Energy Education Science and Technology Part A: Energy Science and Research
2012 Volume (issues) **29(1):** 687-700

Key issues to consider in microalgae based biodiesel production

Anoop Singh[1,*], Deepak Pant[2], Stig Irving Olsen[1], Poonam Singh Nigam[3]

[1] *Technical University of Denmark, Department of Management Engineering, Lyngby, Denmark*
[2] *Flemish Institute for Technological Research (VITO), Separation and Conversion Technology, Mol, Belgium*
[3] *University of Ulster, Faculty of Life and Health Sciences, Coleraine BT52 1SA, Northern Ireland, UK*

Received: 02 July 2011; accepted: 29 August 2011

Abstract

All nations have been confronted with the energy crisis due to depletion of finite fossil fuels reserves, which results an increasing global demand of biofuels for energy security, economic stability and reduction in climate change effects, and generate the opportunity to explore new biomass sources. The production of sustainable bioenergy is a challenging task in the promotion of biofuels for replacing the fossil based fuels to mitigate challenges of fossil based energy consumption. Algae might be a very promising source of biomass in this context as it sequesters a significant quantity of carbon from atmosphere and industrial gases and is also very efficient in utilizing the nutrients from industrial effluents and municipal wastewater. If developed sustainably, the algae biofuel industry may be able to provide large quantities of biofuels with potentially minimal environmental impacts. However, in order to realize this, a complete analysis of full life cycle impact of algal biofuel production in the context of issues such as water resource management, land use impact, energy balance and air emissions are very necessary. The commercial-scale production of algae requires careful consideration of many issues that can be broadly categorized into four main areas: selecting algae species that produce high oil levels and grow well in specified environments, algae growth methods, water sources and related issues, and nutrient and growth inputs.

Keywords: Algae; Biodiesel; Technology; Commercialization; Sustainability

1. Introduction

At present all nations have been confronted with the energy crisis due to depletion of finite fossil fuels reserves. Their continued consumption as energy source is not accepted as sustainable energy source due to depletion of resources and emissions of greenhouse gases (GHGs) in the environment [1] when used. The use of fossil fuels to satisfy the major energy requirements cause increasing anthropogenic GHG emissions and depletion of fossil reserves. Therefore, it is highly important to develop strong abatement techniques and adopt policies to promote those renewable energy sources which are capable of sequestering the atmospheric CO_2 to minimize the dependency on fossil reserves, and to maintain environmental and economic sustainability [2-6].

---

[*] Corresponding author. Tel.: +454-525-4744; fax: +454-593-3435.
E-mail address: apsinghenv@gmail.com (A. Singh).

The biomass based fuels can be a possible solution for all the issues related to energy production and consumption. They are renewable by nature, and there are possibilities to use them for heat, electricity and transportation fuel. Biofuels thus have the capabilities to replace fossil fuels, reduce dependency and provide a number of environmental, economical and social benefits [3-7], and can play a major role in achieving the renewable energy targets set by various countries especially for transport sector. Brazil has already shown the way where the cost of making ethanol from sugar has dropped by a factor of about 3 since they started making ethanol from cane sugar 25 years ago [8]. Energy produced from renewable sources, be it biofuels or bioelectricity [9-10] increasingly becomes a possible fuel option especially in the developed world. The first and second generation biofuels have several constraints (like food-fuel competition, land use change, higher resource use, energy balance, etc.) besides having several other benefits. Algal biofuels could be an answer for those constraints as it can grow very fast, is capable in production of several times higher biomass in comparison to terrestrial crops and trees, requires low and marginal land and other resources, producing higher lipid and carbohydrate, etc.

Keeping in view the benefits of the algal biofuels over first and second generation biofuels and sustainability issues for the production of biofuels, this article reviews the key issues in the sustainable production of algal biofuels.

2. Qualities of sustainable biofuels

World Commission on Environment and Development defined the term ‘sustainability’ as “the development that meets the needs of the present without compromising the ability of future generations to meet their own needs” [11]. Sustainable development encompasses economic, social, and ecological perspectives of conservation and change [12] that can be represented by sustainability matrices and indices (Fig. 1).

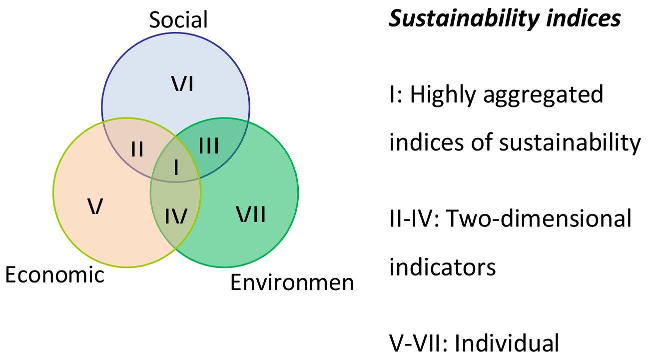

Fig. 1. The sustainability matrix and sustainability indices [12]**.**

Sustainability has become an unavoidable issue in all major planning and undertakings that involve future use of energy, water and other natural resources. In order to ensure sustainable growth, there is a need to satisfy the sustainability criteria and meet the constraints imposed by the finiteness of all natural resources and the dynamics of their natural renewal [13].

The sustainability of biofuels production depends on the net energy gain fixed in the biofuels that depends on the production process parameters, such as land type where the biomass is produced, the amount of energy-intensive inputs and the energy input for harvest, transport and running the processing facilities [14]. Additionally, there can be competition for land use between biomass crops versus food production. These parameters can vary considerably according to the raw material and local conditions, complicating life-cycle assessments and preventing any valid global statement on biofuel sustainability [15-16]. The large-scale biofuel production can only be deemed sustainable if the energy balance of the biofuel is significantly positive [14]. The biofuel production will be sustainable only if it is eco-friendly (less GHG emission, sequester large quantity of carbon, not affect the air, water, soil and biodiversity, etc.), be socially acceptable (provide employment, involved small stack holders, less inputs in the production) and economically viable (Fig. 2).

