Emir. J. Food Agric. 2014. 26 (8): 679-692
doi: 10.9755/ejfa.v26i8.18364
http://www.ejfa.info/

Plant Science

REVIEW ARTICLE

Plants and algae species: Promising renewable energy production source

V. Kumar[1*] and S. Mohan Jain[2]

[1]*Director General, Albert Einstein Science Institute, Bihar, India*
[2]*Department of Agricultural Sciences, PL-27, University of Helsinki 00014, Helsinki, Finland*

Abstract

Rapid increase in fossil energy consumption has raised price and environmental issues such as carbon dioxide emission, environmental pollution, depletion of ozone layer. Attempts are being made to reduce carbon dioxide emission from fossil fuels. Biomass is recognized as an important source for renewable energy production, known for emitting low quantity carbon dioxide and methane. Moreover, carbon dioxide is utilized in algae cultivation for biofuel production. Plants have become major source for biofuel production, which varies widely depending on crop type and yield per hectare, production systems, and conversion efficiency across crops. Certainly, high yielding biomass per hectare producing crops would be well suited in cost effective manner. Different processes of biofuel production from some major energy crops including sugarcane, sorghum, and algae will be discussed, especially bio-ethanol and bio-diesel.

Key words: Algae bio-diesel, Algae cultivation, Biofuel, Bio-ethanol, Sorghum, Sugarcane

1. Introduction

Shortage of fossil fuels, increasing crude oil price, energy security and increasing global warming concerns have resulted in the growing worldwide interest in renewable energy sources such as biofuels. In the recent years, algae have received lots of attention as a new and advanced biomass source for the production of renewable energy (Banerjee et al., 2002). There are some unique properties that set algae apart from other sources of biomass, such properties are their rapid growth rate, high lipid production capacity, CO_2 fixation ability. They do not compete with food or feed crops and can be produced on wastelands. Fresh water is not essential and nutrient can be supplied from wastewater. The first differentiation that needs to be made is between microalgae verses macroalgae. Microalgae, mostly called seaweeds, are organisms that live as single cells or in colonies without any specialization and have many different species with widely varying compositions. Their small size makes subsequent harvesting more complicated, although their cultivation is much easier and controllable. Macroalgae are very less versatile and there are a fewer options of species to cultivate and only one main viable technology to produce renewable energy from them. Microalgae as the name suggests are microscopic photosynthetic organism found in both marine and fresh-water environments. In the recent years, microalgae has become the exclusive focus of research for the production of biodiesel, as microalgae generally produce more of the right kinds of natural oils needed for biodiesel production.

Plant-based biofuel from sweet sorghum for the production of ethanol has generated interest since 1970s. Juice from sweet sorghum can be converted into ethanol using conventional fermentation technology. Sugarcane which is also known as 'miracle biofuel' is also common feedstock used for the production of biofuel. Sugarcane ethanol is made from sucrose found in sugarcane juice and molasses. Brazilians are leading bioethanol production from sugarcane, and routinely used blended with regular fossil fuel (Valdes, 2011). India is the largest producer of castor oil from castor bean producing over 75% of the world supply (Scholz and Da Silva, 2008). This oil is used in over 100 different applications in diverse industries such as paints, lubricants, cosmetics, paper, rubber.

An effort has been made to present an overview of total processes involved in the production of

Received 19 April 2013; Revised 17 March 2014; Accepted 22 March 2014; Published Online 15 June 2014

*Corresponding Author

V. Kumar
Director General, Albert Einstein Science Institute, Bihar, India

Emails: vivek@servenature.in; scientistvivek001@gmail.com

renewable fuel production from plants and algae species in detail.

2. Algae as feedstock for biofuel production

Algae (Singular *alga)* is a term that covers microscopic single or multicellular organisms or simple aquatic plants which do not have root, stem or leaves and have primitive methods of reproduction. They are photosynthetic and use light energy to convert inorganic substances into simple sugars using the captured energy. They live in wide range of aquatic environments and moreover, many are also terrestrial, living in soil, or snow or in association with other organism such as fungi (lichens) and animals.

Algae are among the most robust existing organism with the ability to grow in diverse conditions, common in both aquatic and terrestrial environments. Microalgae, although known to the most primitive form of plant, their photosynthetic mechanism is however closely related to that of higher plants. The simple cellular structure makes them highly efficient in solar energy conversion. As the cells grow in aqueous suspension, they are more accessible to water, CO_2 and other nutrients. Thus, microalgae have the potential to produce 30 times the amount of oil per unit area of land as compared to any other terrestrial oilseed crops (Brune et al., 2009).

Algae strain selection

Algae strain selection is very crucial part of whole system in order to identify and maintain suitable promising algal strain for cultivation and development. As cultivation of algae on mass scale is still facing problems, there is a need of isolation of new strains of algae from a wide variety of environments to provide a wide range in metabolic versatility possible.

Strain isolation, screening and selection

The isolation of algae can be done from a large variety of natural aqueous habitats growing in freshwater, brackish water, marine, soil and hyper-saline environment (Sieracki et al., 2004). Additionally, large-scale sampling should be coordinated to ensure broadest coverage of environments. The specific location can be determined by advanced site selection criteria through the combined use of dynamic maps, Geographical Information System (GIS) data and analysis tools for selection. Ecosystems to be sampled may include aquatic environments such as ocean, lakes, rivers, streams, ponds, geothermal springs which include hyper-saline, fresh ,brackish, acidic and alkaline environments, terrestrial environments in variety of geographical locations in order to maximize the genetic diversity. Moreover, algae are typically found in planktonic and benthic environments within an aqueous habitat. In suspended mass cultures, planktonic algae may be used, whereas biofilm-based production facilities may be achieved in benthic algae. Traditional cultivation techniques such as enrichment cultures may be used for the isolation of new strains from natural habitats (Andersen and Kawachi, 2005). Because of morphological similarities when comparing many algae species, actual strain identification should be based on molecular methods like rRNA sequence comparison, or in the case of closely related strains, other gene markers (Fishman et al., 2010).

Screening criteria and methods

The major three areas should be covered with good screen: growth physiology, metabolite production and strain robustness. A number of parameters such as maximum specific growth rate, maximum cell density, tolerance to environmental variables or/and nutrients requirement should be encompassed by growth physiology as well as all the parameters require significant experimental effort, automated systems would be helpful. It provides information on screening parameters for metabolite production and may involve in the determination of the cellular composition of proteins, lipids and carbohydrate, and measuring organism productivity of metabolites useful for the biofuels production.

Culture collection for database

It is very necessary to preserve the diversity of natural habitats, protect genetic material, and provide basic research and resources, and culture collections. Currently, few major algal collection centers exist in the U.S and some other countries. Organizations collecting and maintaining cultures could be responsible for dissemination and gathering detailed information on potentially valuable strains. The information could include:-strain properties (cytological, biochemical, molecular and screening results); mutants; plasmids and phages; strain administration (number in collection and preservation); practical applications (general and commercial); strain name, growth conditions and germination conditions; biological interactions (symbiosis, pathogenicity and metabolomics; environment and strain history.

