Columbia International Publishing
American Journal of Biomass and Bioenergy
(2016) Vol. 5 No. 3 pp. 111-129
doi:10.7726/ajbb.2016.1009
Review

Isolation Purification and Culturing of Cyanobacteria and Microalgae towards Biodiesel Production: Current Status

Md. Fuad Hossain[1,2], R.R. Ratnayake[2*], and K.L. Wasantha Kumara[1]

Received: 11 September 2015; Published online: 30 July 2016

Abstract

The experimental design and techniques for biofuel production from different feedstock varies depending on geographical region, water sources (fresh water, sea water, waste water or brackish water) and overall, available species variations. Also the sampling, culture conditions, isolation, purification of cultures, harvesting, fatty acid extraction followed by conversion of extracted lipids to biofuel followed different methods in different studies. Therefore, it is a demand to simplify the techniques, improve the methods, and develop the efficiencies with less laborious inputs but rapid and best outcomes. Biofuel production research should focus not only selecting suitable biomass or obtaining high yield of lipids but also look for economically feasible method along with sufficient and continuous supply of biomass. Considering these, cyanobacteria and microalgae creates a great potential for biofuel production. In this review, we focused on compiling different materials and methods used for different studies and their best outputs towards biofuel production from cyanobacteria and micro-algae.

Keywords: Renewable Energy; Biofuel; Micro-algae; Blue-green Algae; Cyanobacteria

1. Introduction

Fossil fuels are non-renewable and will be depleted within next 150 years if this consumption rate stays unchanged (Ferdous et al., 2014). The ultimate crisis we are facing is the rapid increase of atmospheric carbon dioxide and the depleted supplies of fossil fuel which has increased the commercial interest in renewable fuels (Halim et al., 2013) and increasing the demand for alternate fuels (Mutanda et al., 2011).

*Corresponding e-mail: renukaratnayake@yahoo.com, Contact: +94773175850
1 Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Sri Lanka
2 National Institute of Fundamental Studies, Kandy, Sri Lanka

Biofuel used as fuel from biological source which is a gaseous, liquid, or solid substance and biodiesel is defined as alternative diesel fuel which is made from renewable biological sources (Nazir et al., 2014). Biodiesel is biodegradable, nontoxic, renewable, environmentally benign (Encinar et al., 2005; Vicente et al., 2004) and its use in diesel engines also shows a decrease in the emission of CO_2, SO_2, unburned hydrocarbons and particulate matter during the combustion process.

To find out a sustainable source for biofuel industry is the ultimate challenge right now. Since land-based biofuel production competes with conventional food production, a water-based biomass and biofuel production from cyanobacteria offers large potential (Steinhoff et al., 2014). The use of lipids obtained from microalgae biomass has been described as a promising alternative for production of biodiesel to replace petro-diesel (Santos et al., 2014).

Several biofuel candidates were proposed to displace fossil fuels in order to eliminate the vulnerability of energy sector (Korres et al., 2010; Singh et al., 2011). Much of the discussion over biofuels production has focused on higher plants such as corn, sugarcane, soya bean, algae, oil-palm and others (Gnansounou et al., 2008; Pandey, 2009). While most bioenergy options fail on both counts, several microorganism-based options have the potential to produce large amounts of renewable energy without disruptions. Cyanobacteria with their superior photosynthesis capabilities can convert up to 10% of the sun's energy into biomass, compared to the 1% recorded by conventional energy crops such as corn or sugarcane, or the 5% achieved by algae (Li et al., 2008). Microalgae have the ability to grow rapidly, and synthesize and accumulate large amounts (approximately 20–50% of dry weight) of neutral lipid stored in cytosolic lipid bodies (Mutanda et al., 2011). Due to their high biomass productivity, rapid lipid accumulation and high carbohydrate storage capacity, microalgae are viewed as promising feedstock for carbon-neutral biofuels (Halim et al., 2013). These photosynthetic microorganisms can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner and at rates high enough to replace a substantial fraction of our society's use of fossil fuels (Li et al., 2008).
Biofuel research is not just a matter of finding the right type of biomass and converting it to fuel, but it must also find environmentally and economically sound uses for the by-products of biofuel production. Biofuels target a much larger fuel market and so in the future will play an increasingly important role in maintaining energy security (Parmar et al., 2011). In this review, we have attempted in compiling various techniques used in isolating, purifying and culturing of cyanobacteria or blue-green algae (BGA) and micro-algae and their best outputs towards biofuel production in order to facilitate more information in inspiring future research.

2. Current status of Knowledge

Biodiesel production from micro-algae and cyanobacteria involve different but directly related steps such as the sampling, cultivation, biomass harvesting, extraction and trans-esterification of lipids (Santos et al., 2014) and production, concentration, lipid extraction followed by trans-esterification (Ríos et al., 2013) or biomass harvesting, dewatering, pre-treatment, lipid extraction, lipid trans-methylation, anaerobic fermentation (Halim et al., 2013). The biodiesel production process along with steps involved in cultivation of cyanobacteria and microalgae can be summarized by biomass cultivation, harvesting, drying and lipid extraction followed by conversion of extracted lipids to biodiesel and finally purification of the produced biodiesel.

Selection of high lipid producing strain, appropriate media, growth condition and effective fatty acid extraction methods are the key requirement for a successful biofuel industry. Identification of species generally requires a combination of morphological and genetic characterization (Mutanda et al., 2011).

2.1 Sample Collection

Collection of water sample is the first step to obtain cyanobacterial culture. It is important to collect the water sample at day time when the cyanobacteria are expected to be available on photosynthetic area of the water body from the surface. Photosynthetic area is the area from the water surface up to the depth where sunlight can be reached and is measured by Secchi disk. In addition to sample collection, environmental parameters of water such as water temperature, pH, conductivity and dissolved oxygen at the sampling site should be recorded to optimize *in vitro* growth (Mutanda et al., 2011; Sethunge & Manage, 2013). Water samples can be collected using planktonic nets or different samplers i.e., Ruttner sampler. It is necessary to concentrate the samples by filtering through the sieve or filtering followed by centrifugation. The pore size of the planktonic net can vary depending on the water bodies and microorganisms expected to be available and the net size range from 10μm to 55μm was reported from previous studies so far. For example, samples were collected using10 μm pore size plankton net in Sri Lanka (Perera & Yatigammana, 2014), whereas, another study carried out in Greece 50 μm planktonic net was used to remove all zooplankton (Economou et al., 2015). A study in Egypt it was reported that 20 μm mesh size plankton nets were used and filtered through 10 μm polycarbonate filters (PC, Whatman) from the planktonic concentrate. Each filtrate was then passed through 2 μm pore-size PC filter (Ranya Ameret al., 2013). In another study, sample was collected using 55μm planktonic net (Sethunge & Manage, 2013) and collected water samples were then kept in 1 L glass bottle (Pereira et al., 2011) or in sterilized brown glass container at the room temperature and were processed on the spot using vacuum filtering, 0.45 μm pore size membrane filter (Millipore) and using a six place stainless steel vacuum manifold (Alanis, 2013). After collecting the water sample it was transferred to appropriate test tube containing liquid growth medium. In case of marine sample growth media was prepared with sterile sea water supplemented with an algal stock solution (Pereira et al., 2011).

According to Ferris and Chirsch (Ferris & Hirsch, 1991) fresh water and sediment samples were shaken to suspend the sediment and then triplicate. Then 10 μL aliquots are removed and diluted into 100 ml of sterile distilled water which is vacuum filtered through a sterile 47- mm- diameter polycarbonate membrane filter (0.4 μm pore diameter, Nuclepore). The filtrate is then aseptically transferred inoculum side up, on to plates of BG-12 or BG-13 medium containing Nystatin and Cycloheximide.