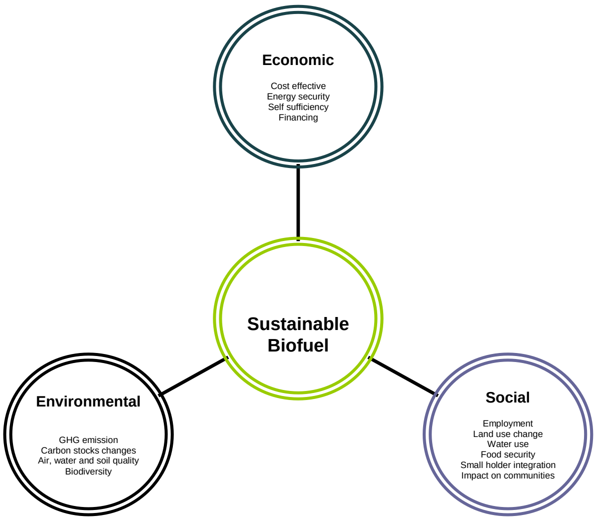

Fig. 2. Economic, social and environmental aspects of sustainable biofuels.

Markevičius et al. [17] found 35 criteria in emerging sustainability assessment frameworks and grouped them into social (15 criteria), economic (4 criteria) and environmental (16 criteria). Energy balance and greenhouse gas balance were perceived as especially critical, social criteria ranked generally low. Although being perceived as important, food security ranked very low.

Silva Lora et al. [18] defined the basic criteria and also provide sustainability indicators for a sustainable biofuels (Table 1). They also stated that the most used indicators to measure the biofuels sustainability are: Life Cycle Energy Balance (LCEB), quantity of fossil energy substituted per hectare, co-product energy allocation, life cycle carbon balance and changes in soil utilization.

Table 1. Criteria and sustainability indicators for sustainable biofuels [18].

Criteria	Sustainability indicators
i. To be carbon neutral, considering the necessity of fossil fuel substitution and global warming mitigation.	i. Economic indicators (cost of production)
ii. Not to affect the quality, quantity and rational use of available natural resources as water and soil.	ii. Output/Input relation (net energy analysis)
iii. Not to have undesirable social consequences as starvation because of high food prices.	iii. Substituted fossil fuel per hectare
iv. To contribute to the society economic development and equity.	iv. Avoided GHG emissions (CO_2 savings)
v. Not to affect biodiversity.	v. Environmental Impacts evaluation using impact categories indicators
	vi. Carbon emissions due to land use changes
	vii. Renewability indicators (exergy or emergy accounting)

3. Algal biodiesel

3. 1. Background

For several years, the research on microalgae focused on combining microalgae cultures that fix CO_2 with photosynthetic bacteria that produce H_2. Though lucrative and seemingly possible, the microalgae biohydrogen production research achieved little progress towards practical and commercial processes [19]. It is only in recent years that algae biofuels is referred as third generation biofuels, also known as ‘oilgae’. The first mention of algae biofuels was in a report published by MIT (Massachusetts Institute of Technology) in the early 1950s, when microalgae were mass cultured for the first time [20]. Proposals to use algae as a means of producing energy started in the late 1950s when Meier [21] and Oswald and Golueke [22] suggested the utilization of the carbohydrate fraction of algal cells for the production of methane via anaerobic digestion. A detailed engineering analysis in 1978 is reported by Benemann and co-workers [23], indicated that algal systems could produce methane at prices competitive with projected costs for fossil fuels.

The discovery that many microalgae species can produce large amounts of lipid as cellular oil droplets under certain growth conditions dates back to the 1940s. Various reports during the 1950s and 1960s indicated that starvation for key nutrients (nitrogen or silicon) could lead to this phenomenon. The concept of utilizing the lipid stores as a source of energy gained serious attention only during the oil embargo of the early 1970s and as the energy price surges through the decade [24].

The Aquatic Species Program (from 1978 until 1996) at DOE-NREL (The US department of Energy - National Renewable Energy Laboratory) represents one of the most comprehensive research efforts to date on microalgae fuels. During the early years, the emphasis was on using algae to produce hydrogen, but the focus changed to liquid fuels (biodiesel) in the early 1980s. Advances were made through algal strain isolation and characterization, studies of algal physiology and biochemistry, genetic engineering, process development, and demonstration-scale algal mass culture [24]. The algal biofuels research is still limited to the laboratories because the high efficiency is not be maintained after scaling-up the technology to a large production plant, while a number of large-scale pilot plants are in operation and focus on CO_2 capture from industrial emitters and demonstrate a good quantity of dry weight production in algal bioreactors [25].

3. 2. Benefits over first and second generation biofuels

The first-generation biofuels have been mainly extracted from food and oil crops as well as animal fats using conventional technology [7]. The increasing energy demand generates the competition in food and fuel crops production for the utilization of arable land, high water and fertilizer requirements, lack of well managed agricultural practices in emerging economies, biodiversity conservation and regionally constrained market structures. The sustainability of many first generation biofuels has been increasingly questioned over concerns such as reported displacement of food crops, effects on the environment and climate change [25].

The increasing criticism of the sustainability of many first-generation biofuels has raised attention to the potential of so called second-generation biofuels produced from lignocellulosic feedstocks, agriculture residues, grasses and municipal wastes, because it produces fewer GHGs and does not compete with food supply needs. Although significant progress continues to be made to overcome the technical and economic challenges, second-generation biofuels production still faces major constraints to execute commercial deployment [26]. The logistics of providing a competitive supply of biomass feedstock to a commercial plant is challenging, as is improving the performance of the conversion process to reduce costs [25]. Recently, it was reported that the cellulosic ethanol have slowed down due to the financial crisis and it is not sure when the growth in this field will pick up [27]. At the same time, the investment in algae as source of biofuel picked up and several big oil companies such as Exxon Mobil, British Petroleum (BP), Dow Chemical Company planned large investments in algal research [28].

The cultivation of algal biomass for the biofuels production has great promise because algae generate higher energy yield and require much less space to grow than conventional feedstocks and also the algal biomass production does not require fertile or arable land. So that algae would not compete with food and could be grown with minimal inputs using a variety of nutrient and carbon sources, GreenFuel Technologies Corporation called algae the fastest growing plant in the world [20]. The various possible energy production routes via microalgal biomass are shown in Fig. 2.

