3 to −12	–	D-2500	–
Cold filter plugging point (◦C)	−25	19	Max. +5	D-6371	EN 14214
Carbon (wt%)	84-87	77	–	–	–
Hydrogen (wt%)	12-16	12	–	–	–
Oxygen (wt%)	0-0.31	11	–	–	–
Water and sediment content (vol%)	0.05	Max. 0.05	Max. 500[b]	D2709	EN ISO 12937
Ash content % (w/w)	0.01	0.02	0.02	–	EN 14214
Sulfur % (m/m)	0.05	Max. 0.05	10[b]	D 5453	EN ISO 20846
Sulfated ash % (m/m)	–	Max. 0.02	Max. 0.02	D-874	EN ISO 3987
Phosphorus content	–	Max. 0.001	10[b]	D-4951	EN 14107
Free glycerin % (m/m)	–	Max. 0.02	Max.0.02	D-6584	EN 14105/14106
Total glycerin % (m/m)	–	Max. 0.24	0.25	D-6584	EN 14105
Monoglyceride % (m/m)	–	0.52	0.8	–	EN 14105
Diglyceride % (m/m)	–	–	0.2	–	EN 14105
Triglyceride % (m/m)	–	–	0.2	–	EN 14105
CCR 100% (mass%)	0.17 (0.1)[d]	Max. 0.05	Max. 0.03	D-4530	EN ISO10370
Distillation temperature (%)	–	Max. 360 ◦C	–	D-1160	–
Oxidation stability (h, 110 ◦C)		3 min	6 min	D-675	EN 14112
Lubricity (HFRR; µm)	685	314	–	–	–

Table 5. Properties of biodiesel (Atabani et al., 2012)

Fuel properties	Jatropha FAME	Sunflower FAME	Rice bran FAME	Microalgal 1 FAME	Mcroalgal 2 FAME	Neem FAME
Density 15°C (kg/m^3)	879.5	880.0	0.872[C,h]	–	–	
Viscosity at 40°C (cSt)	4.8[g]	4.439	4.81[g,i]	4.519	4.624[g]	
Cetane number	51.6	49	51.6	–	—	
Calorific value (MJ/kg)	39.23	–	41.38	–	–	39.81
Pour point (◦C)	2	–	269[j]	–	–	2
Flash point (◦C)	135	>160	430[J]	>160	>160	76
Cloud point (◦C)	2.7	3.4	–	-5.2	3.9	9
Cold filter plugging point (◦C)	0	-3	–	–7	2	11
Carbon (wt%)	—	–	–	–	–	–
Hydrogen (wt%)	—	–	–	–	–	–
Oxygen (wt%)	—	–	–	–	–	–
Water and sediment content (vol%)	<0.005	<0.005	–	<0.005	<0.005	<0.005
Ash content % (w/w)	0.012	–	–	–	–	-
Sulfur % (m/m)	1.2[a]	0.2[a]	11[a]	5.1[a]	0.6[a]	473.8[a]
Sulfated ash % (m/m)	0.009	<0.005	–	<0.005	<0.005	<0.005
Phosphorus content	<0.1	<0.1[a]	–	<0.1[a]	<0.1[a]	<0.1[a]
Free glycerin % (m/m)	0.006	0.007	–	0.009	0.014	0
Total glycerin % (m/m)	0.1	0.121	–	0.091	0.102	0.158
Monoglyceride % (m/m)	0.291	0.387	–	0.265	0.292	0..338
Diglyceride % (m/m)	0.104	0.092	–	0.078	0.07	0.474
Triglyceride % (m/m)	0.022	0.0	0	0.002	0.019	0
CCR 100% (mass%)	0.025	0.035	0.023	0.007	0.042	0.105
Oxidation stability (h, 110 ◦C)	2.3	0.9	–	8.5	11	7.1

[a] ppm. [b] mg/kg. [c] Determined at 25 ◦C. [d] wt.%.. [e] kJ/kg. [f] Determined at 20 ◦C. [g] mm2/s. [h] g/cm3. [i] Determined at 313 K. [j] K. [k]µg g−1.

The advantage of alkali catalyzed transesterification is that it proceeds 4000 times faster than the acid catalyzed reaction (Fukuda *et al*., 2001).