2.2 Culture Media

Once water sample is collected and processed, it is essential to transfer them into specific growth media with some origin water to enhance the growth and enrichment of cyanobacteria and micro-algae. There are number of media available and is been used for cyanobacteria and microalgae culturing. Mostly, efficient growth depends on the right choice of media based on the physicochemical parameters of the origin from where water samples were collected. A study

carried out by Alanis (2013) used solid MN and ASN-III media during the isolation process of the microbial enrichment purposes when samples collected from sea water. . Beside the normal recipe MN and ASN-III with vitamin B_{12} at a concentration of 1.10^{-5} g/L were prepared. Modified MN, F/2 and BG-11 SW (modified by adding sea water instead of deionized water) were used to test preferential growth of isolates (Alanis, 2013). Similarly, Amer *et al.* (2013) used BG11 or BG11- N_0 containing 50 mg/L cyclohexamide media for culturing fresh water cyanobacteria (Ranya Amer et al., 2013) while Da *et al.* 2013 used BG-11, ASM-1 and SWBG-11depending on the type of strain (Rós et al., 2013). Bold's Basal Medium (BBM)(Abou-Shanab et al., 2011), sea water, and MN+ medium (Pandiaraj et al., 2012; Rippka et al., 1979) have also been reported in some studies for culturing cyanobacteria. To separate Blue Green Algae (BGA) from green algae, it is recommended to add 0.02-0.04% (w/v) sodium sulphide (Allen, 1952) which suppress the growth of green algae with some other bacteria and promotes the growth of BGA (Fogget al., 1973). There are some modified media with slight change of composition and ingredients from the conventional media were also reported. Souza Santos (2011) **s**tudied non-axenic strain and used modified liquid medium of BG-11 (3% $NaNO_3$ indicated as BG-11m) (Souza Santos et al., 2011). Prasanna and Kumar (2006) screened the growth potential of 27 strains of *Anabaena* in the N-free BG-11 medium and reported 20 strains were made axenic by repeated antibiotic treatments (Prasanna et al., 2006). Another important issue is to maintain the pH of the media as different media has different pH (Ferris & Hirsch, 1991).

To solidify the media for plate culture, media can be supplemented with 1.0 to 1.5% (w/v) agar (Stanier et al., 1971). It was reported that, for plate culture, agar was added at a concentration of 15 g/L in all cases with liquid media (Alanis, 2013) or 0.8 g/L agarose can be incorporated into liquid media for plate culturing (Kodandoor & Rajashekhar, 2011).

2.3 Growth Conditions

To facilitate suitable growth environment such as temperature, appropriate light and dark period with proper light intensity is mandatory for expected growth. Mostly these parameters are strongly related to the conditions of the origin of the sample. Therefore, these parameters can vary depending upon the climatic zone where the study is been carried out. It was found large variations in individual fatty acid contents according to species, and location of the specific genus (Temina et al., 2007).

The different parameters that were set out for the growth of cyanobacteria sampled from different climatic zones are summarized in Table-1.

2.4 Growth Enhancement

It is reported that cultures with air but for more rapid growth and higher yields were obtainable if the cultures were continuously aerated with N_2 containing 0.5 to 1.0% (v/v) CO_2. A supplement of vitamin B_{12} (final concentration 1µg/L) or NaCl (1%, w/v) can be added for satisfactory growth and media can be supplemented with Cycloheximide and Nystatin (100 µg/ml) to get axenic culture (Ferris & Hirsch, 1991). Several other media which have been used to cultivate BGA are better buffered and provide a larger phosphate supply such as medium C (Kratz & Myers, 1955) and the medium of Van Baalen (Van Baalen, 1962).

Table 1 Sample collection methods and culturing techniques applied for the growth of cyanobacteria sampled from different climatic zones.

Sl	Location	Sample Collection	Culture Media	Growth Condition	Reference
1	Greece	Sample water was filtered through 50 µm net.	Natural waste water with nutrients	Continuous illumination of 12000 lux with atmospheric CO_2, Temp. (23-27^{0}C) and pH was 7-9.	(Economou et al., 2015)
2	Sri Lanka	Water sample was collected using plankton net (pore size 10 µm).	**Not reported**	**Not reported**	(Perera & Yatigammana, 2014)
3	Red Sea	Processed on the spot, vacuum filtering 500 ml, per 0.45 µm filter, placed in agar plate and incubated.	Solid MN and ASN-III with vitamin B12.Andmodified MN, F/2, BG-11	**Not reported**	(Alanis, 2013)
4	Sri Lanka	Samples were collected into sterilized brown glass container, concentrated by centrifugation. An aliquot of 500 µL from the centrifuged pellet and 500 µL from the supernatant and soil samples were inoculated	**Not reported**	Incubated at 28±2^{0}C, under fluorescent light with a 12: 12 dark: light cycle.	(Magana-Arachchi & Wanigatunge, 2013)
5	Egypt	Samples were collected using Planktonic net size 20 µm mesh. From the planktonic concentrate, 15 ml was filtered through 10 µm polycarbonate filters. Each filtrate was then passed through 2 µm PC filter.	BG11 or BG11- $_{N0}$ containing 50 mg/L cyclohexamidewith 0.8 g/L agarose.	100 rpm and 25˚C under 2000 LUX light intensity, incubated for at least 10 days.	(Ranya Amer et al., 2013)

6	Sri Lanka	Water samples were collected using 55µm planktonic net.	**Not reported**	**Not reported**	(Sethunge & Manage, 2013)
7	Brazil	**Not reported**	BG-11, ASM-1 and SWBG-11	Constant aeration, 100 µmol photons m−2•s−1 and at 24 ± 1 °C.	(Rós et al., 2013)
8	India	**Not reported**	sea water, MN+ medium	White fluorescence (1400 lux); 14±10 L/D at 25±2°C.	(Pandiaraj et al., 2012)
9	USA	**Not reported**	**Not reported**	Fluorescent light on 12:12h, L:D, air with an aquarium pump.	(Jones et al., 2012)
10	Portugal	Water was collected and stored in 1 L bottle, kept in room temp, transferred to liquid algal growth medium.	**Not reported**	7 days at 21^0C, 12:12h, D:L, 80 µmol/m2/s.	(Pereira et al., 2011)
11	South Korea	**Not reported**	Bold's Basal Medium (BBM)	Rotary shaker, 27^0C, 150 rpm, constantly 40 µmolm-2s-1 for three weeks.	(Abou-Shanab et al., 2011)
12	India	**Not reported**	BG-11	$26±2^0$C, constant, (14:10h, L:D), 2000 lux.	(Kodandoor & Rajashekhar, 2011)

13	Brazil	**Not reported**	modified liquid medium BG-11 (3% NaNO3 indicated as BG-11m)	pH=9.5, 23±1^{0}C, 14:10h L: D, 40-50 µmol.photons.m-2.s-1	(Souza et al., 2011)
14	USA	**Not reported**	**Not reported**	140 rpm, 180 µmol photons m-2s-1, 14:10h (L:D)	(Wahlen et al., 2011)
15	India	**Not reported**	N-free BG-11 medium	27±2^{0}C, 52-55µmol photon m-2s-1, L:D cycle of 16:8.	(Prasanna et al., 2006a)
16	USA	10 µL aliquots diluted into 100 ml distilled water, vacuum filtered through 47- mm- diameter polycarbonate membrane filter (0.4 µm pore diameter) transferred on to plates of BG-12 or BG-13 medium containing Nystatin and Cycloheximide	BG-12 or BG-13 medium containing Nystatin and Cycloheximide	40W cool white fluorescent lamp, 3k-5klux, 180-200rpm	(Ferris & Hirsch, 1991)
17	USA		modification BG-11	2000-3000 lux	(Stanier et al., 1971)