4. Technological development

4. 1. Strain selection

Approximately 22,000-26,000 species of microalgae exist of which only a few have been identified for successful commercial application [49]. Within the US Department of Energy's Aquatic Species Program (ASP) to develop microalgae as a source of biodiesel more than 3000 strains of microalgae from ponds and oceans have been isolated [50]. The lipid production varies within a vast range among the algal strains (4-80% dry weight basis) and the variation is as result of the environmental conditions. For example, in case of *Botryococcus braunii* Kützing [51], the yield of oil per unit area is estimated to be from 5000 to 20,000 gallons/acre/year which is 7–31 times greater than the next best crop, palm oil (635 gallons/acre/year) [52]. The best performing micro algae strain can be obtained by screening of a wide range of naturally available isolates and the efficiency of those can be improved by selection, adaptation and genetic engineering [25]. Isolation of suitable microalgae from the natural environment is the first critical step in developing oil-rich strains for further exploitation in engineered systems for the production of biodiesel feedstock [53]. Doan et al. [53] found that high-throughput cell sorting, coupled with flow cytometry can be a powerful tool to facilitate the rapid and efficient isolation of microalgae strains for onward axenic culture. The various autofluorescence emitted from microalgae species due to their different photosynthetic pigments have been monitored in flow cytomertry to identify algae [54]. The intracellular neutral lipid of isolated microalgae strains can be measured via fluorescence intensity of Nile Red stained cultures [55] simultaneously to cell growth. Therefore, flow cytometry along with cell sorting, could be a very effective tool for screening and development of microalgal strains for the sustainable production of algal biodiesel.

Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils [56-57]. Not all algal oils are satisfactory for making biodiesel, but suitable oils occur commonly [41]. A list of lipid, carbohydrate and protein composition of some microalgae is shown in Table 2. For microalgae that are able to survive heterotrophically, exogenous carbon sources offer prefabricated chemical energy that often is stored as lipid droplets [62]. Heterotrophically cultivated *Chlorella protothecoides* has accumulated higher lipids (about 55% of dry weight) compared to photoautotrophically (14% of dry weight) grown cells [63]. Another natural mechanism through which microalgae can alter lipid metabolism is the stress response owing to a lack of nitrogen availability [64]. Although nitrogen deficiency appears to inhibit the cell cycle and the production of almost all cellular components, the rate of lipid synthesis remains higher, which leads to the accumulation of oil in starved cells [65] and also promotes the accumulation of the antioxidant pigment astaxanthin in the green alga *Haematococcus pluvialis* [66]. Both of these adaptive responses help to ensure the survival in stress conditions [50].

Table 2. Chemical composition of some microalgae on the basis of % dry matter [36, 41, 58-61]

Algal species	**Proteins**	**Carbohydrates**	**Lipids**
Anabaena cylindrica	43-56	25-30	4-7
Botryococcus braunii	8-17	8-20	25-75
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Euglena gracilis	39-61	14-18	14-20
Isochrysis sp.	31-51	11-14	20-22
Neochloris oleoabundans	20-60	20-60	35-54
Porphyridium cruentum	28-39	40-57	9-14
Prymnesium parvum	28-45	25-33	22-38
Scenedesmus dimorphus	8-18	21-52	16-40
Scenedesmus obliquus	50-56	10-17	12-14
Scenedesmus quadricauda	48	17	21
Spirogyra sp.	6-20	33-64	11-21
Spirulina maxima	60-71	13-16	6-7
Spirulina platensis	46-63	8-14	4-9
Synechoccus sp.	63	15	11
Tetraselmis maculata	52	15	3

The ASP recognized that the key to unlocking profitable commercialization of microalgae lies not only in species selection and optimal cultivation, but also in genetic and metabolic engineering. Manipulation of metabolic pathways can redirect cellular function toward the synthesis of preferred products and even expand the processing capabilities of microalgae. One method of coercing microalgae employs specific environmental factors, such as nutrient regimens, to induce desired fluxes in metabolism. The metabolic engineering allows direct control over the organism’s cellular machinery through mutagenesis or the introduction of transgenes. The development of a number of transgenic algal strains boasting recombinant protein expression, engineered photosynthesis, and enhanced metabolism encourage the prospects of designer microalgae [50].

Another important aspect while selecting the microalgal strains is when microalgae growth is combined with CO_2-biomitigation. This is due the fact that microalgae have much higher growth rates and CO_2 fixation abilities compared to conventional forestry, agricultural and aquatic plants [67-68]. Several such algae species which included species of *Chlorella* and *Scenedesmus* capable of CO_2-biomitigation were listed by Wang et al. [47]. This approach of microalgal biofuel production becomes more attractive when combined with fixing industrial exhaust gases (flue gas) and integrating the cultivation of algae with wastewater treatment.

4. 2. Harvesting technology

Harvesting of algal biomass could be the most energy demanding process due to its concentration, smaller size and surface charge. Cells are more dilute in pond cultures in comparison to bioreactor cultures and natural oceanic conditions. A number of methods are available for harvesting algal biomass and Brentner et al. [69] have compared them in the study ‘Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel’. Centrifugation, filtration, and flocculation and/or settling are the main methods which generally used to harvest the micro-algal biomass.

The choice of method depends on the size and density of algae, target product and the production process used to get the final product. Filtration is the most commonly used method but only suitable for large micro algal strains (>70 μm) and unsuitable for strains having diameter less than 30 μm. Mohn [70] demonstrated that filtration processes can achieve a concentration factor of 245 times the original concentration for *Coelastrum proboscideum* to produce a sludge with 27% solids. For harvesting of algal strain having small size cells membrane microfiltration and ultrafiltration/centrifugation methods can be used [71].

Flocculation and settling are relatively low cost methods that only require energy for a short period to mix the cells with a coagulant. Flocculants neutralized or reduces the negative charge on the algal surface and prevent them sticking together in the suspension [72]. Algae responses differ significantly with certain flocculants and effectiveness of a particular flocculant and dosage varied tremendously from one algal species to another. Some algal species aggregate and settle with an increase in pH that can be controlled with CO_2 aeration or addition of lime [69]. Brennan and Owende [2] reported that multivalent metal salts like ferric chloride, aluminium sulphate and ferric sulphate are suitable flocculants [72-73]. Aluminum sulfate and chitosan could be a promising flocculant as they are produced from crustacean fishery waste and renewable in nature. Kim et al. [74] screened a number of flocculants to harvest *Scenedesmus* sp. and concluded that flocculation method using consecutive treatment with calcium chloride and ferric chloride and a bioflocculant from the culture broth of *Paenibacillus polymyxa* AM49 was found to be effective for the flocculation of a high density *Scenedesmus* sp. and also suggested that a flocculated medium can be effectively reused as a growth supporting medium without compromising with the algal growth and biomass yield, thereby significantly reducing the cost of biodiesel production from algae.