Table 1. Advantages and disadvantages of closed photo bioreactor and open pond algae cultivation.

Algae Cultivation System	Phototrophic Cultivation Systems	Advantages	Disadvantages
Photoautotrophic Cultivation	Closed Photobioreactors	•Water loss is less than open pond •Superior long-term culture maintenance •Higher surface to volume ratio can support higher volumetric cell densities	•Scalability problems •Require temperature maintenance as they do not have evaporative cooling •May require periodic cleaning due to biofilm formation •Need maximum light exposure •Subject to daily and seasonal changes in temperature and humidity
	Open Pond	•Evaporative cooling maintains temperature •Lower capital costs	•Inherently difficult to maintain monocultures •Need maximum light exposure

Algae cultivation

The Algae Culture System is much different between macroalgae (seaweed) and microalgae because of their small (μm) size. The cultivation of microalgae is carried out in a specifically designed system (placed on land or floating on water) (Bird and Benson, 1987). Seaweed can be grown directly in the open sea. Some of the uses of seaweed are: used as a food product, either eaten directly, or used in many processed foods as stabilizers or emulsifiers. With the recent development, it is also being used for making organic fertilizers, pharmaceuticals and waste water treatment.

Photoautotrophic cultivation of algae

Photoautotrophic cultivation of algae requires light to grow and produce biomass. The capital costs are much higher for closed photo-bioreactor construction than open ponds raceways. However, it is important to know both the advantages and disadvantages of photoautotrophic cultivation approaches.

Traditionally, photo-bioreactors have faced problems in scalability, especially in mixing and gas exchange (both CO_2 and O_2). Though photo-bioreactors lose very less water than open ponds due to evaporation, but they do not receive the benefit of evaporative cooling and so temperature must be carefully maintained. However, open ponds, are subject to daily and seasonal changes in temperature and humidity.

The formation of biofilm in photo-bioreactors makes sterilization problematic and may require periodic cleaning. However, long-term culture maintenance is likely to be superior to that in open ponds where contamination and "foreign" algae are readily introduced. Photo-bioreactors also provide a higher surface to volume ratio and so it can support higher volumetric cell densities, reducing the amount of water that must be processed and thus the cost of harvest (Chisti, 2007). Maximizing light exposures the main content in both types of cultivation systems.

Land based open algae culture systems

The mass cultivation of microalgae is carried out in open ponds, which are quite simple and oldest system of cultivation. The depth of shallow pond is about one-foot deep; the advantage of this system that the algae growing condition is identical to the natural environment (Figure 1). The pond design is identical to raceway configuration, and the paddlewheel circulates and mixes both the algal cells and nutrients. The raceways are mainly made from poured concrete or simply dug into the earth and lined with a plastic liner to prevent ground soaking the liquid nutrient medium. Baffles in the channel guide the flow around bends to minimize the space. This system is operated in a continuous mode. The fresh feed, containing nutrients including nitrogen, phosphorus and inorganic salts, is added in front of the paddlewheel. The algal broth is harvested behind the paddlewheel after its circulation through the loop. Different sources of wastewater, such as dairy/swine lagoon efficient and municipal wastewater, can be used for algal culture, e.g. Marine-type microalgae growth is ideal in seawater or high saline water.

Figure 1.Open raceway-type culture ponds of Earthrise in California, U.S. (Source: www.atlanticgreenfuels.com)

Algae cultivation using photo bioreactors

Photo bioreactor or PBR is a controlled system that incorporates some type of light source (Figure 2). A photo bioreactor is a closed system as opposed to an open pond. In this system, all growth requirements of algae are introduced into the system and controlled according to the requirements. Photo-bioreactor provides better control of culture environment such as CO_2 supply, water supply, optimal temperature, efficient exposure to light, culture density, pH levels, gas supply rate, mixing regime, (Kunjapur and Eldridge, 2010).

Flow description

The flow progresses to the diaphragm pump from the feeding vessel, moderating the flow rate of algal culture into the actual tube. This tube is built into the pump, which is the CO_2 inlet valve. The tubes are made of acrylic, and designed to receive light and dark intervals to improve the algal growth rate. The photo bioreactor has built in cleaning system for cleaning tubes internally without interruption of the production. Algal cultures feed back to the Feeding Vessel after completion of circulation through the photo bioreactor. The oxygen sensors define the amount of oxygen build up in the plant which is released in the feeding vessel. However, at this stage the optical Cell Density sensor determines the harvesting rate. Algae are passed through the filtering system as soon as they are ready to harvesting, and processing; and finally fed back to the feeding vessel and the process continues to grow algal cultures.

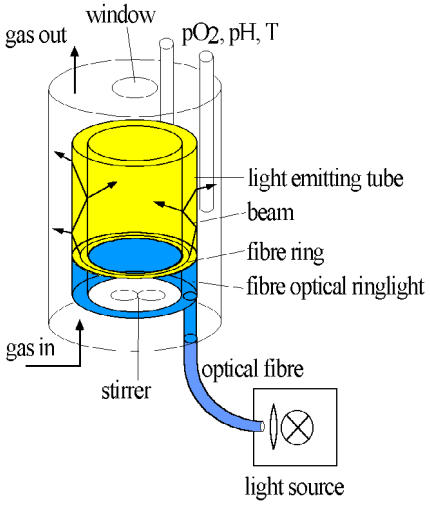

Figure 2. The components of a tubular photo bioreactor (Source: www.oilgae.com).

Biochemistry and physiology of algae

Most of the algae are photosynthetic in nature using energy from the sun to convert CO_2 and water to oxygen and macromolecules like carbohydrates and lipids. Some microalgae form lipids like Triacylglycerol's (TAG) as the chief carbon storage compounds under unusual stressful conditions such as increased sunlight or nutrient deficiency. While few others naturally build up high amount of TAG (up to 60% of dry weight), show highly efficient photosynthesis and production of lipid higher than that produced by terrestrial crops (Zhu et al., 2008). Generally carbohydrates accumulation dominates in macro algae and cyanobacteria while lipid is less than 5% of total dry (Mcdermid and Stuercke, 2003); although 50% lipid is reported in few

species. Among biologically generated hydrogen and alcohols, lipids and carbohydrates are probable biofuels and biofuel precursors. Hence, metabolic pathways and production enhancements should be significantly clarified.