2.5 Isolation and Purification

To get an axenic culture, still there is no report found having single step or automated technology. It consists mostly the conventional methods of repeated culturing, serial dilution, streaking the uni-culture from agar plate followed by liquid culturing. It is reported that, axenic microalgae were pinched from algal plates and streaked into agar slants and were stored at appropriate temperature, photoperiod and light intensity (Alanis, 2013). According to Rayna Amer et al., (2013) cyanobacterial growth on each plate was carefully examined under the microscope and colonies were repeatedly transferred to fresh plates to obtain uni-algal cultures (Ranya Amer et al., 2013). A study done by Abou-Shanab and others (2011) reported that sub cultures were made by inoculating 50 µL culture solution on to petri plates containing media solidified with 1.5% (w/v) bacteriological agar. Further, 50 µL aliquots of the same dilution were placed in the wells of 96 microtiter plates containing media. These procedures were repeated for each of the original flaks. Both the petri and microtiter were incubated at 27 ^{0}C under continuous illumination for two weeks. The purity of the culture was confirmed by repeated plating and by regular observation under microscope (Abou-Shanab et al., 2011). Ferris and Hirsch (1991) reported that, contaminated cyanobacteria colonies were picked and transferred in to 20 ml of sterile BG-12 medium in a 50 ml Delong flask and the mixed cultures were incubated for 3 to 4 weeks under the proper conditions to obtain sufficient biomass and then 400 µL of sterile nutrient solution (SNS) consisting of 2.5% (w/v) sucrose, 0.5% (w/v) Difco yeast extract and 0.5% (w/v) Difcobacto-Peptone was added along with 400 µL of sterile antibiotic solution to give a final antibiotic concentration of 100 µg/ml. The culture was then incubated for 18- 24 hours in the dark at 180 to 200 rpm under an atmosphere of 5% (v/v) CO_2 in air. After incubation the cyanobacteria were harvested by centrifugation 17000 rpm for 15 minutes at 20 ^{0}C. The cells were washed twice and pipetted and plated into agar containing nystatin and cyclohexide. The plates were incubated for 2 to 4 weeks and observed at weekly intervals for the growth of cyanobacteria. Under the dissecting microscope (10X to 50X magnification) purified colonies or filaments of cyanobacteria were picked and transferred to plates of BG-12 agar (Ferris & Hirsch, 1991).

3. Morphological Identification

Identification of species generally requires a combination of morphological and genetic characterization (Mutanda et al., 2011). Morphological identification was done from each sample using compound light microscope (Magana-Arachchi & Wanigatunge, 2013) which was done using standard method described by Desikachary (1959).

4. Fatty acid Extraction

Once sufficient biomass is grown, there are some conventional methods for fatty acid extraction. Beside these conventional methods several studies have been conducted to make the procedure easier, time saving, economically feasible, single step scale up and effective. Mostly used fatty acid extraction methods are Folch method, Bligh and Dyer method (Bligh & Dyer, 1959; Folch et al., 1957). It is also reported and proposed some of the modified methods of those as well. For example a study carried by Keshini Beetul and others used modified Folch method with chloroform:

methanol 1:1 (v/v) for the extraction of total lipids (Keshini Beetul et al., 2014). Abou-Shanab (Abou-Shanab et al., 2011) reported that the total lipids were extracted from the fresh microalgal biomass using the modified methods of Bligh and Dyer and fatty acids were analyzed by the modified method of Lepage and Roy (Lepage & Roy, 1986). Rodríguez-Ruizet al (1998) carried out the fatty acid analysis by a method adopted from Lepage and Roy (1986). In this method, samples were put in test tubes with 1 mL of the methylation mixture (Methanol/ Acetyl Chloride 20:1 v/v) and 0.5 mL hexane and heated for 10 minutes at 100^0C. A single methanol hexane phase was formed. After cooling to room temperature 1 mL distilled water was added. Two phases established very rapidly. The upper one (hexanic) was extracted and placed into chromatograph vial for injection (Rodríguez-Ruiz et al., 1998).

For different studies it was used some direct methods such as *in-situ* alkaline transesterification procedure (Nazir et al., 2014) or direct transesterification (Yaniv et al., 1996). It was reported that biodiesel of good quality can be produced from microalgae in the reaction condition of 3.4:1 methanol to dry biomass (v/w) ratio with 0.6:1 catalyst loading to dry algae (v/w) temperature at 75 ^{0}C in 35 minutes and fatty acid were analysed using GC (Thao et al., 2013). In this study, algal oil extraction procedure was adapted from the protocol described by Bligh and Dyer in 1959 (Bligh & Dyer, 1959). Another study reported that, cells were harvested by centrifugation and lyophilized. Total lipids were extracted using the methodology described by Bligh and Dyer (Rós et al., 2013). In this study, total lipids were measured gravimetrically and yields were calculated. The lipid extracted was dried in a rotary evaporator to remove remaining residues of solvent and subsequently dried at 60°C to constant weight. Analysis of fatty acid composition was performed in a capillary gas chromatography (CGC Agilent 6850 Series GC System) according to AOCS procedure(American Oil Chemists' Society, 2004). Kodandoor Sharathchandra (2011) extracted total lipids by Chloroform/ Methanol extraction methods (Řezanka et al., 1982) and Fatty Acid Methyl Esters (FAME) were prepared by total lipid fraction using HCl-Methanol and analyzed by GC(Kodandoor Sharathchandra & Rajashekhar, 2011). One of the efficient and one step scale up process is direct transesterification. A study in case of seed fatty acid extraction was done by Yaniv (1996) using this direct method(Yaniv et al., 1996).

5. Cryopreservation

Several methods have been applied for cryopreservation and thawing of algae, blue-green algae or micro-algae using Dimethyl Sulphoxide (DMSO), Sorbitol, Glycerol, Ethylene Glycol (EG) and L-proline as Cryoprotectant (Mori & Watanabe, 2002; Müller et al., 2007; Nakanishi et al., 2012).

Figure 1 shows the schematic comparison between algal biomass and direct photosynthetic processes. The direct process, developed by Joule and called Helioculture™, combines an engineered cyanobacterial organism supplemented with a product pathway and secretion system to produce and secrete a fungible alkane diesel product continuously in a Solar Converter™ designed to efficiently and economically collect and convert photonic energy. The process is closed and uses industrial waste CO_2 at concentrations 50–100× higher than atmospheric. The organism is further engineered to provide a switchable control between carbon partitioning for biomass or product. The algal process is based on growth of an oil-producing culture in an industrial pond on atmospheric CO_2, biomass harvesting, oil extraction, and chemical esterification to produce a biodiesel ester (Robertson et al., 2011).