The centrifugation method is only feasible for relatively high value products [72] as it is very energy intensive process, though continuous centrifugation has been explored which might be more economic if systems are built on a large scale [75]. Gentle acoustically (ultrasound) induced aggregation followed by enhanced sedimentation can also be used to harvest microalgae biomass [2], this method is successfully used by Bosma et al. [76] and achieved 92% separation efficiency and a concentration factor of 20 times. Bruton et al. [60] reported that some micro algal strains naturally float at the surface of the water as lipid content increased and can be harvested by flotation method, while the evidence of its technical and economical effectiveness is very limited [2]. Gravity settling is only suitable for large micro algal strains (>70 μm)

4. 3. Oil extraction

Each algal cell has a sturdy cell wall which makes oil extraction complicated. The algae also have to be dried out before the oil extraction [77]. Lipid or oil extraction from microalgae may be one of the least developed areas among the algal biodiesel production processes [69]. Widjaja et al. [78] found that the drying temperature during lipid extraction from algal biomass affect both the lipid composition and content. Freeze drier retain the original composition of microalgal lipid, while higher temperature drying decreased the content of TAG. Drying at 60 °C still retained lipid composition with slight decrease in lipid content. Ultrasonication has no significant effect on extraction, while sufficient pulverization help to extract entire lipid from the cells.

There are several approaches to extract lipids from harvested algal biomass, including solvent extraction, osmotic shock, ultrasonic extraction, critical point CO_2 extraction, etc. Some common extraction methods explored in the last decade and their effectiveness at recovering lipids and lipid products are summarized by Mercer and Armenta [79]. Solvent extraction is a quick and efficient extraction method, applied directly on dried biomass [80]. Solvent extraction entails extracting oil from microalgae by repeated washing or percolation with an organic solvent. A number of solvents (hexane, ethanol or mixture of hexane–ethanol, benzene, cyclohexane, etc.) can be used and possible to obtain up to 98% fatty acids extraction [81]. Hexane is a popular choice due to its relatively low cost and high extraction efficiency [79]. Osmotic shock is a sudden reduction in osmotic pressure that can rupture the cells in a solution. Ultrasonic waves are used to create cavitation bubbles in a solvent and when these bubbles collapse near the cell walls, it creates shock waves and liquid jets that break cell wall and release their contents into the solvent. Supercritical fluid extraction involves the use of substances that have properties of both liquids and gases (i.e. CO_2) when exposed to increased temperatures and pressures. This property allows them to act as an extracting solvent, leaving no residues behind when the system is brought back to atmospheric pressure and room temperature [79].

Lee et al. [82] compared five methods (viz. autoclaving, bead-beating, microwaves, sonication, and a 10% NaCl solution) to identify the most effective cell disruption method. The total lipids from *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. were extracted using a mixture of chloroform and methanol (1:1) and identified microwave oven method as the most simple, easy and effective for lipid extraction from microalgae among the tested methods. Heger [77] reported that OriginOil (an algal biofuel company in Los Angeles) has developed a simpler and more efficient way to extract oil from algae by combining ultrasound and an electromagnetic pulse to break the algal cell walls. Then the algae solution is force-fed carbon dioxide, which lowers its pH and separates oil from the algal biomass.

Cravotto et al. [83] employed ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) techniques to extract oils from vegetable sources and a cultivated marine microalga rich in docosahexaenoic acid (DHA) and concluded that either alone or combined techniques can greatly improve the extraction of bioactive substances, achieving higher efficiency and shorter reaction times at low or moderate costs, with minimal added toxicity. Samori et al. [84] proposed switchable-polarity solvents (SPS) method to extract hydrocarbons from dried and water-suspended samples of microalga *Botryococcus braunii* based on 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) and an alcohol. The high affinity of the non-ionic form of DBU/alcohol SPS towards non-polar compounds exploited to extract hydrocarbons from algae, while the ionic character of the DBU-alkyl carbonate form (obtained with CO_2 addition) can be used to recover hydrocarbons from the SPS. On the basis of the results Samori et al. [84] concluded that SPS also have the advantage to be recyclable non-volatile/non-inflammable systems, therefore suited for non-hazardous small plants for biofuel production located nearby algal cultivation sites.

Widjaja et al. [78] found that cultivation of algal strains in nitrogen deficient media not only result in higher lipid accumulation but also gradually change the lipid composition from free fatty acid-rich lipid to lipid mostly contained TAG. Since accumulation of lipid occurs at nitrogen depletion condition under which the growth slow down or even no growth. Therefore, compromising between increasing lipid content and harvesting time is necessary to obtain higher lipid content and productivity. At higher CO_2 concentration under normal nutrition get higher lipid productivity and this can also be obtained by varying not only the length of starvation but also the length of normal nutrition. Relatively low-grade heat (such as waste heat) could be employed to separate solvents from oil in some circumstances, greatly increasing the overall economics. Osmotic shock, though requiring low-energy input, is probably the method with the lowest efficiency and creates a further issue for some downstream processes, as water content can be a problem requiring energy input to overcome. In one project under the ASP, solvent extraction costs were three times higher for algal oil than for soybean oil due to high moisture content of the paste in the experiment [65]. Mechanical dewatering (pressing and filtration) can be cheaper than heating [72], but the real key is to have as few steps as possible and simple scalable extraction.