Utilization of photosynthesis and light

Photosynthetic efficiency is a significant determinant of production in photo synthetically cultivated algae influencing rate of development, production of biomass and biomass percentage that is the potential fuel precursor. Theoretically production of biomass varies in 100-200 g/m^2/day (Chisti, 2007), however, the real maximum limit of algae production is not proved yet. Theoretical production may be applied to set targets for cultivation processing design and improvement of stains. Light utilization is still a restraint in algal photosystems. Most of light received in algal culture larger than laboratory level is not used. Incoming light is completely absorbed by cells closer to the source leaving no light reachable for further cells in high density cultures (Chisti, 2007). Algal photosynthesis has natural mechanism that prevents excessive light absorption that can cause oxidative damage when exposed to high level of light. The majority of incoming light is released as heat also considered as 'wasted'. However in certain condition, damage of light can still lead to photo inhibition or photosynthesis reduction (Long et al., 1994; Foyer et al., 1994; Niyogi, 1999). To prevent this occurrence, the size of chlorophyll antenna can be reduced to increase light utilization efficiency (Polle et al., 2003). The dynamics and regulation of photosynthetic apparatus need more understanding (Eberhard et al., 2008). These processes should be studied more to enhance light utilizations for biomass production.

Partitioning and metabolism of carbon

It would be very beneficial if we come to know about the carbon partition in a cell into lipids and/or carbohydrates. It would help us in biofuel strain development and designing cultivation strategies. We will require extensive knowledge of metabolic pathways for underestimating carbon partitioning. Several researches studied in plants system to understand carbon flux in synthetic and degradative pathways (Allen et al., 2009). Nonetheless, carbon partitioning in algae is less understood. Research on how algae cells control the flux and partitioning of photo synthetically fixed carbon into various groups of major macromolecules (combinations of carbohydrates, protein and lipids) if needed. Link between starch and lipid metabolism has been established. Starch (a common carbon) is also an energy storage compound in plants and algae. Arabidopsis seeds and Brassica embryos constantly accumulated starch and starch metabolism function better earlier in the lipid accumulation phase; signifying starch as an important storage compound if which synthesis leads oil accumulation. Studies show that when starch is impaired or reserved, plant embryos accumulated 40% less oil. This results show that starch synthesis is related to lipid synthesis.

Carbohydrates in algae

Algae are incredibly diverse in the simple and complex carbohydrates used for carbon storage and cell storage .Many algae commonly used for energy storage which include red algae and dinoflagellates. Many brown algae and diatoms use carbohydrates as laminarin, mannitol, and fuccoidon as food storage. Cyanobacteria mainly store heavy quantity of glycogen. This storage signifies potential biochemical feedstock's for conversion to liquid fuels. Many microorganisms are also identified which are capable for fermenting laminarin and mannitol from *Laminaria hyperborean* to ethanol. Many others polysaccharides like Alginate in many brown algae are less suitable for fermentation. These polysaccharides, nevertheless, may prove useful as intermediates to other conversion process and final fuels. Composition and structure of the polysaccharide cell wall is also important in algal strains and a source of carbohydrates. But those from plants should be broken into simpler forms before conversion to biofuel. The diversity of algal polysaccharides and cell wall, the technical challenges of those structures that may present in strain manipulation, feedstock potential, and extraction processes are important information to be identified.

Synthesis and regulation of lipid

Some algae, under stressed conditions or naturally, collect significant quantity of neutral storage lipids such as triacylglycerols (TAG), an important potential fuel precursor. The major pathway for TAG formation in plants involves de novo fatty acids in the stroma of plastids. TAG use 16 or 18 carbon fatty acids as precursors, TAG is formed by incorporation of fatty acids in glycerol backbone through three sequential acyl transfers (from acyl CoA) in the endoplasmic reticulum (IR). Through the above projected Kennedy pathway in plants TAG biosynthesis in algae (Eltgroth et al., 2005). In chloroplast, fatty acids produced are sequentially transferred from CoA to positions 1

and 2 of glycerol -3-phostphate, which result in the formation of the central metabolic phosphatidic acid (PA)

Dephosphorylation of PA releases diacylglycerol (DAG). DAG is usually present in high amounts for growing cultures, which is of great interest to research these TAG intermediates. In the last step TAG synthesis, a third fatty acid is transferred to the vacant position 3 of DAG by diacylglycerol acyltransferase, an enzyme which is unique to TAG biosynthesis. Acyltransferases which are involved in TAG synthesis may exhibit preferences for specific acyl CoA molecules and therefore may play an important role in determining the final acyl composition at TAG. Different pathways to convert membrane lipids or carbohydrates to TAG have been demonstrated in bacteria, plants and yeast in an acyl CoA independent way (Stahl et al., 2004). Moreover, PA and DAG can also use directly as substitutes for synthesis of polar lipids, such as phosphatidylcholine (PC) and galactolipids.

Synthesis of fatty acids and TAG in algae is comparatively less understood. And lack of understanding may contribute to why the lipid yields obtained from algal mass culture efforts fall short of their high values (50 to 60%) observed in the laboratory. Underestimating of lipid regulation can help in maximizing of scenarios for lipid production and strain movement (Grima et al., 1994).

Since fatty acids are common precursors for the synthesis of both membrane lipids and TAG, how the algal cell coordinates the distribution of the precursors to distinct destination. And the interaction between the two types of lipids occurs needs to be clarified. If the capability to control the chance of fatty acids differ among the algal groups or even between isolates or strains then inferring known information about lipids biosynthesis and regulation in laboratory strains to produced strains could be challenging.

Oxidative stress and lipids storage

In unfavorable conditions such as in the absence of nutrient, certain algal cells stops cell division and TAG is accumulated as the primary carbon storage. Under stressful conditions TAG synthesis and deposition into cytosolic liquid bodies may be the default pathway in certain algae (Zhu et al., 2008). In response to stress, TAG may also influence a very active and diverse task. With high stress level of light, additional electrons accumulated in photosynthetic transportation of electrons stimulates excess production of reactive oxygen species causing photosynthesis inhibition and harm membranes of lipids, proteins and other macromolecules. Secondary carotenoid synthesis may often be associated along with TAG synthesis (Rabbani et al., 1998). Molecules like β carotene formed in the pathway are isolated into cytosolic lipid bodies. Lipid with rich amount of carotenoid acts as 'sunshield' preventing and reducing excessive amount of light from reaching chloroplast under stressed condition. Synthesis of TAG uses phosphatidylcholine, phatidylethanolamine or excluded toxic fatty acids from the membrane as acyl donor thus detoxifying these compounds and depositing them in the form of TAG.

Direct biofuel production from algae

Direct biofuel production by heterotrophic fermentation and growth from algae is advantageous with regards to cost as many processing steps are avoided. High controlled conditions are well maintained through this process which could be adjusted firstly while producing biomass followed by production of oil. This system produces considerably high amount of biomass with hundreds of grams per liter and above 60% in the form of lipid. This system is easily established with the availability to utilize variety of carbon fixed feedstock that would even further lessen production cost. Algae can directly produce different fuel types such as alcohols, alkanes and hydrogen (Kumar, 2012).