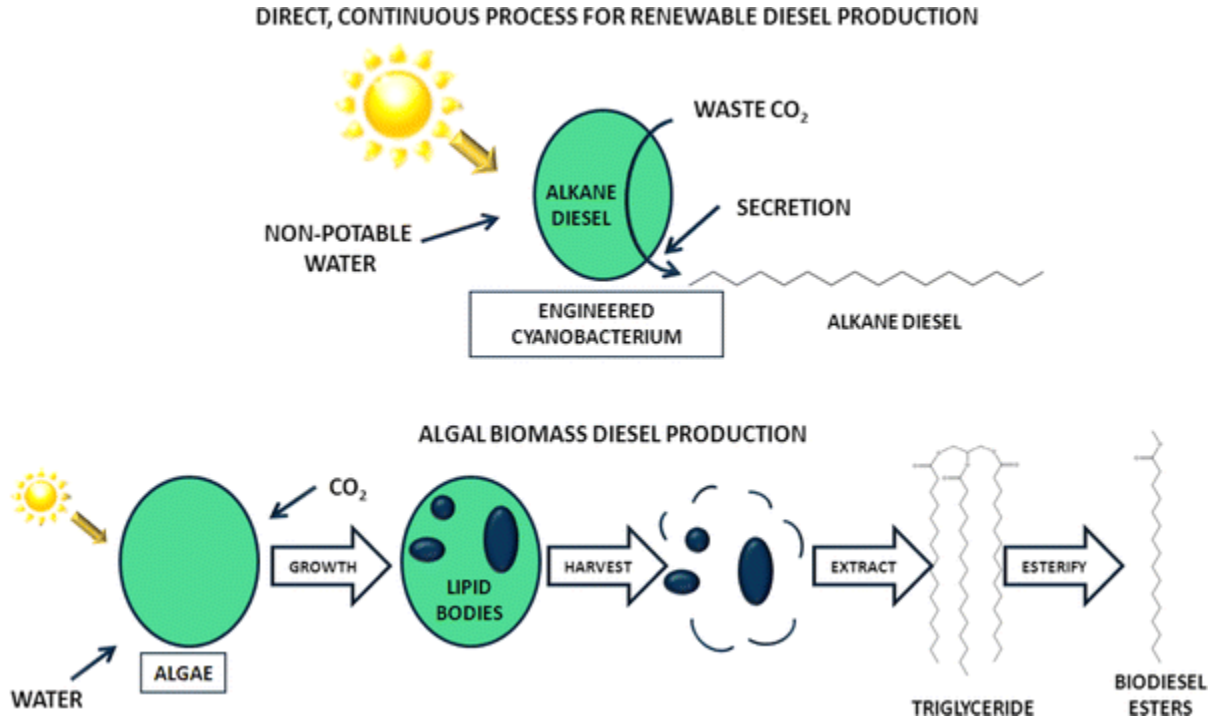

Fig. 1. The schematic comparison between algal biomass and direct photosynthetic processes Adopted from Robertson (Robertson et al., 2011).

6. Best Outputs of Different Studies

Some studies have been carried out to compare different methods of fatty acid and total lipid extraction based on Folch method, Bligh and Dyer method, Lepage and Roy method, Hilditch method and direct transesterification to select the best efficient one. A study carried out by Hall (2012) compared fatty acid methyl ester (FAME) yield from microalgae of two *in situ* transesterification methods to a typical Folch extraction method (Folch et al., 1957) followed by transesterification using the Hilditch procedure (Hilditch & Williams, 1964). A method based on Park and Goins, (1994) (Park & Goins, 1994), utilizing 0.5 N NaOH in methanol, then 14% BCl_3 in methanol, was found to be superior to the method based on Lepage and Roy(1986) (Lepage & Roy, 1986), utilizing acetyl chloride in methanol. The Park and Goins method(Park & Goins, 1994) was equivalent to the traditional method. Further studies showed that in establishing the parameters of the method, water contents up to 0.55 mL was not found to inhibit the reaction within the maximum lipid load, conservatively assessed at ~1 mg. The reaction time and temperature required to produce a maximum FAME yield was 10 min at 90°C for the BCl_3-catalyzed reaction, while the NaOH-catalyzed reaction happened instantaneously at ambient temperature (Hall, 2012). Griffiths and Van et al., (2010) compared the effectiveness of direct transesterification (DT) in quantifying the total fatty acid content in three species of microalgae to extraction using the Folch method (Folch et al., 1957), the Bligh and Dyer method (Bligh & Dyer, 1959) and the Smedes and Askland method (Smedes, 1999) followed by transesterification and gas chromatography (GC). DT proved a convenient and more accurate method than the extraction techniques for quantifying total fatty acid content in microalgae (Griffiths et al., 2010). This finding is also supported in several

other studies. Rios and Castaneda (2013) carried out a study to identify the best method of lipid extraction to undergo the potentiality of some micro-algal biomass obtained from two different harvesting path ways. One step lipid extraction–transesterification reached the same fatty acid methyl ester yield as the Bligh and Dyer and soxhlet extraction with *n*-hexane methods with the corresponding time, cost and solvent saving (Ríos et al., 2013). It was further reported that, one step transesterification (Direct Transesterification or *in situ* transesterification) reaction was effective compare to other conventional methods. To determine the effect of alcohol, methanol was reported as more effective adding that while high volume of methanol are needed for optimal direct conversion of lipids in algae to biodiesel, the direct method completely eliminates the needs for n-hexane extraction prior to transesterification. It was also reported that reaction temperature and catalyst concentration has also effect on fatty acid methyl ester (FAME) yield (Wahlen et al., 2011). The best output is dependent not only on the methods but also the physical and chemical parameters such as reaction solvent used and its ratio, pH, temperature, biomass condition and some others. It is reported that, when wet biomass was used as feedstock, the one-stage method resulted in a much-lower biodiesel yield (Johnson & Wen, 2009). According to this experiment different biodiesel preparation methods were studied, including oil extraction followed by transesterification (a two-stage method), only chloroform-based transesterification and a direct transesterification of algal biomass (a one-stage method). It was reported that the alga *Schizochytrium limanicum* is a suitable feedstock for producing biodiesel via direct transesterification method (Johnson & Wen, 2009). When wet biomass was used as feedstock, the one-stage method resulted in a much-lower biodiesel yield (Johnson & Wen, 2009). It has been tested the possibility of avoiding the drying process, and extracting the lipid directly from the wet concentrated cells, using enzymatic disruption to enhance the extraction. From wet samples, highest lipid extraction yield of 16.6% achieved using lysozyme was reported. On the other hand, 12.5% lipid was achieved using supercritical CO_2 (SC-CO_2) and lysozyme. In addition, a two-step culturing process was applied, using *Scenedesmus* sp., to combine both high biomass growth and lipid content. The strain was able to increase its biomass productivity in the first stage with almost constant lipid content. In the second stage, the lipid content was enhanced by six-fold after three weeks of nitrogen starvation, but with lower biomass productivity (Taher et al., 2014). Jones (2012) reported that 2-ethoxyethanol (2-EE) was more effective at extracting lipids from wet biomass rather than dried algal pellets (Jones et al., 2012). In this study they compared different lipid extraction methods using 2-ethoxyethanol (2-EE), chloroform: methanol and hexane as solvent from dry and wet algal biomass. It was reported that 2-ethoxyethanol (2-EE) provides superior lipid recovery (>150–200 %). Analysis of crude lipid extracts showed that all major lipid classes could be identified and quantified and revealed a surprisingly large amount of saturated hydrocarbons (HC) (Jones et al., 2012).