4. 4. Biodiesel production

Bio-oils (derived from crop seed, animal feed, algal biomass, etc.) have high viscosity, high molecular weights, higher flash point (above 200 °C), low volumetric heating values compared to diesel fuels. It has been found that the use of such bio-oils as diesel fuels in conventional diesel engines leads to a number of problems due to significant difference in the injection, atomization and combustion characteristics of these oils in diesel engines. The problems with substituting triglycerides for diesel fuels are mostly associated with their high viscosities, low volatilities and polyunsaturated character [85]. Therefore, refinement is essential in order to turn bio-oils into quality fuel (biodiesel) [86]. Considerable efforts have been made to develop vegetable oil derivatives that approximate the properties and performance of the hydrocarbon-based diesel fuels and it can be changed mainly by four processes, viz. pyrolysis (thermal cracking), microemulsification, dilution and transesterification [85]. Balat [85] have described and Lin et al. [86] have summarized the pros and cons of all these processes. Both studies concluded that transesterification is the most promising solution to the high viscosity problem and biodiesel produced from transesterification with methanol quite similar to the conventional diesel in its main characteristics and compatible with conventional diesel that can be blended in any proportion. Currently, transesterification is the most accepted process for production of biodiesel from bio based oils, due to its high conversion efficiency and low cost. Fig. 3 shows energy production routes via microalgal biomass.

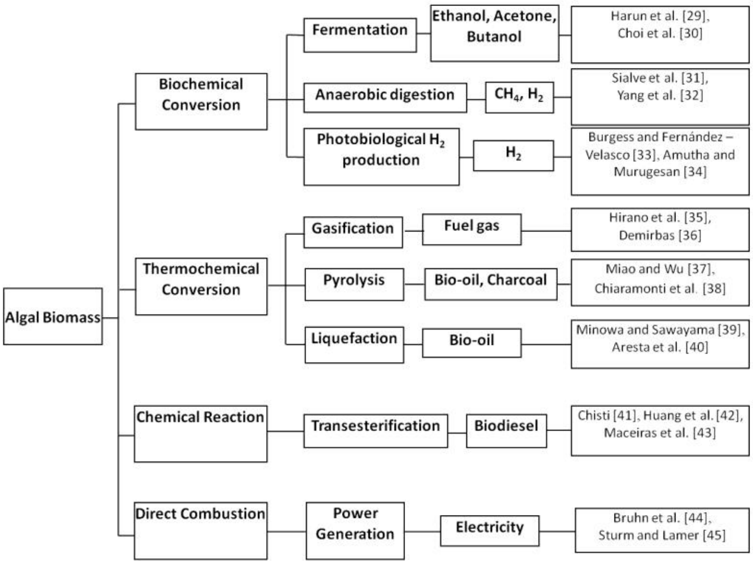

Fig. 3. Energy production routes via microalgal biomass.
(Modified from Tsukahara and Sawayama [49], Wang et al. [47], Brennan and Owende [2], Singh et al. [48]).

The extracted oil from algal biomass can be converted into biodiesel through transesterification that is a chemical reaction between triacylglycerides (TAGs) and alcohol in the presence of a catalyst to produce mono-esters that are termed as biodiesel [87]. Transesterification is a multiple step reaction, including three reversible steps in series, where triglycerides are converted to diglycerides, then diglycerides are converted to monoglycerides, and monoglycerides are then converted to esters (biodiesel) and glycerol (by-product) [80].

Microalgae biodiesel produced from transesterification has been found to have analogous properties (e.g. viscosity, density, flash point, cold filter plugging point, solidifying point and heating value) to the conventional diesel [88] and most of these parameters comply with the limits established by American Society for Testing and Materials (ASTM) [89] and International Biodiesel Standard for Vehicles (EN14214) [90].

Some microalgae produce high levels of TAGs but the growth of such strain might be slow and many marine species produce higher levels of phospholipids than TAGs, which do not behave optimally during transesterification. There is also the issue to utilize the byproducts (remaining biomass and glycerol). Glycerol has been used in the soap industry in the past but the increased amounts being presently produced are not being absorbed in this way, representing the difficulty of economics of co-products matching the scale of commodities like transport fuels. New ways of using this by-product, such as for plastic production are becoming economic. Proteinaceous and polysaccharide remnants can be used in a variety of ways, the integrative approach of consuming this in anaerobic digesters is one pathway [91] and protein-rich residues can be used as fertilizers.

5. Major constraints in commercialization of algal biodiesel

Biodiesel is currently produced from plant and animal oils, but not from microalgae [92]. One of the biggest challenges in this regard is to reproduce laboratory conditions on a large scale. In the lab, it is easier to control algal growth and find strains that produce large quantities of oil [27]. This situation is however likely to change as several companies are attempting to commercialize microalgal biodiesel [27-28]. Oil productivity, that is the mass of oil produced per unit volume of the microalgal broth per day, depends on the algal growth rate and the oil content of the biomass. Microalgae with high oil productivities are desired for producing biodiesel. These algae can even be nourished on recycled sources and can play a role in the treatment of wastewater and avoid the disposal problem [93]. However, producing microalgal biomass is generally more expensive than growing crops. Photosynthetic growth requires light, carbon dioxide, water and inorganic salts. Temperature must remain generally within 20 to 30 °C. To minimize expense, biodiesel production must rely on freely available sunlight, despite daily and seasonal variations in light levels [41]. It has been argued that growing algae in bioreactors requires fossil fuels for the building of these bioreactors and for their operational activities [94].

Manipulation of metabolic pathways can redirect cellular function toward the synthesis of products and even expand the processing capabilities of microalgae [93]. The crucial economic challenge for algae producers is to discover low cost oil extraction and harvesting methods [95]. Singh and Gu [95] suggested that the utilization of fatter algae with higher oil content (about 60%) in comparison to lower oil content algae can reduce up to half of the size and footprint of algae biofuels production systems and reduces the capital and operating costs and a cheaper and easier process can provided a better ground to commercialize the algal biodiesel. The algal micro-refineries can avoid the harvesting, extraction and refining systems by excreting lipids directly from the cells using non-lethal extraction known as milking. Carotenoids, high value lipids, have also been selectively extracted from the green alga *Chlorella* sp. using decane [96]. Such methods have the capability to reduce the production cost significantly and simplify the biodiesel production process from algal biomass.

6. Future promises

Ongoing advances in cultivation techniques coupled with genetic manipulation of crucial metabolic networks will further promote microalgae as an attractive platform for the production of numerous high value compounds [50]. Besides growing algae in well defined conditions and provided with ideal growth substrates, algae can be grown on industrial waste or by-product. Recently it was shown that alga *Schizochytrium limacinum* could be grown on crude glycerol, which is a major byproduct of commercial biodiesel production. The algae produced from biodiesel-derived glycerol had a composition similar to the commercial algae [97].