Properties of Biodiesel

Biodiesel is produced in different countries from different sources. So it is necessary to set a standard of fuel quality for better engine performance (Balat and Balat, 2010). Many countries approved the standard for biodiesel such as Germany, France and United States (Meher *et al*., 2006). International biodiesel standards for testing are (ASTM 6751-3) or the European Union Standard (EN14214) (Atabani *et al*., 2012). Physiochemical properties of diesel and biodiesel produced from different sources are shown in table 4 and table 5. Some of these properties include density, ash content, water content, sulfur content, glycerin, and oxidation stability. The physiochemical properties of biodiesel depends on fatty acid composition and type of feedstock

Flash point

Flash point of fuel is defined as temperature at which fuel will ignite when fuel is exposed to spark. The flash point of biodiesel is higher than the base diesel fuel (Atabani *et al*., 2012). Thus biodiesel and its blends are safer to handle and

store (Atabani *et al*., 2012). The flash point of base diesel is 55-60^{0}C (Atabani *et al*., 2012) While that of biodiesel is more than 150^{0}C. The flash point limit is prescribed by ASTM D93 is 93^{0}C and in EN ISO 3679 is 120^{0}C (Atabani *et al*., 2012).

Cloud point, Pour point and Cold filter plugging point

At low temperature solidification of fuel cause blockage of fuel lines, and filters leading to various problems. Cloud point is the temperature at which cloud (wax crystal) appear in the fuel. When the fuel is cooled generally biodiesel has higher cloud point. Pour point is the lowest temperature at which fuel can flow. The cloud point and pour point are measured using ASTM D2500 procedure (Atabani *et al*., 2012). Biodiesel has higher pour point and cloud point compared to diesel (Atabani *et al*., 2012). It is proposed to specify pour point for diesel and specify cold filter plugging point (CFPP) for biodiesel. The CFPP indicates the limit of filterability at low temperature. The filter starts to plug due to crystallizing some component of fuel. At low temperature fuel gets thicker and may not flow properly affecting fuel supply in pump, injector and fuel lines. CFPP is measured using ASTM D6371 (Meher *et al*., 2006 and Moser and Vaughn, 2010).

Kinematic viscosity

Viscosity is the ability of the material to flow. Higher the viscosity the fuel it is difficult to inject and flow the fuel in fuel lines especially in winters. The kinematic viscosity of biodiesel is generally 10 – 15 times greater than diesel fuel because of its larger molecular mass and larger chemical structure. Improper viscosity may also leads to poor combustion, which results in excessive smoke with loss of power. If the viscosity of fuel is very low it may not provide sufficient lubrication for the closely fit pumps and injectors. They can promote wear and cause leakage in injector pump which leads to loss of power as fuel supply is reduced. So the viscosity must be in limit. The allowable limit according to EN ISO 3104 (3.5-5.0mm^2/s) and (1.9-6.0 mm^2/s) in ASTM D445 (Atabani *et al*., 2012).

Density and Specific gravity

Density is the mass per unit volume. Biodiesel is slightly heavier than petro diesel (specific gravity is 0.859 compared to 0.850 for diesel fuel). For making biodiesel blended biodiesel should be added at the top of diesel fuel. If biodiesel is put at the bottom then diesel is added it will not mix. The oil with high density contains more energy (Atabani *et al*., 2012). Density is measured according to EN ISO 3675/12185 and ASTM D1298. Density is tested at reference temperature 15 ^{o} or 20^{o}C (Atabani *et al*., 2012). Specific gravity is the density of component compared to the density of water. It is used to make mass to volume conversions, calculate flow and viscosity properties.

Cetane number

Cetane number is the ability of fuel to auto ignite quickly when injected. Higher the cetane number means the fuel has better ignition quality. Higher cetane number indicates shorter time between the initiation of fuel injection and the ignition. This results in higher combustion efficiency and smoother combustion. Biodiesel has higher cetane number than diesel fuel (Moser and Vaughn, 2010). The cetane number of diesel specified by EN ISO 5165 is 51.0 min and ASTM D613 is 47 min (Atabani *et al*., 2012).

Conclusion

In order to cut carbon emission to reduce dependence on fossil fuel and to achieve clean environment, biodiesel produced from algae taking lead over other sources of biodiesel. Algae appear to be a significant source of biodiesel because they have the potential of high yield oil. The microalgae has the great ability to fix the CO_2 thus reducing the green house gases. But still it has some technical and economical constraints which it has to overcome for large scale production. Some advancements in cultivation and harvesting techniques are required in order reduce the cost. This state of the art highlights the various methods like cultivation, harvesting, and lipid extraction for the production of biodiesel fro microalgae.

REFERENCES

Khan S.A., Rashmi H., Hussain M.Z., Prasad S. and Banerjee U.C., 2009. "Prospects of Biodiesel Production from Microalgae in India", *Renewable & Sustainable Energy Review*, Vol. 13(9), .pp. 2361-2372.