A high degree of coherence between cyanobacterial growth and nutrient uptake kinetics was observed, as well as a strong dependence on the change of the two parameters- light intensity and temperature (Dechatiwongse et al., 2014). A study carried out to analyze the effect of light intensity and temperature, on the photoautotrophic growth of *Cyanothece* and showed that nitrogen depletion was confirmed as a trigger, which transforms an exponential into a stationary growth phase. A non-linear relationship between the maximum specific growth rate and the irradiance up to 320 $\mu E\ m^{-2}\ s^{-1}$ was identified and found to be dominated by light saturation rather than photo inhibition. The relationship between the specific growth rate and the temperature was found to be linear until a remarkable drop in the final biomass productivity and cyanobacterial photosynthetic

capability was observed at 40 °C (Dechatiwongse et al., 2014). Biodiesel production from microalgae through experimental investigation of transesterification conditions reported that 3.4:1 methanol dry algal biomass ratio with 35 minutes reaction time and 0.6:1 catalyst to dry biomass ration was the most efficient in experimental process. In this condition, 60% lipids could be achieved from dry algal biomass (Thao et al., 2013).

Table 2 Highest lipid yield recorded from microalgae and blue green algae in different study

Sl	Species	Lipid yield	Comments	Reference
1	*Scenedesmus* sp.	16.6%	Wet cells, using enzymatic disruption (Lysozyme)	(Taher et al., 2014)
2	Microalgae	60%	Dry weight of biomass	(Thao et al., 2013)
3	*Chlorella vulgaris*	19%	Solvents chloroform:methanol (2:1) assisted by ultrasound	(Santos et al., 2014)
4	*Spirulina*	85%	Three stage extraction (20 min/stage), sample- solvent ratio of 1:5 at 60 °C.	(Chaiklahana et al., 2008)
5	*Chlamydomonas mexicana*	33 ± 3%		(Abou-Shanab et al., 2013)
6	*Isochrysis galbana*	23.15%	Dry cell weight	(Lee et al., 2011)
7	*Scenedesmus obliquus*YSR01	58±1.5%	Not Reported	(Abou-Shanab et al., 2011)

A comparison between different methods of extracting total lipids in biomass of *Chlorella vulgaris* with the solvents ethanol, hexane and a mixture of chloroform: methanol in ratios 1:2 and 2:1 was carried out by (Santos et al., 2014). Among the tested methods, the mixture of chloroform: methanol (2:1) assisted by ultrasound was most efficient, extracting an average of 19% of total lipids, of which 55% were triglycerides. Another study carried out on lipid extraction from *Spirulina* using single or multi-stage extraction at 30°C or 60 °C with different sample-solvent ratios and found that a three-stage extraction (20 min/stage) using a sample- solvent ratio of 1:5 at 60 °C was the best procedure. Lipid extracted from *Spirulina* contained approximately 21% linoleic acid and 18% γ-linolenic acid with the total fatty acid (TFA) recovery of 85% (Chaiklahana et al., 2008). Inclusion of isopropanol as a co-solvent for hexane extraction, continuous operation with a Soxhlet apparatus can enhance lipid yields from either dried micro-algal powder or wet micro-algal paste. In case of $SCCO_2$ extraction, decreasing temperature and increasing pressure resulted in increased lipid yields(Halim et al., 2011).

Selvan (2013)(Selvan et al., 2013)carried out a study on possibility of biodiesel production from marine cyanobacteria *Lyngbya* sp. and *Synechococcus* sp. in different media, different photo bioreactor, different salinity and quantification of extracted lipid in different sample: solvent ratio. Out of three media such as ASNIII, sea water enrichment medium and BG11, the sea water

enrichment medium was found superior in enhancing the growth rate of these cyanobacteria. The total biomass yield was higher in tubular LED photo-bioreactor than the fluorescent flat plated photo-bioreactor. Increase in salinity from 0.5-1.0 M showed an increase in the lipid content to 2.0 and 0.8 % in these strains; but a salinity of 1.5 M showed a total inhibitory effect on growth. Lipid extraction was obtained maximum at 60 ^{0}C in 1:10 sample: solvent ratio. Biodiesel production was found maximum in *Synechococcus* sp. than *Lyngbya* sp. A study carried out on *Anabaenopsis elenkinii* CCIBT1059 strain isolated from one of the alkaline lakes of the Brazilian Pantanal reported that in relation to growth rate and cell yield the higher values were observed at pH 10.5. Morphologically, the longest trichomes were found at pH 7 (maximum 45 cells) in comparison with pH 9.5 (maximum 32 cells) and pH 10.5 (maximum 23 cells). The occurrence of heterocytes was observed in all treatments, but akinetes were never formed. Results indicate that *A. elenkinii* is typical of alkaline systems and also that in lower pH values the growth limitation can occur in terms of number of cells and biomass (Souza Santos et al., 2011).

Research on cyanobacteria and microalgae in relation to its effectiveness on biodiesel production, extraction of other value added compound along with its effectiveness for waste water treatment is now emerging in all over the world. Different countries and research institutes are now keeping keen sight on this field. It is reported that, cultivation and harvest of bloom forming cyanobacteria for fuel and by-product production are feasible in Scandinavia, but strongly depends on the desired compounds and biomass. According to their study, three Baltic cyanobacteria strains (*Aphanizomenon flos-aquae*, *Dolichospermum lemmermannii* and *Nodularia spumigena*) in full nutrient levels, as well as phosphorus and nitrogen depleted medium was inoculated and being monitored for 14 days. It was observed a strong negative relationship between lipid content, growth and nutrient availability, resulting in high lipid and pigment production in combination with a limited growth rate in nutrient depleted treatments (Steinhoff et al., 2014). A study carried out on different microalgae found in Mauritian marine water reported that highest amount of lipid was recorded in the *Symbiodinium* clade C. The presence of biodiesel was detected also by alkaline trans-esterification reaction (Keshini Beetul et al., 2014). Potentiality of biodiesel production in Bangladesh from non-edible oil has recently been focused (Ferdous et al., 2014). Six species of freshwater microalgae (*Desmodesmus* sp. DRLMA7, *Scenedesmus* sp. DRLMA5, *Chlorella* sp. DRLMA3, *Chlorococcum macrostigmatum* DRLMA1, *Desmodesmus elegans* DRLMA13 and *Scenedesmus* sp. DRLMA9) were isolated from freshwater bodies in Assam, India. Experiments were carried out to compare fatty acid composition between the late exponential and stationary growth phases among the two species stated later (*D. elegans* DRLMA13 and *Scenedesmus* sp. DRLMA9) in BG11 medium. All six microalgae showed similar fatty acid profiles 16:0, 16:4, 18:1, 18:2, and 18:3 as major components (Kaur et al., 2012**)**. In Southern Karnataka, it was isolated 13 species of freshwater cyanobacteria isolated from different aquatic habitats which showed fatty acid profile as palmitic acid C16:0 for all the isolates followed by linoleic acid C18:2. In some, the long chain fatty acids (C20:1 and C24:0) were found in lower concentrations. Of the 13 species investigated, two toxic bloom forming species such as *Microcystis aeruginosa* and *Nostoc linckia*were also involved (Sharathchandra & Rajashekhar, 2011).

Six microalgal species *Ourococcus multisporus*, *Nitzschia cf. pusilla*, *Chlamydomonas mexicana*, *Scenedesmus obliquus*, *Chlorella vulgaris*, and *Micractinium reisseri* were examined to determine their effectiveness in the coupling of piggery wastewater treatment and biodiesel production. It was reported that *C. mexicana* is one of the most promising candidates for simultaneous nutrient

removal and high-efficient biodiesel production. The highest lipid productivity and lipid content (0.31 ± 0.03 g/L and 33 ± 3%, respectively) were found in *C. mexicana*. The fatty acid compositions of the studied species were mainly palmitic, linoleic, α-linolenic, and oleic (Abou-Shanab et al., 2013). Beside this, feasibility to couple the removal of nitrogen and phosphorus from wastewater to algal biomass and biofuel production has also been reported for the species *Platymonas subcordiformis*(Guo et al., 2013). The effectiveness of micro-algal wastewater treatment and production of biofuels is supported by another study (Peccia et al., 2013).