The sustainability of a fuel product depends on its environmental, economic and social impacts throughout the products entire life cycle. The complete life cycle of the fuel product includes everything from raw material production and extraction, processing, transportation, manufacturing, storage, distribution and use. A fuel chain and its life cycle stages cause various harmful impacts on the environment. In addition, the life cycle stages can have harmful effects or benefits of different economic and social dimensions. For this reason, the total management of complete fuel chains (cradle-to-grave) from different perspectives is of crucial importance in order to achieve sustainable fuel products and systems in our society. For this purpose LCA appears to be a valuable tool and its use for the assessment of the sustainability of not only fuel products, but also of other commodities has increased dramatically in recent years [17]. Several LCA-studies has been performed on algal biofuels (biodiesel, biohydrogen and biogas) [69, 98-101]. The studies suggest that algal biofuel can be an environmentally friendly solution compared to other types of fuel. The LCA studies can further be used for identification of main environmental improvements in the technology development (e.g. recirculation of the sewage and reuse of the remains for animal feed). In addition to the technological challenges described above the studies indicate that further development of the technologies from an environmental point of view should focus on reusing all byproducts and on reducing electricity use during processing and growing.

7. Conclusions

Biodiesel from microalagae has the potential to replace fossil-based petroleum; seems technically feasible and conversion of extracted lipid to biodiesel is relatively easy. The commercial-scale production of algal biofuels requires careful consideration of several issues that can be broadly categorized as: selection of high oil and biomass yielding algal species, cultivation and harvesting technology, water sources, and nutrient and growth Inputs [101-106]. The cultivation of algal biomass provides dual benefit, i.e. biomass for the production of biofuels and also save our environment from air and water pollution and minimizes the waste disposal problems by utilizing wastewater and flue gases for the growth. The life cycle assessment, energy balance, biofuels yield per unit area, carbon balance, land use, water and nutrient sources are very important factors to decide the sustainability of algal biofuels.

References

[1] Demirbas A. Use of algae as biofuel sources. Energy Convers Manage 2010;51:2738–2749.
[2] Brennan L, Owende P. Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 2010;14:557–577.
[3] Prasad S, Singh A, Jain N, Joshi HC. Ethanol production from sweet sorghum syrup for utilization as automotive fuel in India. Energy Fuels 2007;21:2415–2420.
[4] Prasad S, Singh A, Joshi HC, Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour Conserv Recycl 2007;50:1–39.
[5] Singh A, Pant, D, Korres, NE, Nizami, AS, Prasad, S and Murphy, JD. Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: challenges and perspectives. Bioresour Technol 2010;101:5003–5012.
[6] Singh A, Smyth BM, Murphy JD. A biofuel strategy for Ireland with an emphasis on production of biomethane and minimization of land-take. Renew Sustain Energy Rev 2010;14:277–288.

[7] Nigam, PS, Singh A. Production of liquid biofuels from renewable resources. Prog Energ Combus Sci 2011;37:52–68.
[8] Dale B. Biofuels: Thinking clearly about the issues. J Agric Food Chem 2008;56:3885–3891.
[9] Pant D, Bogaert GV, Diels L, Vanbroekhoven K. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 2010;101:1533–1543.
[10] Pant D, Singh A, Bogaert GV, Diels L, Vanbroekhoven K. An introduction to the life cycle assessment (LCA) of bioelectrochemical systems (BES) for sustainable energy and product generation: relevance and key aspects. Renew Sustain Energ Rev 2011;15:1305–1313.
[11] UNCED. Report of The United Nation Conference on Environment and Development, Vol. 1, Chapter 7, June, 1992.
[12] Lapkin A. Sustainability Performance Indicators. Dewulf J, Langenhove, HV (Eds.), Renewables-based technology sustainability assessment. John Wiley & Sons, Ltd, England 2006.
[13] Afgan NH. Sustainability Concept for Energy,Water and Environment Systems. In: K. Hanjali´c, R. Van De Krol, A. Leki'c (Eds.), Sustainable Energy Technologies: Options and Prospects, 25-49. Springer, The Netherlands 2008.
[14] Haye S, Hardtke CS. The Roundtable on Sustainable Biofuels: plant scientist input needed. Trends Plant Sci 2009;14:409–412.
[15] Davis SC, Anderson-Teixeira KJ, DeLucia EH. Life-cycle analysis and the ecology of biofuels. Trends Plant Sci 2009;14:140–146.
[16] Farrell AE, Pelvin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM. Ethanol can contribute to energy and environmental goals. Science 2006;311:506–508.
[17] Markevičius A, Katinas V, Perednis E, Tamasauskiene M. Trends and sustainability criteria of the production and use of liquid biofuels. Renew Sustain Energ Rev 2010;4:3226–3231.
[18] Silva Lora EE , Escobar Palacio JC, Rocha MH, Grillo Renó ML, Venturini OJ, del Olmo OA. Issues to consider, existing tools and constraints in biofuels sustainability assessments. Energy 2011;36:2097–2110.
[19] Benemann JR. Hydrogen production by microalgae. J Appl Phycol 2000;12:291–300.
[20] Girardet H, Mendonça M. A renewable world energy, ecology, equality: a report for the world future council. Green Books Ltd. UK. 2009.
[21] Meier RL. Biological cycles in the transformation of solar energy into useful fuels. Solar Energy Research, Daniels F, Duffie JA, editors, Madison University Wisconsin Press, pp 179–183, 1955.
[22] Oswald WJ, Golueke CG. Biological transformation of solar energy. Adv Appl Microbiol 1960; 11:223–242.
[23] Benemann JR, Pursoff P, Oswald WJ. Engineering design and cost analysis of a large-scale microalgae biomass system. Final Report to the US Energy Department, NTIS# H CP/ T, 1605(UC-61), 91, 1978.
[24] USDOE. National Algal biofuels technology roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program, 2010.
[25] Singh, A., Nigam, PS and Murphy, JD. Mechanism and challenges in commercialisation of algal biofuels. Bioresour Technol 2011;102:26–34.
[26] Sims REH, Mabee W, Saddler JN, Taylor M. An overview of second generation biofuel technologies. Bioresour Technol 2010;101:1570–1580.
[27] Kwok R, Cellulosic ethanol hits roadblock. Nature 2009;461:582–583.
[28] Mascarelli AL, Gold rush for algae. Nature 2009;461:460–461.
[29] Harun R, Jason WSY, Cherrington T, Danquah MK. Exploring alkaline pre-treatment of microalgal biomass for bioethanol production, Appl Energ 2011;88:3464–3467.
[30] Choi SP, Nguyen MT, Sim SJ. Enzymatic pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production, Bioresour Technol 2010; 01:5330–5336.
[31] Sialve B, Bernet N, Bernard O, Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable, Biotechnol Adv 2009;27:409–416.
[32] Yang Z, Guo R, Xu X, Fan X, Luo S. Fermentative hydrogen production from lipid-extracted microalgal biomass residues. Appl Energ 2011;88:3468–3472.