Schuchardt, U., R. Sercheli and R.M. Vargas 1998. Transesterification of vegetable oils: A review. *Journal of Brazilian Chemical society*, 9:199-210.

Al-Widyan, M.I. and A.O. Al-Shyoukh, 2002. Experimental evaluation of the transesterification of waste palm oil into biodiesel. *Bioresource Technology*, 85:253-6.

Mata, T.M., N. Cardoso, M. Ornelas, S. Neves and N.S. Caetano. 2011. Evaluation of two purification methods of biodiesel from beef tallow, pork lard, and chicken fat. *Energy Fuels*, 25:4756-62.

Radmer, R.J. 1994. Commercial applications of algae: Opportunities and constraints. *Journal of Applied Phycology*, 6:93-98.

Ranganathan, S.V., S.L. Narasimhan and K. Muthukumar. 2008. An overview of enzymatic production of biodiesel. *Bioresource Technology*, 99:3975-81.

Nayak, MM., J. Jena, S. Bhakta, S.S. Rath, C. Sarika, B.V.S.K. Rao,, N. Pradhan, M. Thirunavoukkarasu, S.K. Mishra, P.K. Panda, R.B.N. Prasad, L.B. Sukla and B.K. Mishra, 2011. Screening of fresh water microalgae from eastern region of India for sustainable biodiesel production. *International Journal of Green Energy* 8:669-83.

Chisti, Y., 2007. "Biodiesel from microalgae", *Biotechnology Advances*, Vol.25, pp.294–306.

Banerjee, A., Sharma, R., Chisti, Y. and Banerjee, UC. 2002. Botryococcus braunii: a renewable source of hydrocarbons and other chemicals. *Crit. Rev. Biotechnol*.; 22:245–79.

Sudhakar, K. and M. 2012. Premalatha Micro-algal Technology for Sustainable Energy Production: State of the Art. *Journal of Sustainable Energy & Environment*, 3, 59-62

Atabani, A.E., Silitonga, A.S., Badruddin Irfan Anjum, Mahlia, T.M.I., Masjuki, H.H. and Mekhilef, S., 2012. "A comprehensive review on biodiesel as an alternative energy resource and its characteristics", *Renewable and Sustainable Energy Reviews*, Vol.16, pp.2070– 2093.

Grima Molina, E., Fernandez, F.G.A., Camacho Garcia, F. and Chisti, Y., "Photo bioreactors: light regime, mass transfer and scale up", *Journal of Biotechnology*, Vol.70, pp.231–247, 1999.

Terry Kenneth, L. and Raymond Lawrence, P., 1985. System Design for the autotrophic production of microalgae. *Enzyme and Microbial Technology*, 7,474-487.

Tredici, MR. 1999. Bioreactors, photo. In: Flickinger MC, Drew SW, editors. Encyclopedia of bioprocess technology: fermentation, *biocatalysis and bioseparation Wiley*;. p. 395–419.

Chisti, Y. 1999a. Shear sensitivity. In: Flickinger MC, Drew SW, editors. Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation, vol. 5. Wiley. p. 2379–406.

García Camacho, F., Molina Grima, E., Sánchez Mirón, A., González Pascual, V. and Chisti, Y. 2001. Carboxymethyl cellulose protects algal cells against hydrodynamic stress. *Enzyme. Microb. Technol*., 29: 602–10.

García Camacho, F., Gallardo Rodríguez, J., Sánchez Mirón, A., Cerón García, MC., Belarbi, EH., Chisti, Y. et al. 2007. Biotechnological significance of toxic marine dinoflagellates. *Biotechnol. Adv*.; 25:176–94.

Bernhardt, H. and Clasen, J. 1991. Flocculation of micro-organisms. *Aqua- J Water Supply: Res Technol*.; 40:76–87.

Danquah, MK., Ang, L., Uduman, N., Moheimani, N. and Forde, GM. 2009. Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. *J. Chem. Technol. Biotechnol*.; 84:1078–83.

Kumar, H., Yadava, P. and Gaur, J. 1981. Electrical flocculation of the unicellular green alga Chlorella vulgaris Beijerinck. *Aquat Bot*, 1981; 11:187–95.

Shelf, G., Sukenik, A. and Green, M. 1984. Microalgae harvesting and processing: a literature review. Haifa (Israel): Technion Research and Development Foundation Ltd.

Mohn, F.H., 1980b. Experiences and strategies in the recovery of biomass from mass cultures of microalgae. In : Shelef G, Soeder CJ (eds) Algae biomass, *Elsevier Amsterdam*, 547-571.