Whole algae processing

Whole algae can be utilized as fuels in contrary to direct production that includes firstly extraction and post processes. Such process minimizes cost related to extraction and another advantage is the ability to utilize varieties of algae, although a little dewatering process is needed. Macroalgae is very much considered as a gasification feedstock (Ross et al., 2008). The primary biochemical feedstocks, the polysaccharides like mannitol, lamarin and flucoidin are used to form liquid fuels (McHugh, 2003). The content of lipid in most of the macroalgal species is not more than 5% of total dry weight which is very low for biodiesel production, even though some species are believed to produce around 20% (McDermid and Stuercke, 2003). The methods that can process whole algae are pyrolysis, gasification, supercritical processing and anaerobic digestion (Kumar, 2012).

Pyrolysis

This is the chemical decomposition of compressed substance through heat. Although, it does not include any reaction with oxygen and

other reagents but can occur in their existence. Thermo chemically treated algae or other biomass produce variety of products according to the reaction variables. Liquid products allow short time of residence, rapid rates of heating, and balanced temperature (Huber and Dumesic, 2006). Pyrolysis is more advantageous than others as it is considerably fast with the time of reaction within seconds and minutes. Better efficiency can be reached by rapid heating of finely ground feedstock to 350-500˚C in 2 seconds by a process known as 'flash pyrolysis' (Bridgewater, 2004; Miao and Wu, 2004). This is another advantage why algae are considered over other sources due to its naturally small units and fibreless tissues.

Gasification

Algal biomass gasification can offer readily method in producing varieties of liquid fuels, particularly through Fisher- Tropsch Synthesis (FTS) or mixed alcohol synthesis of consequent syngas. Gasification of Lignocellulose in the synthesis of mixed alcohol is much matured (Phillips, 2007), and as long as content of water is maintained, this process of gasification can be very simple. In FTS method, syngas constituents like CO, CO_2, H_2O and other impurities are easily removed and upgraded to usable fuels by a water gas-shift and CO hydrogenation (Okabe et al., 2009; Srinivas et al., 2007; Balat, 2006).

This method is advantageous to other bio-syngas conversion. Firstly, variety of fuels with agreeable and known properties can be achieved. Also, bio-syngas is a flexible feedstock that can generate different products enhancing the feasibility of this process. Integration ability of feedstock into current thermochemical infrastructure is another benefit. It may also be feasible to feed algae into coal gasification plant to lessen capital infrastructure, consider the accessibility of biomass plants and enhancing efficiency of the process economically. As FTS process is exothermic it is possible to utilize heat partially to dry algae during harvesting and dewatering with regenerative heat exchanger.

The main barriers to the FTS technology for algae are the same as that of coal (Yang et al., 2005), excluding the upstream process that can be the origin of contaminants, requiring removal before approaching FT catalyst. For higher efficiency, this method should involve large scale production. But the main problem with this method is the cost of cleaning up and reforming the tar.

Reports suggest that thermal efficiency, the ratio of heating values of bio-crude products and feedstock, and input of external heat, can reach up to 75%. Liquefaction of *Dunaliella tertiolecta,* with moisture content 78.5 wt. %, generates around 38% oil, at 300˚C and 10 Moa (Minowa et al., 1995). Oil attained at 340˚C and holding the time for 60min, shows up to 330mPas viscosity and 3kJ /g of calorific value relative to that of fuel oil. Algal liquefaction is taken as a potential method, however as a limit knowledge is available in current algal hydrothermal liquefaction, further studies are necessary prior to commercial development.

Supercritical processing

Supercritical processing is a recent technology that can extract and convert oils to biofuels at the same time. Extracting algal oil with this method is highly proficient to traditional solvent separation methods. It has also been projected that this method is considerably influential in extracting other algal constituents. Supercritical Tran's esterification may also be used in extraction of oil form algae (Fajardo et al., 2007). Supercritical fluids are selective giving highly purified and concentrated products. Also, organic solvent deposits are absent in the extract or spent biomass (Demirbas, 2009a). Moreover, such fluids can utilize whole algae without the need of dewatering, which, thus, improves efficiency.

The supercritical extraction can be combined with transesterfication reaction to allow a 'one pot' concept in production of biofuel (Anitescu et al., 2008). Though it is only a demonstration of combined extraction and transesterfication of vegetable oils, it is predicted as being employable in algae as well. Supercritical methanol, or ethanol, is used as extraction medium, as well as a catalyst for transesterfication. With the use of catalyst free supercritical ethanol, water tolerance is achieved with a production similar to that of anhydrous process used in vegetable oils. Though the presence of water in the medium is like a factor in efficiency of the process, however the degradation of fatty acids is the prime factor that restricted achievable ester content (Brunner, 2005).

Anaerobic digestion of whole algae

Anaerobic digestion of algae like *Laminaria hyperbore* and *Laminaria saccharina* for biofuel production is quite remarkable however there is less consideration in the United States. Using this method removes most of the challenges related to high infrastructures on producing biofuel, such as drying, extracting, and fuel conversion. A production of 180.5ml/g-d can be achieved in a two-stage anaerobic digestion method with many algal strains with a 65% methane concentration, as

estimated in recent study (Vergara- Fernández et al., 2008). Further enhancement of this method can very effective in terms of waste water treatment integration, where algae are cultivated in conditions that are uncontrolled utilizing strains not optimized for production of lipid.

Algal extracts conversion

Conversion of extracts from algal sources is the common method of producing algal biofuel. There is however a crucial link between extraction type and product constituents, hence primary and comprehensive knowledge of conversion method inputs should be realized. The very common extracts used are lipids content such as tricylglycerides for biodiesel conversion. The following will describe each process that can be used in algal extracts conversion such as chemical, biochemical, and catalytic methods.

Chemical transesterification

This process involves conversion of triacylglycerols to FAMEs (fatty acid methyl esters), which simply displaces alcohol group from an ester by another alcohol (Demeirbas, 2009b). This process can either be used in catalytic or non-catalytic reactions by involving various heating systems needed to start the reaction. This method is very mature and has been indicated as the 'gold standard' for the conversion vegetable oils into diesel. Besides the classic base-catalyzed methanol approach, it is projected that ethanol can serve the purpose in algal oil production, being sodium ethanolate used as the catalyst. The products formed are segregated by mixing ether and salt water to the solution. Vaporizer at high vacuum separates biodiesel from ether.

It is also known that another method using liquid acid catalyst such as H_2SO_4, HCl or H_3PO_4 is another alternative that can be used. Acidic catalysts are less susceptible to the presence of water and free acids, hence minimize saponification and emulsification, and consequently enhance recovery of product. Although acid catalysts have such advantages, they however are not preferred due to lower activity compared to conventional alkaline catalysts and, thus, required higher temperature and longer reaction times. Heteropolyacids are known to show these necessary requirements (Alsalme et al., 2008).