[33] Burgess G, Fernández-Velasco JG. Materials, operational energy inputs, and net energy ratio for photobiological hydrogen production, Int J Hydrogen Energ 2007;32:1225–1234.

[34] Amutha KB, Murugesan AG. Biological hydrogen production by the algal biomass *Chlorella vulgaris* MSU 01 strain isolated from pond sediment, Biores Technol 2011;102:194–199.

[35] Hirano A, Hon-Nami K, Kunito S, Hada M, Ogushi Y. Temperature effect on continuous gasification of microalgal biomass: theoretical yield of methanol production and its energy balance, Catalysis Today 1998;45:399–404.

[36] Demirbas MF. Biofuels from algae for sustainable development. Appl Energy 2011;88:3473–3480.

[37] Miao X, Wu Q. High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*, J Biotechnol 2004;110:85–93.

[38] Chiaramonti D, Oasmaa A, Solantausta Y. Power generation using fast pyrolysis liquids from Biomass. Renew Sustain Energ Rev 2007;11:1056–1086.

[39] Minowa T, Sawayama S. Novel microalgal system for energy production with nitrogen cycling, Fuel 1999;78:1213–1215.

[40] Aresta M, Dibenedetto A, Carone M, Colonna T, Fragale C. Production of biodiesel from macroalgae by supercritical CO_2 extraction and thermochemical liquefaction. Environ Chem Lett 2005;3:136–139.

[41] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306.

[42] Huang G, Chen F, Wei D, Zhang X, Chen G, Biodiesel production by microalgal biotechnology, Appl Energy 2010;87:38–46.

[43] Maceiras R, Rodrıguez M, Cancela A, Urréjola S, Sánchez A. Macroalgae: Raw material for biodiesel production. Appl Energy 2011;88:3318-3323.

[44] Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Bo Rasmussen M, Markager S, Olesen B, Arias C, Jensen PD. Bioenergy potential of *Ulva lactuca*: Biomass yield, methane production and combustion. Biores Technol 2011; 102:2595-2604.

[45] Sturm BSM, Lamer SL. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Applied Energy 2011;88:3499-3506.

[46] Tsukahara K, Sawayama S. Liquid fuel production using microalgae. J Jpn Petrol Inst 2005;48:251–259.

[47] Wang B, Li Y, Wu N, Lan CQ. CO_2 bio-mitigation using microalgae. Appl Microbiol Biotechnol 2008;79:707–718.

[48] Alp D, Cirak B. Biofuels from micro- and macroalgae. Energ Educ Sci Technol Part-A 2012;28:719-726.

[49] Norton TA, Melkonian M, Andersen RA. Algal biodiversity. Phycologia 1996;35:308–326.

[50] Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Curr Opin Biotechnol 2008;19:430–436.

[51] Smittenberg R, Baas HM, Schouten S, Damste JSS. The demise of the alga, *Botryococcus braunii* from a Norwegian fjord was due to early eutrophication. Holocene 2005;15:133–140.

[52] Raja R, Hemaiswarya S, Kumar NA, Sridhar S, Rengasamy R. A perspective on the biotechnological potential of microalgae. Crit Rev Microbiol 2008;34:77–88.

[53] Doan TTY, Sivaloganathan B, Obbard JP. Screening of marine microalgae for biodiesel feedstock. Biomass Bioenergy 2011; 35:2534–2544.

[54] Davey HM, Kell DB. Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analyses. Microbiol Rev 1996;60:641–696.

[55] Cooksey KE, Guckert JB, Williams SA, Callis PR. Fluorometric determination of the neutral lipid-content of microalgal cells using Nile Red. J Microbiol Meth 1987;6:333–345.

[56] Banerjee A, Sharma RYC, Banerjee UC. *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. Crit Rev Biotechnol 2002;22:245–279.

[57] Guschina IA, Harwood JL. Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 2006;45:160–186.

[58] Vazquez-Duhalt R, Arredondo-Vega BO. Haloadaptation of the green alga *Botryococcus braunii* (race a). Phytochemistry 1991;30:2919–2925.

[59] Renaud SM, Van Thinh L, Lambrinidis G, Parry DL. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 2002;211:195-214.

[60] Bruton T, Lyons H, Lerat Y, Stanley M, BoRasmussen M. A review of the potential of marine algae as a source of biofuel in Ireland. Sustainable Energy Ireland, 2009.

[61] Pruvost J, Van Vooren G, Le Gouic B, Couzinet-Mossion A, Legrand J. Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application. Bioresour Technol 2011;102:150–158.

[62] Konur O. The evaluation of the research on the biodiesel: a scientometric approach. Energ Educ Sci Technol Part-A 2012;28:1003–1014.

[63] Wu Q, Miao X. Biodiesel production from heterotrophic microalgal oil. Bioresour Technol 2006;97:841–846.

[64] Tornabene TG, Holzer G, Lien S, Burris N. Lipid composition of the nitrogen starved green alga *Neochloris oleoabundans*. Enzyme Microb Technol 1983;5:435–440.

[65] Sheehan J, Dunahay T, Benemann J, Roessler P. A Look Back at the U.S. Department of Energy’s Aquatic Species Program: Biodiesel from algae golden, Colorado: TP-580-24190, National Renewable Energy Laboratory, 1998.

[66] Boussiba S. Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. Physiol Plant 2000;108:111–117.

[67] Borowitzka MA. Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 1999;70:313–321.

[68] Li Y, Horsman M, Wu N, Lan CQ. Dubois-Calero N, Biofuels from microalgae. Biotech Prog 2008;24:815–20.

[69] Demirbas A. High quality water supply for the production of algae. Energ Educ Sci Tech-A 2011;27:467–476.