Oswald, w.J., 1988b. Large-scale algal culturing systems . In: Micro-algal Biotechnology. Borowitzka, M.A. & Borowitzka, L.J. (Ed.). Cambridge University Press New York, 357-394.

Borowitzka Michael, A. and Borowitzka Lesley, J., 1988 Micro-algal biotechnology, Press Syndicate of the University of Cambridge.

Brennan, L. and Owende, P. 2010. Biofuels from microalgae—A review of technologies for production, processing, and extraction of biofuels and co-products. *Renewable and sustainable energy reviews*, 14:557-577.

Pal, S., Mal, D. and Singh, R., 2005. "Cationic starch: an effective flocculating agent", *Carbohydrate Polymers*, Vol.59, pp.417–423.

Grima Molina, E., Belarbi, E., Fernandez Acien, F.G., Medina Robles, A. and Chisti Y., 2003. "Recovery of microalgal biomass and metabolites: process options and economics", *Biotechnology Advances*, Vol.20, pp.491–515.

Becker, E.W., 1994. Microalgae Biotechnology and microbiology, Cambridge University Press.

Lee, J.Y., C. Yoo, S.Y. Jun, C.Y. Ahn and H.M. Oh. 2010. "Comparison of Several Methods for Effective Lipid Extraction from Microalgae." *Bio resource Technology* 101.1: S75-77. Print.

DOE, U.S. 2010. National Algal Biofuels Technology Roadmap (Technology Roadmap). U.S. Department of Energy, Office of Energy Efficency and Renewable Energy, Biomass Program.

Mercer, P. and Armenta, RE. 2010. Developments in oil extraction from microalgae. *Eur J Lipid Sci Technol* 2011; 113, 539–547.

Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W. and Flynn, K.J., 2009. "Placing microalgae on the biofuels priority list: a review of the technological challenges", *Journal of Royal Society Interface*.

Doucha, J. and Livansky, K., 2008. "Influence of processing parameters on disintegration of Chlorella cells in various types of homogenizers", *Applied Microbiology and Biotechnology*, Vol.81, pp.431–440.

Russin, TA., Boye, JI., Arcand, Y. and Rajamohamed, SH. 2010. Alternative Techniques for Defatting Soy: A Practical Review. *Food Bioprocess Technol* 2010; 4, 200–223.

Ahmad, AL., Yasin, NHM., Derek, CJC. and Lim, JK. 2011. Microalgae as a sustainable energy source for biodiesel production: A review. *Renewable and Sustainable Energy Rev*.; 15, 584–593.

Halim, R., Gladman, B., Danquah, M.K., Webley, P.A., 2011. "Oil extraction from microalgae for biodiesel production", Bioresource Technology, Vol.02, pp.178–185.

Bligh, E.G. and Dyer, W.J., 1959. "A rapid method of total lipid extraction and purification", Canadian Journal of Biochemistry and Physiology, Vol.37, pp.911–917.

Folch, J., Lees, M. and Sloane Stanley, GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem*., 226, 497–509.

Gloria, RD., Ankney, CD. and David, G. 1985. The effect of apparatus, extraction time, and solvent type on lipid extractions of snow geese. *Can. J. Zool*.; 63.

Enssani, E. 1987. Fundamental Parameters in Extraction of Lipids from Wastewater-Grown Microalgal Biomass, Ph.D. Dissertation. Berkeley, California: University of California, Berkeley.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P. and Borghi, M., 2009. "Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production", *Chemical Engineering and Processing*, Vol.48(6), pp.1146–1151.

Young, G., Nippgen, F., Titterbrandt, S. and Cooney, MJ. 2010. Lipid extraction from biomass using co-solvent mixtures of ionic liquids and polar covalent *molecules. Sep. Purif. Technol*.; 72, 118–121.

Lardon, L., H lias, A., Sialve, B., Steyer, J.-P. and Bernard, O. 2009. Life-Cycle Assessment of Biodiesel Production from Microalgae. *Environmental Science & Technology*, **43**, 6475–6481.

Lewis, T., Nichols, P.D. and McMeekin, T.A., 2000. "Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs", *Journal of Microbiological Methods*, Vol.43, pp.107–116, 2000.

Dufreche, S., Hernandez, R., French, T., Sparks, D., Zappi, M. and Alley, E., 2007. "Extraction of Lipids from Municipal Wastewater Plant Microorganisms for Production of

Biodiesel", *Journal of the American Oil Chemists Society*, Vol.84, pp.181–187, 2007.