Besides, alternatives catalysts there are other potential variations such as the use of heating system that can improve transesterifcation kinetics using microwaves, thereby accelerating the reaction and provides short reaction time. Microwave process may be cost competitive with present mature conversion method.

In ultrasonic reactor, bubbles are produced and subsided regularly by ultrasonic waves. This allows concurrent mixing and heating needed for transesterification (Armenta et al., 2007). Ultrasonic reactor minimizes reaction time, temperature and the energy input. The industrial scale ultrasonic method provides processing of thousands of barrels each day, nevertheless, yet more modification for large scale production is still necessary (Stavarache et al., 2007).

Conversion by biochemical (enzymatic)

High triacylglycerols conversion is achieved by chemical conversion compared to esters but the disadvantages could be their high energy intensity, difficult glycerol removal and also needs to remove alkaline catalyst from the product and alkaline waste water treatment. However, the use of biocatalyst like lipases proves to be the solutions to these drawbacks and also provides an environmental friendly provision to the conventional methods. Large scale production has not been established, mostly due to the high cost of lipases and short operational time resulted from excessive methanol and glycerol.

The solvent and temperature tolerance of enzymes for efficient biocatalyst processing should be taken into account. Solvents are required for better solubility of triacylglycerols during extraction process and the enzymes used should perform well even in the presence of these solvents for cost effectiveness of biofuel production (Fang et al., 2006). The use of the solvent engineering method to improve lipase catalyzed methanolysis of triacylglycerol has been reported (Liao et al., 2003). The use of a co-solvent mixture can be advantageous in many ways: elimination of negative effects caused by excess methanol andthe co-product glycerol; provides high reaction rates ad conversion; catalyst regeneration for lipase reuse is avoided; high operational stability of the catalyst. The conversion of algal oil extracts at large industrial scale, and at competing prices, are the drawbacks that need further consideration. Others factors to be taken into account are development of enzymes that can dissolve algal cell walls; maximizing particular enzyme activity; identifying essential enzyme reactions for cell wall deconstruction and autolysin; conversion of carbohydrates to sugar; catalyzing nuclei acid hydrolysis; and lipids conversion to suitable diesel substitutes (Demirbas, 2009b).

Conversion of renewable diesel, gasoline and jet fuel

Current processes in petroleum refineries are typically classified into separation and modification of the constituents in crude oil to give varieties of end products. These products might vary according to input stream and process steps, mostly characterized by their specifications than by sum of specific molecules. Gasoline, jet fuel and diesel should achieve great performance specifications, including volatility, initial and final boiling point, auto ignition features, flash point and cloud point. Though crude oil is the primary feedstock, other resources such as oil shale and tar sands are however widely considered. Petroleum industry original started with whale oil and at present is again looking for biological feedstock to meet the demand.

If produced from biological feedstock, gasoline, jet fuel and diesel are classified as renewable or green. The significant feature of fuels produced from petroleum is its high energy level with almost zero oxygen content. Biological feedstock has more oxygen content than crude oil therefore removing oxygen and increasing resulting energy content are significant.

Several refiners and catalyst manufacturers have started to find ways in converting vegetable oil and waste animal fats to renewable fuels. Fatty acids are very well adapted to generate diesel and jet fuel with less processing steps. This method has already offered renewable jet fuel mix generated from jatropha and algae oil that is being used in commercial jet test flights. Straight chain alkanes are not well suited for gasoline production as they give low octane numbers. Algal lipids can be developed by hydrothermal treatment known as hydro treating where carboxylic acid moiety is converted to a combination of water, carbon dioxide or carbon monoxide, and reduce double bonds to give hydrocarbons. Glycerin can be transformed to propane that could be used as liquefied petroleum gas. Catalyst development is the main issue in using algae oil to form renewable fuels. It is needed to modify catalyst such that impact on oxygen bearing carbon atoms will reduce the loss of CO and CO_2 and also that of used H_2. Catalysts used in refineries are made to operate at desirable range of chemical constituents such as metals, sulfur and nitrogen etc., presence in petroleum streams without becoming toxic. Crude algal oil can consist of high phosphorus from phospholipids, nitrogen rom extracted proteins and metals from chlorophyll. It is thereby essential to enhance both level of lipid purification and catalyst tolerance for the contaminants to form at a very cost effective method.

Biodiesel preparation by direct transesterification

One gram of algal biomass that is freeze dried or wet algal biomass equivalent to one gram dry weight in a glass tube was mixed with 3.5ml methanol and 0.7ml sulfuric acid. According to the experimental design, 4.0 mL solvent was added; if there was no solvent used, an extra 4.0ml methanol was added for consistent volume reaction. At 90˚C for about 45 min the samples undergone reaction and were properly mixed. After completion, the tubes were kept away from the water bath to cool at room temperature. 2mL distilled water was added followed by 50min mixing. Tubes were separated forming biphasic solution. For rapid separation, the tubes were subjected to centrifuge at 7232g for about 9 min. The organic layer containing FAME was collected and shifted to a pre weighed glass vial. The solvent was evaporated using N_2 and the biodiesel mass was determined by weighing.

Climate and geographic regions preferred for algae production

Climatic factors such as solar radiation, temperature, precipitation, evaporation and severe weather influence photoautotrophic algae production especially that of terrestrial microalgae. Seasonal and annual temperature and amount of sunlight will directly influence productivity, while evaporation, precipitation and extreme weather will have an influence on water supply and quality in open systems. However closed photo bioreactors have a much regulated system, thus are unlikely to be affected by climate elements. What is common for both open and closed photoautotrophic systems is the presence of ample sunlight. Heterotrophic algae in closed bioreactors at industrial level used advanced technology providing clean and regulated environment to maximize growth condition for better biomass density cultures. This method can be up to 2 orders of magnitude higher in biomass density as compared to open photoautotrophic system which is only a gram per liter culture density. It also uses lesser amount of water and energy. Using expensive system eliminates the variations in outdoor climatic conditions and day/ night light cycles. For the case of marine macroalgae production, the encompassing environment acts as a regulator that controls temperature differences. Storms and ocean dynamics that cause waves, currents and

transportation of nutrients can indirectly have an impact on productivity.

Photoautotrophic microalgae productivity depends on selective factors such as mean yearly climatic conditions, non-fresh water availability and presence of CO_2 sources. The criteria used to limit locations geographically are: annual average cumulative sun hour's ≥ 2800, annual average daily temperature ≥ 55°F, and annual average freeze-free day's ≥ 200. It was known that the interrelation among these factors with which could be acquired an annual mean algae production in wide scale systems may only be considered as gross indicator.