Out of 9 *Nannochloropsis* species, the best strain was *N. oceanica* IMET1, with lipid productivity, TAG production, favorable fatty acid profiles as well as suitable biodiesel properties of higher cetane number, lower iodine number and relative low cloud point (Ma et al., 2014). When the *Nannochloropsis gaditana* was cultured in two batches it was able to transform up to 97.0% of extracted Saponifiable lipids (SLs) into Fatty Acid Methyl Esters (FAMEs) by acid catalyzed *trans* esterification. Saponifiable lipids (SLs) were extracted with hexane from wet biomass (86 wt% water).Higher Saponifiable lipids (SLs) and neutral saponifiable lipids (NSLs) were obtained from Batch 2 which was cultivated in the same conditions of Batch-1, but once the steady state was reached, the culture was centrifuged and the pellet was re-suspended in nitrate-free culture medium (nitrogen starvation conditions) (Jiménez Callejón et al., 2014). Afterwards, the reactor was operated in batch mode for 12 days (San Pedro et al., 2013).

However, by combining biomass and lipid productivity parameters, the greatest potential was found for *Synechococcus* sp. PCC7942, *M. aeruginosa* NPCD-1 and *Trichormus* sp. CENA77 out of five non-toxin producing cyanobacterial isolates from the genera *Synechococcus, Trichormus, Microcystis, Leptolyngbya* and *Chlorogloea* (Rós et al., 2013). Another study confirms the growth traits and potential for the production of renewable biomass, biofuels, or other products from *Leptolyngbya*. *Leptolyngbya* sp. strain BL0902 with robust growth at temperatures from 22°C to 40°C and tolerated up to 0.5 M NaCl, 32 mM urea, high pH, and high solar irradiance. It was reported that *Leptolyngbya* BL0902 accumulated higher FAME content and a higher proportion of mono-unsaturated fatty acids, preferable for a biodiesel feedstock, than two strains of *Arthrospira* sp (Taton et al., 2012). Another study on five marine micro-algae (*Tetraselmis suecica, Phaeodactylum tricornutum, Chaetoceros calcitrans, Isochrysis galbana*, and *Nannochloropsis oculata*) reported that, *T. suecica* showed the shortest culture period of 9 days to reach the stationary phase. Therefore, the highest biomass production of 0.58 g/L was obtained and *I. galbana* and oil content of 23.15% of dry cell weight .*I. galbana* showed 417.33 mg of palmitic acid per g oil and *T. suecica* showed 235.61 mg of oleic acid per g oil (Lee et al., 2011). Abou-Shanab (2011) (Abou-Shanab et al., 2011) carried out a study on biodiesel production from micro-algae and selected five cultures (*Scenedesmus obliquus* YSR01, *Nitzschia cf. pusilla* YSR02, *Chlorella ellipsoidea* YSR03, *S. obliquus* YSR04, and *S. obliquus* YSR05) isolated from river and three cultures (*S. obliquus* YSW06, *Micractinium pusillum* YSW07, and *Ourococcus multisporus* YSW08) isolated from waste water out of total of 33 isolated cultures based on their morphology and ease of cultivation for biodiesel production. It was reported that *S. obliquus* YSR01 can be a possible candidate species for producing oils for biodiesel, based on its high lipid and oleic acid contents. *S. obliquus* YSR01 reached a growth rate of 1.68 ± 0.28 day^{-1} at 680_{nm} and a biomass concentration of 1.57 ± 0.67 g dwt L^{-1}, with a high lipid content of 58 ± 1.5%. The fatty acid compositions of the studied species were mainly myristic, palmitic, palmitoleic, oleic, linoleic, g-linolenic, and linolenic acids.

It has been described a new selection method based on BODIPY (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene) staining, fluorescence activated cell sorting (FACS) and microplate-based isolation of lipid-rich microalgae from an environmental sample. It was reported that direct sorting onto solid medium upon FACS can save about 3 weeks during the scale-up process as compared with the growth of the same cultures in liquid medium. This approach enabled us to isolate a biodiverse collection of several axenic and uni-algal cultures of different phyla (Pereira et al., 2011).

7. Conclusion

Research on biofuel production from cyanobacteria and micro-algae depends on the appropriate selection of materials and methods. The present study will be a baseline for this selection which will also be an effective guideline for new researchers.

References

Abou-Shanab, R. A., Hwang, J. H., Cho, Y., Min, B., & Jeon, B. H. (2011). Characterization of microalgal species isolated from fresh water bodies as a potential source for biodiesel production. Applied Energy, 88(10), 3300-3306. http://dx.doi.org/10.1016/j.apenergy.2011.01.060

Abou-Shanab, R. A., Ji, M. K., Kim, H. C., Paeng, K. J., & Jeon, B. H. (2013). Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. Journal of environmental management, 115, 257-264. http://dx.doi.org/10.1016/j.jenvman.2012.11.022

Alanis, P. A. L. (2013). Isolation, Characterization and Purification of Microalgae from the Red Sea. King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia.

Allen M.B. (1952). The cultivation of Myxophycae. Archiv fur Mikrobiologie, 17, 34-53. http://dx.doi.org/10.1007/BF00410816

American Oil Chemists' Society. (2004). In Official Methods and Recommended Practices of the AOCS. AOCS Press: Boulder, CO, USA, 5th edition.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37(8), 911-917. http://dx.doi.org/10.1139/o59-099

Chaiklahana, R., Chirasuwana, N., Lohab, V., & Bunnag, B. (2008). Lipid and fatty acids extraction from the cyanobacterium Spirulina. Sci. Asia, 34, 299-305. http://dx.doi.org/10.2306/scienceasia1513-1874.2008.34.299

Souza Santos, K. R., Jacinavicius, F. R., & Leite, C. (2011). Effects of the pH on growth and morphology of Anabaenopsis elenkinii MILLER (Cyanobacteria) isolated from the alkaline shallow lake of the Brazilian Pantanal. Fottea, 11(1), 119-126. http://dx.doi.org/10.5507/fot.2011.012

Dechatiwongse, P., Srisamai, S., Maitland, G., & Hellgardt, K. (2014). Effects of light and temperature on the photoautotrophic growth and photoinhibition of nitrogen-fixing cyanobacterium Cyanothece sp. ATCC 51142. Algal Research, 5, 103-111. http://dx.doi.org/10.1016/j.algal.2014.06.004

Desikachary, T. (1959). Cyanophyta. Indian Council of Agricultural Research. New Delhi.

Santos, R. R., Moreira, D. M., Kunigami, C. N., Aranda, D. A. G., & Teixeira, C. M. L. L. (2014). Comparison Between Several Methods of Total Lipid Extraction From Chlorella vulgaris Biomass. Ultrasonics Sonochemistry.

Economou, C. N., Marinakis, N., Moustaka-Gouni, M., Kehayias, G., Aggelis, G., & Vayenas, D. V. (2015). Lipid production by the filamentous cyanobacterium Limnothrix sp. growing in synthetic wastewater in suspended-and attached-growth photobioreactor systems. Annals of Microbiology, 1-8. http://dx.doi.org/10.1007/s13213-014-1032-7

Encinar, J. M., Gonzalez, J. F., & Rodríguez-Reinares, A. (2005). Biodiesel from used frying oil. Variables affecting the yields and characteristics of the biodiesel. Industrial & Engineering Chemistry Research, 44(15), 5491-5499. http://dx.doi.org/10.1021/ie040214f

Ferdous, K., Uddin, M. R., Islam, R., Khan, M. R., & Islam, M. (2014). Potentiality of Biodiesel Production From Non-Edible Oil: Bangladesh Perspective. Journal of Chemical Engineering, 27(2), 1-5. http://dx.doi.org/10.3329/jce.v27i2.17763

Ferris, M. J., & Hirsch, C. (1991). Method for isolation and purification of cyanobacteria. Applied and Environmental Microbiology, 57(5), 1448-1452.