Carrapiso, A. and Garcia C., 2000. "Development in lipid analysis: Some new extraction techniques and insitu transesterification", *Lipids*, Vol.35, pp.1167–1177.

Herrero, M., Cifuentes, A. and Ibanez, E., 2006. "Sub and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review", *Food Chemistry*, Vol.98, pp.136–148.

Gong, Y. and Jiang, M., 2011. "Biodiesel production with microalgae as feedstock: from strains to biodiesel", *Biotechnology Letters*, Vol.33, pp.1269–1284.

Gouveia, L. and Oliveira, A.C., 2008. "Microalgae as a raw material for biofuels production", *Journal of Industrial Microbiology and Biotechnology*, Vol.36, pp.269–274.

Singh, J. and Gu, S., 2010. "Commercialization potential of microalgae for biofuels production" *Renewable and Sustainable Energy Review*, Vol.14, pp.2596–2610.

Widjaja, A., Chien, C.C. and Ju, Y.H., 2009. "Study of increasing lipid production from fresh water microalgae Chlorella vulgaris", *Journal of Taiwan Institute of Chemical Engineers*, Vol.40, pp.13–20, 2009.

Chaplin, M. 2004, December 20. London South Bank University. Retrieved July 9, 2009, from Ultrasonic cell disruption:

U.S. Department of Energy, 2009. National Algal Biofuels Technology Roadmap. Washington, D.C.: Department of Energy.

Chisti, Y. and Moo-Young, M., 1986. "Disruption of microbial cells for intracellular products", *Enzyme and Microbial Technology*, Vol.8, pp.194-204.

Meher, L.C., Vidya Sagar, D. and Naik, S.N., 2006. "Technical aspects of biodiesel production by transesterification-a review", *Renewable and Sustainable Energy Review*, Vol.10, pp.248–268.

Vyas, A.P., Verma, J.L. and Subrahmanyam, N., 2010. "A review on FAME production processes", Fuel, Vol.89, pp.1–9.

Demirbas, A., 2006. "Biodiesel production via non-catalytic SCF method and biodiesel fuel characteristics", Energy Conversion and Management, Vol.47, pp.2271–2282.

Huang, G., Chen, F., Wei, D., Zhang, X. and Chen, G., 2010. Biodiesel production by microalgal biotechnology Appl. Energy, 87(1), 38–46.

Singh, A., B. He, J. Thompson, J. Van Gerpen, 2006. Process optimization of biodiesel production using alkaline catalysts applied engineering in agriculture vol. 22(4): 597-600. *American Society of Agricultural and Biological Engineers*, ISSN 0883−8542

Marinkovic Siler, S. and Tomasevic, A., 1998. "Transesterification of sunflower oil in situ", Fuel, Vol.77, pp.1389-1391.

Canakc, M. and Van Gerpen, J.H., 2003. "A pilot plant to produce biodiesel from high free fatty acid feed stocks", Transactions of the ASAE (American Society of Agricultural Engineers) Vol.46, pp.945–954.

Marchetti, J.M., Miguel, V.U. and Errazu, A.F. 2007. Renewable and Sustainable Energy Rev., 11(6), 1300–1311.

Freedman, B., Pryde, E. and Mounts, T., 1984. "Variables affecting the yields of fatty esters from transesterified vegetable oils", *Journal of the American Oil Chemists Society*, Vol.61, pp.1638-1643.

Georgogianni, K.G., Kontominas, M.G., Pomonis, P.J., Avlonitis, D. and Gergis, V., 2008. "Conventional and in situ transesterification of sunflower seed oil for the production of biodiesel", *Fuel Processing Technology*, Vol.89, pp.503-509.

Fukuda, H., Kondo, A. and Noda, H., 2001. "Biodiesel fuel production by transesterification of oils", Journal of Bioscience and Bioengineering, Vol.92, pp.405-416, 2001.

Balat, M. and Balat, H., 2010. "Progress in biodiesel processing", *Applied Energy*, Vol.87(6), pp.1815–1835, 2010.

Meher, L.C., Sagar Vidya, D. and Naik, S.N., 2006. "Technical aspects of biodiesel production by Transesterification a review", *Renewable & Sustainable Energy Reviews*, Vol.10(3), pp.248–268.

Moser, BR. and Vaughn, SF. 2010. Coriander seed oil methyl esters as biodiesel fuel: unique fatty acid composition and excellent oxidative stability. *Biomass Bioenergy*; 34(4):550–8.

Demirbas, A. 2005. Biodiesel production from vegetable oils by supercritical methanol. *J. Sci. Ind. Res*.; 64:858–65.

****