Other factors may overcome the unfavorable site and resource conditions for algae production. For example, location of inland microalgae production at higher latitudes may be feasible with industrial operations, capable of providing excess heat and power for cost-effective environmental regulation. This, however, require a much refined analysis for systems that would likely be closed and highly integrated with co-located industries that could utilize waste, heat and energy. These analyses must include evaluation of the monthly or seasonal solar radiation and ambient temperature ranges; it should also be able to use low cost operational requirements for heating in the winter, for closed reactors, cooling in the summer.

Seasonal factors and water requirement

The advantages of microalgae as potential sources of biofuel are that of their species varieties and capability to accommodate varying temperatures. Appropriate conditions of climate for the most time of the year are essential for high algae production of a particular species cultivated photoautotrophic ally (Maxwell et al., 1985). A crucial climate for both open and closed photo bioreactor systems is the time span of economically feasible growing season(s) for the particular species available for dynamic cultivation. For outdoor ponds, the conventional crop analog is the length of time between the last killing frost in the spring and the first killing frost in the fall. For closed photo bioreactor systems, the conventional crop analog is the greenhouse and the limiting energy and cost needed to regulate internal temperature throughout the seasonal duration. Availability and rotation of wide ranges of algal species that are more productive in cold and hot seasonal conditions, correspondingly, enhance adaptability or could expand the restricted periods of economically possible algae production. Evaporation, discussed later in this section, is closely related to climate and will amplify water contents for an open algae production system. Evaporative loss can be a critical factor to consider when choosing locations for open pond production. Evaporation is not of a concern in closed photo bioreactors, although evaporative cooling as a means of reducing culture temperature.

For open pond systems in arid environments with high rates of evaporation, salinity and water chemistry alter with evaporative water loss, thus altering the culture conditions. This will need intermittent blow-down of ponds after salinity increases, addition of non-saline make-up water to dilute the salinity, use of desalination treatment to control salinity build-up, or well adaptive algae that withstand these conditions.

Desalination would require extra capital, energy, and operational costs. Water balance and salt build-up problems, from resources and algal cultivation aspects are matters to be considered in future research, modeling, and field assessment.

Carbon dioxide: The opportunity of carbon capture in algae production

Algae act as a sink of global CO_2 emissions. For producing algae efficiently an enriched source of CO_2 better than the atmospheric CO_2 is necessary. Flue gas from fossil fuel power plants can be a better source of CO_2. Algae production has a dual benefit by utilizing fossil carbon emissions thus balancing subsurface sequestration. Even though, algae production does not sequester fossil carbon in a lateral sense, however they provide a capture carbon opportunity by reusing it in the form of biofuels and other derivatives produced from algae biomass. Greenhouse gas mitigating credits would be advantageous from the replacement of renewable bio fuels and their co-products with the use of fossil fuel (Brune et al., 2009).

Land factors

Availability of land is important for algae production since both open and closed systems require considerably vast areas as likely needed by any photosynthesis-based biomass feedstock. Physical characteristics, such as topography and soil could hinder accessibility for open pond algae farming. Soil properties may have an impact on construction costs and design of open systems. Topography would be a limiting factor as large shallow ponds will require a considerable flat topography. Furthermore, likewise any form of biomass, algae productivity is limited by available energy density in sunlight and the relatively low efficiencies of photosynthetic processes along with other systems deficiencies. Contributing to productivity limits per unit of illuminated surface

area is the fact that algal cells nearest the illuminated surface absorb the light and shade their neighbors farther from the light source.

Assessing the recent economic analyses for algae biomass and projected oil production, the cost of land is not typically taken into account or can be considerably minimal in comparison to other expenses. Important land for development may not be appropriate for algae production. Likewise, land necessary for higher-value agricultural use is not considered. Beyond economical aspect, this also abstain the perception and possible issue of conflict on fuel against food and feed supply.

3. Biofuel from sorghum

Sorghum an annual type of grass used as a grain and fodder not uncommon to farmers has been currently sought out to be among the viable options for the production of biofuel. Sorghum is easily and highly adaptable, generating starch, sugar and lignocelluloses with a maturation period of just under months. Well known for its natural drought tolerance, sorghum can be grown in most part of the world of varying climatic conditions though is less cold tolerant with soil condition above 65 degree Fahrenheit. Sorghum is considered to be a high energy crop. Sorghum for biofuel production is grown in the United States, Brazil and India.

Sorghum may be used for starch-based, sugar-based and cellulosic ethanol production. Compared to its competitor, Grain sorghum produced equivalent amount of energy from biomass with quarter less of water than that used by corn. Energy sorghum can produce 1400 gallons of ethanol compared to 499 gallons by corn per acre. Sweet sorghum can produce 446 gallons of ethanol per acre per harvest. Sweet sorghum is although more advantageous biofuel feedstock compared to grain sorghum as it has higher reducing sugar content and water efficiency, stalks are used without the grain.

3.1. Steps for extraction of biofuel from sweet sorghum

3.1.1. Juice extraction

The extraction of juice from the stalks is carried out by a series of mills (Almodares et al., 2008). Consequently, the juice is screened and sterilized by heating to 100°C (Almodares and Hadi, 2009). The grimy juice is transferred to a rotary vacuum filter while the clear filtrate juice is transferred to the evaporation unit for concentration (syrup to ethanol). The juice can also be directly transferred to fermentation unit for straightaway conversion of juice to ethanol. According to the chosen method, the juice can be concentrated by evaporators to reach various brix. If fermented directly from juice to ethanol it is recommended to slightly increase concentration to 18 brix. The syrup to be stored in future use should be concentrated to at least 65 brix.

3.1.2. Fermentation

Fermentation is carried out in various procedures according to the chemistry, biochemistry and microbiology of the feedstock. The conversion of juice or syrup to ethanol is carried out by yeast *Saccharomyces cerevisiae*. Sugar is transformed to ethanol, carbon dioxide and yeast biomass along with smaller quantity of end products like aldehydes, ketones and glycerols (Flickinger and Drew, 1999; Almodares and Hadi, 2009).

3.1.3. Distillation and dehydration

Alcohol from fermentation method is further concentrated to at least 99.6% v/v in the distillation step. Treatment of the formation of vinase from this step is done by concentration of vinase to 25% solid and composting of the same using available press mud and concentration of the other part to be used as liquid fertilizer.

3.1.4. Processing ethanol production from grain sorghum

After washing, crushing and milling of the grain, the starchy substance is gelatinized and liquefied and saccharified using enzymes α-amylase and glucoamylase to form glucose. Fermentation, distillation and dehydration are similar to the methods used in the case of sorghum stalks.

Sorghum has many advantages over its competitors, maize, sugarcane and the like. Stakeholders are considering its potential as a feasible bioenergy crop. Sorghum certainly stands to be the most sustainable bioenergy crop. It does not compromise food security and does not compete with other food crops and prices. It uses less nitrogen and cost of production is considerably lesser than that of maize and sugarcane. As per the analysis of EPA (Environmental Protection Agency, US), ethanol produced from sorghum shows 53% reduction of greenhouse gases qualifying it as an advanced biofuel under the Renewables Fuels Standard.