Folch, J., Lees, M., & Sloane-Stanley, G. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem, 226(1), 497-509.

Fogg, G. E., Stewart, W. D. P., Fay, P. & Walsby, A. E. (1973). THE BLUE-GREEN ALGAE. Academic Press-London and New York, 130.

Gnansounou, E., Larroche, C., & Pandey, A. (2008). Biofuels II. Special Issue of J. Sci. Ind. Res., 67, 837-1040.

Griffiths, M., Van Hille, R., & Harrison, S. (2010). Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. Lipids, 45(11), 1053-1060. http://dx.doi.org/10.1007/s11745-010-3468-2

Guo, Z., Liu, Y., Guo, H., Yan, S., & Mu, J. (2013). Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. Journal of Environmental Sciences, 25, S85-S88. http://dx.doi.org/10.1016/S1001-0742(14)60632-X

Halim, R., Gladman, B., Danquah, M. K., & Webley, P. A. (2011). Oil extraction from microalgae for biodiesel production. Bioresource technology, 102(1), 178-185. http://dx.doi.org/10.1016/j.biortech.2010.06.136

Halim, R., Harun, R., Webley, P. A., & Danquah, M. K. (2013). Bioprocess engineering aspects of biodiesel and bioethanol production from microalgae Advanced Biofuels and Bioproduct, Springer, 601-628.

Hall, J. (2012). Development of a Rapid in Situ Transesterification Method for Fatty Acid Analysis in Microalgae. http://dalspace.library.dal.ca//handle/10222/14790

Hilditch, T. P., & Williams, P. N. (1964). The chemical constitution of natural fats. The chemical constitution of natural fats.(4th edition).

Jiménez Callejón, M. J., Medina, A. R., Macías Sánchez, M. D., Pe-a, E. H., Cerdán, L. E., González Moreno, P. A., & Grima, E. M. (2014). Extraction of saponifiable lipids from wet microalgal biomass for biodiesel production. Bioresource technology. http://dx.doi.org/10.1016/j.biortech.2014.06.106

Johnson, M. B., & Wen, Z. (2009). Production of biodiesel fuel from the microalga Schizochytrium limacinum by direct transesterification of algal biomass. Energy & Fuels, 23(10), 5179-5183. http://dx.doi.org/10.1021/ef900704h

Jones, J., Manning, S., Montoya, M., Keller, K., & Poenie, M. (2012). Extraction of algal lipids and their analysis by HPLC and mass spectrometry. Journal of the American Oil Chemists' Society, 89(8), 1371-1381. http://dx.doi.org/10.1007/s11746-012-2044-8

Kaur, S., Sarkar, M., Srivastava, R. B., Gogoi, H. K., & Kalita, M. C. (2012). Fatty acid profiling and molecular characterization of some freshwater microalgae from India with potential for biodiesel production. New biotechnology, 29(3), 332-344. http://dx.doi.org/10.1016/j.nbt.2011.10.009

Keshini Beetul, Shamimtaz Bibi Sadally, Nawsheen Taleb-Hossenkhan, Ranjeet Bhagooli, & Puchooa, D. (2014). An investigation of biodiesel production from microalgae found in Mauritian waters. Biofuel Research Journal, 2, 58-64.
http://dx.doi.org/10.18331/BRJ2015.1.2.5

Kodandoor Sharathchandra, & Rajashekhar, M. (2011). Fatty acid composition in some fresh water cyanobacteria. Journal of Algal biomass utilization., 2(2), 83-97.

Korres, N. E., Singh, A., Nizami, A. S., & Murphy, J. D. (2010). Is grass biomethane a sustainable transport biofuel? Biofuels, Bioproducts and Biorefining, 4(3), 310-325.
http://dx.doi.org/10.1002/bbb.228

Kratz, W. A., & Myers, J. (1955). Nutrition and growth of several blue-green algae. American Journal of Botany, 282-287.
http://dx.doi.org/10.2307/2438564

Lee, S. J., Go, S., Jeong, G. T., & Kim, S. K. (2011). Oil production from five marine microalgae for the production of biodiesel. Biotechnology and Bioprocess Engineering, 16(3), 561-566.
http://dx.doi.org/10.1007/s12257-010-0360-0

Lepage, G., & Roy, C. C. (1986). Direct transesterification of all classes of lipids in a one-step reaction. Journal of Lipid Research, 27(1), 114-120.

Li, Y., Horsman, M., Wu, N., Lan, C. Q., & Dubois-Calero, N. (2008). Biofuels from microalgae. Biotechnology Progress, 24(4), 815-820.
http://dx.doi.org/10.1021/bp070371k

Perera, M. B. U., & Yatigammana, S. K. (2014). Distribution and Composition of Cyanobacteria in Sri Lankan Reservoirs. Symposium Proceedings, Third International Symposium on Water Quality and Human Health: Challenges Ahead, PGIS, Peradeniya, Sri Lanka(14).

Ma, Y., Wang, Z., Yu, C., Yin, Y., & Zhou, G. (2014). Evaluation of the potential of 9 Nannochloropsis strains for biodiesel production. Bioresource technology.
http://dx.doi.org/10.1016/j.biortech.2014.06.047

Magana-Arachchi, D., & Wanigatunge, R. (2013). First report of genus Chroococcidiopsis (cyanobacteria) from Sri Lanka: a potential threat to human health. Journal of the National Science Foundation of Sri Lanka, 41(1), 65-68.
http://dx.doi.org/10.4038/jnsfsr.v41i1.5334

Mori, F., Erata, M., & Watanabe, M. M. (2002). Cryopreservation of cyanobacteria and green algae in the NIES-Collection. Microbiol. Cult. Coll, 18, 45-55.

Müller, J., Day, J. G., Harding, K., Hepperle, D., Lorenz, M., & Friedl, T. (2007). Assessing genetic stability of a range of terrestrial microalgae after cryopreservation using amplified fragment length polymorphism (AFLP). American Journal of Botany, 94(5), 799-808.
http://dx.doi.org/10.3732/ajb.94.5.799

Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A., & Bux, F. (2011). Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. Bioresource technology, 102(1), 57-70.
http://dx.doi.org/10.1016/j.biortech.2010.06.077

Nakanishi, K., Deuchi, K., & Kuwano, K. (2012). Cryopreservation of four valuable strains of microalgae, including viability and characteristics during 15 years of cryostorage. Journal of Applied Phycology, 24(6), 1381-1385.
http://dx.doi.org/10.1007/s10811-012-9790-8

Nazir, N., Mangunwidjaja, D., Setyaningsih, D., Yuliani, S., Yarmo, M. A., Salimon, J., & Ramli, N. (2014). In-situ Alkaline Transesterification of Jatropha curcas seed Oil for Production of Biodiesel and Nontoxic Jatropha seed Cake. International Journal on Advanced Science, Engineering and Information Technology, 4(1), 07-13.
http://dx.doi.org/10.18517/ijaseit.4.1.353

Pandey, A. (2009). Handbook of plant-based biofuels: CRC Press Boca Raton.