[70] Mohn FH. Experiences and strategies in the recovery of biomass from mass cultures of microalgae. In: Shelef G, Soeder CJ, editors. Algae biomass. Elsevier, Amsterdam, 1980.

[71] Petrusevski B, Bolier G, Van Breemen AN, Alaerts GJ. Tangential flow filtration: a method to concentrate freshwater algae. Water Res 1995;29:1419–1424.

[72] Molina Grima E, Acién Fernández, FG, García Camacho F, Chisti Y. Photobioreactors: light regime, mass transfer, and scaleup. J Biotechnol 1999;70:231–247.

[73] Divakaran R, Pillai VNS. Flocculation of algae using chitosan. J Appl Phycol 2002;14:419–422.

[74] Kim DG, La HJ, Ahn CY, Park YH, Oh HM. Harvest of *Scenedesmus* sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. Bioresour Technol 2011;102:3163–3168.

[75] Briggs M. Widescale Biodiesel Production from Algae. Biodiesel Group, Physics Department, University of New Hampshire, US, 2004.

[76] Bosma R, van Spronsen WA, Tramper J, Wijffels RH. Ultrasound, a new separation technique to harvest microalgae. J Appl Phycol 2003;15:143–153.

[77] Heger M. A New Processing Scheme for Algae Biofuels, 2009. http://www.technologyreview.com/energy/22572/page1/.

[78] Widjaja A, Chien CC, Ju YH. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. J Taiwan Inst Chem Engineers 2009;40:13–20.

[79] Mercer P, Armenta RE. Developments in oil extraction from microalgae. Eur J Lipid Sci Technol 2011;113:539–547.

[80] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. Renew Sustain Energ Rev 2010;14:217–232.

[81] Richmond A. Handbook of microalgal culture: biotechnology and applied phycology, Blackwell Science Ltd, 2004.

[82] Lee JY, Yoo C, Jun SY, Ahn CY, Oh HM. Comparison of several methods for effective lipid extraction from microalgae. Bioresour Technol 2010;101:S75–S77.

[83] Cravotto G, Boffa L, Mantegna S, Perego P, Avogadro M, Cintas P. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. Ultrasonics Sonochemistry 2008;15:898–902.

[84] Samori C, Torri C, Samori G, Fabbri D, Galletti P, Guerrini F, Pistocchi R, Tagliavini E. Extraction of hydrocarbons from microalga *Botryococcus braunii* with switchable solvents. Bioresour Technol 2010;101:3274–3279.

[85] Balat M. Challenges and opportunities for large-scale production of biodiesel. Energ Educ Sci Tech-A 2011;27:427–434.

[86] Lin L, Cunshan Z, Vittayapadung S, Xiangqian S, Mingdong D. Opportunities and challenges for biodiesel fuel. Appl Energ 2011;88:1020–1031.

[87] Altun S. Fuel properties of biodiesels produced from different feedstocks. Energ Educ Sci Tech-A 2011;26:165–174.

[88] Xu H, Miao X, Wu Q. High quality biodiesel production from a microalgae *Chlorella protothecoides* by heterotrophic growth in fermenters. J Biotechnol 2006;126:499–507.

[89] Ilkilic C, Aydin S, Behcet R. Production of biodiesel from safflower oil. Energ Educ Sci Tech-A 2011;27:295–300.

[90] Ahmad AL, Mat Yasin NH, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: A review. Renew Sustain Energy Rev 2011;15:584–593.

[91] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends Biotechnol 2008; 26:126–131.

[92] Hussain K, Nawaz K, Majeed A, Lin F. Economically effective potential of algae for biodiesel production. World Appl Sci J 2010;9:1313–1323.

[93] Singh, A., Nigam, PS and Murphy, JD. Renewable fuels from algae: An answer to debatable land based fuels. Bioresour Technol 2011;102:10–16.

[94] Reijnders L. Do biofuels from microalgae beat biofuels from terrestrial plants? Trends Biotechnol 2008;26:349–350.

[95] Singh J, Gu S. Commercialization potential of microalgae for biofuels production. Renew Sustain Energy Rev. 2010;14:2596–2610.

[96] Hejazi MA, Kleinegris D, Wijffels RH. Mechanism of extraction of beta-carotene from microalga *Dunaliellea salina* in two-phase bioreactors, Biotechnol Bioeng 2004;88:593–600.

[97] Pyle, DJ, Garcia RA, Wen Z. Producing docosahexaenoic acid (DHA)-rich algae from biodiesel-derived crude glycerol: Effects of impurities on DHA production and algal biomass composition. J Agric Food Chem 2008;56:3933–3939.

[98] Collet P, Hélias A, Lardon L, Ras M, Goy RA, Steyer JP. Life-cycle assessment of microalgae culture coupled to biogas production. Bioresour Technol 2011;102:207–14.

[99] Clarens AF, Resurreccion EP, White MA, Colosi LM. Environmental life cycle comparison of algae to other bioenergy feedstocks. Environ Sci Technol 2010;44:1813–1819.

[100] Soratana K, Landis AL. Evaluating industrial symbiosis and algae cultivation from a life cycle perspective. Bioresour Technol 2011;102:6892–6901.

[101] Campbell PK, Beer T, Batten D. Life cycle assessment of biodiesel production from microalgae in ponds. Bioresour Technol 2011;102:50–56.

[102] Sahin Y. Environmental impacts of biofuels. Energ Educ Sci Tech-A 2011;26:129–142.

[103] Ilkilic C. Performance and emissions characteristics of biofuel blend in a CI engine. Energ Educ Sci Tech-A 2011;28:369-378.

[104] Singh, A., Olsen SI. And Nigam, PS. A viable technology to generate third generation biofuel. Journal of Chemical Technology and Biotechnology. 2011;86:1349-1353.

[105] Konur O. The evaluation of the research on the biofuels: a scientometric approach. Energ Educ Sci Tech-A 2012;28:903-916.

[106] Aksoy L. Process optimization for biodiesel production from *Nigella sativa* oil. Energ Educ Sci Tech-A 2011;27:359–366.

[107] Ekim I, Ak A. Use of vegetable oils and animal fats in Diesel engines. Energ Educ Sci Technol Part-A 2012;28:683-688.

[108] Demirbas MF. Educational approach to the future potential of energy and food crisis. Energ Educ Sci Tech-B 2011;3:423–430.