Research for genome sequencing is undergoing for better improvement of biofuel production from sorghum in particular use of the stalk rather than the grain. Large scale commercial production of biofuel from sorghum is a challenge to be taken into account due to unavailability of efficient harvester. Research for efficient result on cropping system, enhancement of cellulose content, fast

growing and reducing seeds quantity through genetic engineering are being studied.

4. Biofuel from sugarcane

With the recent concern raised on climate change, biofuels have been sought out to combat the rising levels of greenhouse gases. Sugarcane known as a 'miracle biofuel' is the most common feedstock used for biofuel production. Sugarcane a tropical crop and perennial belongs to the genus *Sacchurum*. It requires minimum rainfall of 24 inches annually. It can be harvested up to five times before it produces seeds. Sugarcane ethanol is made from the sucrose found in sugarcane juice and molasses although this method captures only a quarter of the energy that sugarcane can provide.

Biofuel from sugarcane is significantly sustainable and is believed to reduce greenhouse gases up to 90% more than any other ethanol renewable resources. It is cheap and clean with high efficient compared to biofuel from maize. Moreover, with sugarcane, the same plant can be grown again without uprooting for up to 8 years without reducing its yielding capacity. The leftover bagasse can be used to heat the distillation process. With this new technique, ethanol in the form of cellulosic ethanol can be obtained from any leftover parts of the plant but with a high cost (McLaren, 2009).

Brazil is currently the leading producer of biofuel from sugarcane with 40% of the same is used in automobiles. In Brazil sugarcane produces 622 gallons of ethanol per acre compared to only 354 gallons an acre from maize in the United States.

4.1. Steps involving the production of ethanol

Four major steps are involved in ethanol production from sugarcane, including milling, fermentation, evaporation distillation and dehydration.

After harvesting, sugarcane stalks are crushed in the mills for the extraction of the juice also known as garappa in Brazil that contains about 15% sucrose, bagasse and fibrous residue. The juice is filtered before evaporation. The juice is then precipitated by crystallization forming crystals surrounded by molasses. Separated by centrifugation, the crystals are used as sugar and molasses is sent to another unit for the production of ethanol. The molasses is sent for clarification whereby any impurity is removed. This is followed by fermentation in the presence of yeast *Saccharomyces cerevisiae.* Fermentation results a 15% alcohol this is sent for centrifugation to recover the yeast. At varying boiling points alcohol is separated and hydrated ethanol is formed.

Sugarcane has many advantages over maize and other similar energy crops being a more sustainable energy crop with high yield, low carbon, ability to reuse by products and its cost is considerably low. The only challenge could really be that biodiesel is still more efficient compared to ethanol. A method that can produce biodiesel from sugarcane is significant.

5. Conclusion and prospects

Plants have become a major source for biofuel production and avoid food crops for this. Otherwise food price will rise in the international market may lead to the shortage of food supply. In addition, expansion of arable land is difficult in feeding the ever-growing population worldwide, and growing the bio-energy crops will certainly effect negatively on overall food production worldwide. Therefore, selected bio-energy crops should be grown on the marginal land, and improve their efficiency with mutation (Jain, 2012), transgenic approach (Gressel, 2008) and breeding for higher sugar content e.g. in sugarcane, sweet sorghum; develop specific agronomical practices for large-scale biomass production.

Algae represent the third generation feedstock for biodiesel, with much higher yields than second generation crops. Algae can yield more than any of the biodiesel feedstock available today. Oil yields per unit area from algae can be even further increased, and it is one of the most researched topics currently. Moreover, for algae cultivation, waste land is used. Algae use CO_2 for massive production, which is a plus point (Brune, 2009). The production of algae can be integrated with waste water treatment facility (Downing et al., 2002).With the increasing human population, algae can stand as the promising source of biofuel.

References

Allen, D. K., J. B. Ohlrogge and Y. Shachar-Hill. 2009. The role of light in soybean seed filling metabolism. Plant J. 58:220–234.

Almodares, A and M. R. Hadi. 2009. Production of bioethanol from sweet sorghum – a review. Afr. J. Agric. Res. 4(9):772-780.

Almodares, A., R. Taheri, S. Adeli. 2008. Stalk yield and carbohydrate composition of sweet sorghum [*Sorghum bicolor* (L.) Moench] cultivars and lines at different growth stages. J. Malesian Appl. Biol. 37:31-36.

Alsalme, A., E. F. Kozhevnikova and I. V. Kozhevnikov. 2008. Heteropoly acids as catalysts for liquid-phase esterification and

transesterification. App. Catal. A: Gen. 349(1-2):170-176.

Andersen, R. A. and M. Kawachi. 2005. Traditional microalgae isolation techniques, In: Andersen R.A. (Ed.), pp.83-100. Algal Culturing Techniques 1st Edition, Elsevier, New York.

Anitescu, G., A. Deshpande and L. L. Tavlarides. 2008. Integrated technology for supercritical biodiesel production and power cogeneration. Energy Fuels 22(2):1391-1399.

Armenta, R. E., M. Vinatoru, A. M. Burja, J. A. Kralovec and C. J. Barrow. 2007. Transesterification of fish oil to produce fatty acid ethyl esters using ultrasonic energy. J. Amer. Oil Chem. Soc. 84(11):1045-1052.

Balat, M. 2006. Sustainable transportation fuels from biomass materials. Energy Edu. Sci. Tech. 17(1-2):83-103.

Banerjee, A., R. Sharma, Y. Chisti and U. Banerjee. 2002. *Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals. Crit. Rev. Biotech. 22(3):243–279.

Bird, K. T. and P. H. Benson. 1987. Seaweed Cultivation for Renewable Resources, Elsevier Science Ltd. pp. 285-303.

Bligh, E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Physiol. Pharm. 37(8):909–917.

Bridgewater, A. V. 2004. Biomass fast pyrolysis. Thermal Sci. 8(2):21–50.

Brune, D. E., T. J. Lundquist and J. R. Benemann. 2009. Microalgal biomass for greenhouse gas reductions: Potential for replacement of fossil-fuels and animal feeds. J. Environ. Engin. 135(11):1136-1144.

Brunner, G. 2005. Supercritical fluids: technology and application to food processing. J. Food Eng. 67(1-2):21-33.

Chisti, Y. 2007. Biodiesel from microalgae. Biotech. Adv. 25(3):94–306.

Demirbas, A. 2009a. Biodiesel from waste cooking oil via base-catalytic and supercritical methanol transesterification. Energy Conver. Manage. 50(4):923-927.

Demirbas, A. 2009b. Production of biodiesel from algae oils. Energy Sources, Part A: Recov. Utiliz. Env. Effects 31(2):163-168.