Pandiaraj, D., Mubarak, A., Praveen, K., Ravikumar, S., & Thajuddin, N. (2012). Molecular characterization and phylogeny of Marine cyanobacteria from Palk Bay region of Tamil Nadu, India. Ecologia, 2, 23-30. http://dx.doi.org/10.3923/ecologia.2012.23.30

Park, P., & Goins, R. (1994). In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. Journal of Food Science, 59(6), 1262-1266. http://dx.doi.org/10.1111/j.1365-2621.1994.tb14691.x

Parmar, A., Singh, N. K., Pandey, A., Gnansounou, E., & Madamwar, D. (2011). Cyanobacteria and microalgae: a positive prospect for biofuels. Bioresource technology, 102(22), 10163-10172. http://dx.doi.org/10.1016/j.biortech.2011.08.030

Peccia, J., Haznedaroglu, B., Gutierrez, J., & Zimmerman, J. B. (2013). Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends in Biotechnology, 31(3), 134-138. http://dx.doi.org/10.1016/j.tibtech.2013.01.010

Pereira, H., Barreira, L., Mozes, A., Florindo, C., Polo, C., Duarte, C. V., . . . Varela, J. (2011). Microplate-based high throughput screening procedure for the isolation of lipid-rich marine microalgae. Biotechnology for biofuels, 4(1), 1-12. http://dx.doi.org/10.1186/1754-6834-4-61

Prasanna, R., Kumar, R., Sood, A., Prasanna, B., & Singh, P. (2006a). Morphological, physiochemical and molecular characterization of Anabaena strains. Microbiological Research, 161(3), 187-202. http://dx.doi.org/10.1016/j.micres.2005.08.001

Prasanna, R., Kumar, R., Sood, A., Prasanna, B., & Singh, P. (2006b). Morphological, physiochemical and molecular characterization of Anabaena strains. Microbiological Research, 161(3), 187-202. http://dx.doi.org/10.1016/j.micres.2005.08.001

Ranya Amer, Rehab Shehawy, Saraa Saad El-Dien, Marwa Abu Serie, & Shaker, K. (2013). Isolation and Characterization of Cyanobacterial Community Including a Microcystin-Producing Nostoc sp. Strain in the Nile River, Egypt. Advances in Microbiology, 3(6A), 38-46. doi: DOI:10.4236/aim.2013.36A005 http://dx.doi.org/10.4236/aim.2013.36A005

Řezanka, T., Zahradnik, J., & Podojil, M. (1982). Hydrocarbons in green and blue-green algae. Folia Microbiologica, 27(6), 450-454. http://dx.doi.org/10.1007/BF02876458

Ríos, S. D., Casta-eda, J., Torras, C., Farriol, X., & Salvadó, J. (2013). Lipid extraction methods from microalgal biomass harvested by two different paths: screening studies toward biodiesel production. Bioresource technology, 133, 378-388. http://dx.doi.org/10.1016/j.biortech.2013.01.093

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology, 111(1), 1-61. http://dx.doi.org/10.1099/00221287-111-1-1

Robertson, D. E., Jacobson, S. A., Morgan, F., Berry, D., Church, G. M., & Afeyan, N. B. (2011). A new dawn for industrial photosynthesis. Photosynthesis research, 107(3), 269-277. http://dx.doi.org/10.1007/s11120-011-9631-7

Rodríguez-Ruiz, J., Belarbi, E.-H., Sánchez, J. L. G., & Alonso, D. L. (1998). Rapid simultaneous lipid extraction and transesterification for fatty acid analyses. Biotechnology techniques, 12(9), 689-691. http://dx.doi.org/10.1023/A:1008812904017

Rós, P., Da, C., Silva, C. S., Silva-Stenico, M. E., Fiore, M. F., & Castro, H. F. D. (2013). Assessment of chemical and physico-chemical properties of cyanobacterial lipids for biodiesel production. Marine drugs, 11(7), 2365-2381. http://dx.doi.org/10.3390/md11072365

San Pedro, A., González-López, C., Acién, F., & Molina-Grima, E. (2013). Marine microalgae selection and culture conditions optimization for biodiesel production. Bioresource technology, 134, 353-361. http://dx.doi.org/10.1016/j.biortech.2013.02.032

Selvan, B. K., Revathi, M., Piriya, P. S., Vasan, P. T., Prabhu, D., & Vennison, S. J. (2013). Biodiesel production from marine cyanobacteria cultured in plate and tubular photobioreactors.

Sethunge, S., & Manage, P. M. (2013). Nuisance algae in water supply projects in Sri Lanka.
Sharathchandra, K., & Rajashekhar, M. (2011). Total lipid and fatty acid composition in some freshwater cyanobacteria. J. Algal Biomass Util, 2, 83-97.
Singh, A., Nigam, P. S., & Murphy, J. D. (2011). Renewable fuels from algae: an answer to debatable land based fuels. Bioresource technology, 102(1), 10-16. http://dx.doi.org/10.1016/j.biortech.2010.06.032
Smedes, F. (1999). Revisiting the development of the Bligh and Dyer total lipid determination method. Marine Pollution Bulletin, 38(3), 193-201. http://dx.doi.org/10.1016/S0025-326X(98)00170-2
Stanier, R., Kunisawa, R., Mandel, M., & Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriological Reviews, 35(2), 171.
Steinhoff, F. S., Karlberg, M., Graeve, M., & Wulff, A. (2014). Cyanobacteria in Scandinavian coastal waters—A potential source for biofuels and fatty acids? Algal Research, 5, 42-51. http://dx.doi.org/10.1016/j.algal.2014.05.005
Taher, H., Al-Zuhair, S., Al-Marzouqi, A. H., Haik, Y., & Farid, M. (2014). Effective extraction of microalgae lipids from wet biomass for biodiesel production. Biomass and Bioenergy, 66, 159-167. http://dx.doi.org/10.1016/j.biombioe.2014.02.034
Taton, A., Lis, E., Adin, D. M., Dong, G., Cookson, S., Kay, S. A., . . . Golden, J. W. (2012). Gene transfer in Leptolyngbya sp. strain BL0902, a cyanobacterium suitable for production of biomass and bioproducts. PloS one, 7(1), e30901. http://dx.doi.org/10.1371/journal.pone.0030901
Temina, M., Rezankova, H., Rezanka, T., & Dembitsky, V. M. (2007). Diversity of the fatty acids of the Nostoc species and their statistical analysis. Microbiological Research, 162(4), 308-321. http://dx.doi.org/10.1016/j.micres.2006.01.010
Thao, N. T. P., Tin, N. T., & Thanh, B. X. (2013). Biodiesel Production from Microalgae by Extraction–Transesterification Method. Waste Technology, 1(1), 6-9. http://dx.doi.org/10.12777/wastech.1.1.2013.6-9
VAN B. C. (1962). Studies on marine blue-green algae. Botanica marina, 4(1-2), 129-139.
Vicente, G., Martınez, M., & Aracil, J. (2004). Integrated biodiesel production: a comparison of different homogeneous catalysts systems. Bioresource technology, 92(3), 297-305. http://dx.doi.org/10.1016/j.biortech.2003.08.014
Wahlen, B. D., Willis, R. M., & Seefeldt, L. C. (2011). Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Bioresource technology, 102(3), 2724-2730. http://dx.doi.org/10.1016/j.biortech.2010.11.026
Yaniv, Z., Schafferman, D., Zur, M., & Shamir, I. (1996). Matthiola incana: Source of omega-3-linolenic acid. Progress in new crops. ASHS Press, Alexandria, VA, 368-372.