Downing, J. B., E. Bracco, F. B. Green, A. Y. Ku, T. J. Lundquist, I. X. Zubieta and W. J. Oswald. 2002. Low cost reclamation using the advanced integrated wastewater pond systems® technology and reverse osmosis. Water Sci. Tech. 45(1):117–125.

Eberhard, S. G. Finazzi and F. A. Wollman. 2008. The dynamics of photosynthesis. Ann. Rev. Genet. 42:463–515.

Eltgroth, M. L., R. L. Watwood and G. V. Wolfe. 2005. Production and cellular localization of neutral long-chain lipids in the haptophyte algae *Isochrysis galbana* and *Emiliania huxleyi*. J. Appl. Phycol. 41(5):999-1009.

Fajardo, A. R., L. E. Cerdan, A. R. Medina, F. G. A. Fernandez, P. A. G. Moreno and E. M. Grima. 2007. Lipid extraction from the microalga *Haeodactylum tricornutum*. Eur. J. Lipid Sci. Tech. 109(2):120–126.

Fang, Y., Z. Lu, F. Lv, X. Bie, S. Liu, Z. Ding and W. Xu. 2006. A newly isolated organic solvent tolerant *Staphylococcus saprophyticus* M36 produced organic solvent-stable lipase. Curr. Microb. 53(6):510-515.

Fishman, D., R. Majumdar, J. Morello, R. Pate and J. Yang. 2010. US Department Of Energy, National Algal Biofuels Technology Roadmap, 8-10.

Flickinger, M. C. and S. W. Drew. 1999. encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation. Vol. 1. New York- Wiley. pp.393–419.

Foyer, C. H., M. Lelandais and K. J. Kunert. 1994. Photooxidative stress in plants. Physiol. Plant. 92(4):696-717.

Gressel, J. 2008. Trangenics are imperative for biofuel crops. Plant Sci. 174:246-263.

Grima, E. M., E.-H. Belarbi, Acie´nFerna´ndez, F. G., Medina, A. R. and Y. Chisti. 1994. Comparison between extraction of lipids and fatty acids from microalgal biomass. J. Amer. Oil Chem. Soc. 71(9):955–959.

Huber, G. W. and J. A. Dumesic. 2006. An overview of aqueous-phase catalytic processes for production of hydrogen and alkanes in a biorefinery. Catal. Today 111(1-2):119–132.

Jain, S. M. 2012. *In vitro* mutagenesis for improving date palm (*Phoenix dactylifera* L.). Emir. J. Food Agric. 24(5):386-399.

Kumar, V. 2012. Biodiesel From Algae. Lambert Publishing, Germany. pp. 68-80.

Kunjapur, M. A. and R. B. Eldridge. 2010. Photobioreactor design for commercial biofuel production from microalgae. Ind. Eng. Chem. Res. 49:3516–3526.

Liao, J. C., R. Boscolo, Y. L. Yang, L. M. Tran, C. Sabatti and V. Roychowdhury. 2003. Network component analysis: Reconstruction of regulatory signals in biological systems. Proc. Natl. Acad. Sci. USA.100:15522–15527.

Long, S. P., S. Humphries and P. G. Falkowski. 1994. Photoinhibition of photosynthesis in nature. Ann. Rev. Plant Physiol. Plant Mol. Biol. 45(1):633-662.

Maxwell, E. L, A. G. Folger and S. E. Hogg. 1985. Resource evaluation and site selection for microalgae production systems. (SERI/TR-215-2484). Golden, CO. Solar Energy Research Institute.

McDermid, K. J. and B. Stuercke. 2003. Nutritional composition of edible Haawaiian seaweeds. J. Appl. Phycol. 15:513–524.

McHugh, D. J. 2003. A Guide to Seaweed Industry, FAO Fisheries Technical paper No. 441,105.

McLaren, J. 2009. Sugarcane as a Feedstock for Biofuels. NCGA; www.ncga.com

Miao, X. and Q. Wu. 2004. High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. J. Biotech. 110(1):5-93.

Minowa, T., S. Y. Yokoyama, M. Kishimoto and T. Okakurat. 1995. Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction. Fuel 74:1735-1738.

Niyogi, K. K. 1999. Photoprotection revisited: Genetic and molecular approaches. Ann. Rev. Plant Physiol. Plant Mol. Biol. 50(1):333-359.

Okabe, K., K. Murata, M. Nakanishi, T. Ogi, M. Nurunnabi and Y. Liu. 2009. Fischer-Tropsch synthesis over Ru catalysts by using syngas derived from woody biomass. Catal. Lett. 128(1-2):171-176.

Phillips, S. D. 2007. Technoeconomic analysis of a lignocellulosic biomass indirect gasification process to make ethanol via mixed alcohols synthesis. Indus. Eng. Chem. Res. 46(26):8887-8897.

Polle, J. E. W., S. Kanakagiri and A. Melis. 2003. tla1, a DNA insertional transformant of the green alga *Chlamydomonas reinhardtii* with a truncated light-harvesting chlorophyll antenna size. Planta 217:49-59.

Rabbani, S., P. Beyer, J. V. Lintig, P. Hugueney, H. Kleinig. 1998. Induced b-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. Plant Physiol. 116:1239–1248.

Ross, A., J. Jones, M. Kubacki and T. Bridgeman. 2008. Classification of microalgae as fuel and its thermochemical behavior. Biores. Tech. 99(14):6494-6504.

Scholz, V. and J. N. Da Silva. 2008. Prospects and risks of the use of castor oil as a fuel. Biomass Bioener. 32(2):95-100.

Sieracki, M. E., N. J. Poulton and N. Crosbie. 2004. Automated isolation techniques for microalgae. Algal culture techniques. Academic Press, New York. (7):103-116.

Srinivas, S., R. K. Malik, Mahajani and M. F. T. Sanjay. 2007. Synthesis using bio-syngas and CO_2. Energy Sust. Devel. 11(4):66-71.

Stahl, U., A. S. Carlsson, M. Lenman, A. Dahlqvist, B. Q. Huang, W. Banas, A. Banas and S. Stymne. 2004. Cloning and functional characterization of a phospholipid: diacylglycerol acyltransferase from *Arabidopsis*. Plant Physiol. 135:1324–1335.

Stavarache, C., et al. 2007. Ultrasonically driven continuous process for vegetable oil transesterification. Ultrasonics Sonochem. 14(4):413-417.

Valdes, C. 2012. Brazil's Ethanol Industry: Looking Forward / BIO-02. Econ. Res. Service/USDA.

Vergara-Fernandez, A., G. Vargas, N. Alarcon and A. Velasco. 2008. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Biomass Bioener. 32:338-344.

Yang, Y., et al. 2005. A highly active and stable Fe-Mn catalyst for slurry Fischer-Tropsch synthesis. Catal. Today 106(1-4):170-175.

Zhu, X. G., S. P. Long and D. R. Ort. 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Curr. Opin. Biotech. 19(2):153–159.