

Potential of the Micro and Macro Algae for Biofuel Production: A Brief Review

Renganathan Rajkumar,* Zahira Yaakob, and Mohd Sobri Takriff

The world seems to be raising its energy needs owing to an expanding population and people's desire for higher living standards. Diversification biofuel sources have become an important energy issue in recent times. Among the various resources, algal biomass has received much attention in the recent years due to its relatively high growth rate, its vast potential to reduce greenhouse gas (GHG) emissions and climate change, and their ability to store high amounts of lipids and carbohydrates. These versatile organisms can also be used for the production of biofuel. In this review, sustainability and the viability of algae as an up-coming biofuel feedstock have been discussed. Additionally, this review offers an overview of the status of biofuel production through algal biomass and progress made so far in this area.

Keywords: Microalgae; Macroalgae; Biomass; Lipid; Biofuel; Oil production; Bioconversion; Algaculture; Wastewater treatment; Malaysia

Contact information: Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, University Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia;

** Corresponding author: micro_rajkumar@yahoo.co.in*

INTRODUCTION

The energy requirements of the global community are rising year by year. Currently, fossil fuels are a prominent source of transportation fuels and energy. The world's demand for oil is expected to rise 60% from the current level by 2025 (Khan *et al.* 2009). In view of the increasing oil demand and the depleting oil reserves, development of innovative techniques for the production of biofuels from novel renewable biomass feedstock sources are gaining importance all over the world. Production of biofuels from traditional agricultural crops such as corn, oil palms, and soybeans using arable lands and fresh water will greatly impact food production.

Biomass, whether terrestrial or aquatic, is considered a renewable energy source. Relative to alternative energy sources, the aquatic biomass represents the strategy that is most ready to be executed on a large scale without any economic or environmental penalty (Aresta *et al.* 2005). Among these, algae are endowed with a unique adaptability to grow in diverse habitats, either in marine or fresh waters (IEA Report 1994). In the past, research mainly focused on their usage as food, animal feed, bio-fertilizer, and in aquaculture.

Algae have received a great deal of attention as a novel biomass source for the generation of renewable energy. Apart from other biomass sources, algae contains a high biomass yield per unit of light and area, can have a lot of starch or oil content, does not require fresh water or agricultural land, and the requirements for nutrients can be fulfilled by either wastewater or seawater. Algae produce an array of organic molecules, particularly carbohydrates and lipids. These biomolecules can be used to extract a fuel

known as biofuel. Algae are both unicellular and multicellular autotrophic aquatic life forms.

Microalgae can provide several different kinds of renewable biofuel. These include methane produced by the anaerobic digestion of the algal biomass (Spolaore *et al.* 2006), biodiesel synthesized from the micro algal oil (Thomas 2006), and biohydrogen produced by a photobiological mechanism (Gavrilescu and Chisti 2005). The idea of producing microalgal biofuel is not a new one (Kapdan and Kargi 2006), but it is now being viewed seriously in view of the increasing price of petroleum. Serious interest is also motivated by concern about global warming that is associated with the use of fossil fuels (Sawayama *et al.* 1995).

Macroalgae are generally fast growing and are able to reach sizes up to 60 m in length (McHugh 2003). Growth rates of macroalgae far exceed those of terrestrial plants. For example, brown algae biomass of the average productivity was approximately 3.3 to 11.3 kg dry weight $\text{m}^{-2} \text{yr}^{-1}$ for non-cultured algae and up to 13.1 kg dry weight m^{-2} over 7 month for cultured algae compared with 6.1 to 9.5 kg fresh weight $\text{m}^{-2} \text{yr}^{-1}$ for sugar cane, a most productive land plant (Kraan 2010). They are seasonally available in the natural water basins. Cultivation of macroalgae at sea, which does not require arable land and fertilizer, offers a possible solution to the energy crisis. Macroalgae are mainly utilized for the production of food and the extraction of hydrocolloids, and it is possible to produce ethanol from algae (Goh and Lee 2010). Macroalgal biomass contains high amounts of sugars (at least 50%), which can be used in ethanol fuel production (Wi *et al.* 2009).

This review explores the opportunities for energy products, encompassing both fresh and marine habitat macro- and microalgae. This paper also discusses the variety of algal resources and their environment, along with the manufacture systems that have been demonstrated for use, as well as algal mass cultivation.

BACKGROUND

History and the Prospects of Research for Algal Biofuel Production

Biofuel production and the environment have been crucial issues in today's world. Several researchers have described the need for biofuels and the kinds of materials that can serve this purpose (Naika *et al.* 2010; Antoni *et al.* 2007). Based on productivity per unit area, algae constitute one of the most effective raw materials that could be exploited for the biofuel production. Algal biomass is capable of producing a host of end products including energy, chemicals, food, cosmetics, fertilizer, and agents for wastewater treatment and/or CO_2 sequestration. This could reduce production costs, since there would be a variety of products to serve as sources of revenue, as the cost-effectiveness of these is crucial for the economic and commercial viability of these algal products.

Algal biomass can be used as raw material for biofuel production *via* pyrolysis (bio-oil), or for bio-gas and bio-ethanol generation through fermentation. Macro and micro algae for bioenergy production should satisfy several criteria as listed below (Carlsson *et al.* 2007): i) they should be highly productive; ii) they should be easily harvestable; iii) they should be able to withstand water currents in the open ocean; and iv) they should be produced at a cost that is equal or lower than the other available sources. Scientific research has been started on the utilization of the various species of algae in waste water/seawater treatment in order to transform them into biofuels by means of

various technological processes ranging from the esterification to anaerobic digestion (Kraan 2010).

Macroalgae

Macroalgae constitute the most important component in the marine ecosystems that serve for the marine bioresources preservation by preventing eutrophication and pollution (Notoya 2010). Macroalgae belong to the lower plants, in that they do not have roots, stems, and leaves. Instead, they are composed of a thallus (leaf-like) and sometimes a stem and a foot. Some species enclose gas-filled structures to help in buoyancy. They can grow very fast and in sizes of up to tens of meters in length (Luning and Pang 2003). Macroalgae differ in various aspects, such as morphology, longevity, and ecophysiology. Based on their pigmentation, they are classified into Phaeophyta (brown), Rhodophyta (red), and Chlorophyta (green) algae (Chan *et al.* 2006). In their natural environment, macro-algae grow on rocky substrates and form stable, multi-layered, perennial vegetation, capturing almost all available photons. Approximately 200 species of macroalgae are used worldwide, about ten of which are intensively cultivated, such as the Phaeophyta, *Laminaria japonica* and *Undaria pinnatifida*, the Rhodophyta, *Euclima*, *Gracilaria*, *Porphyra* and *Kappaphycus*, and the Chlorophyta, *Enteromorpha* and *Monostroma* (Luning and Pang 2003). Figure 1 shows examples of some commercially exploited macroalgae.

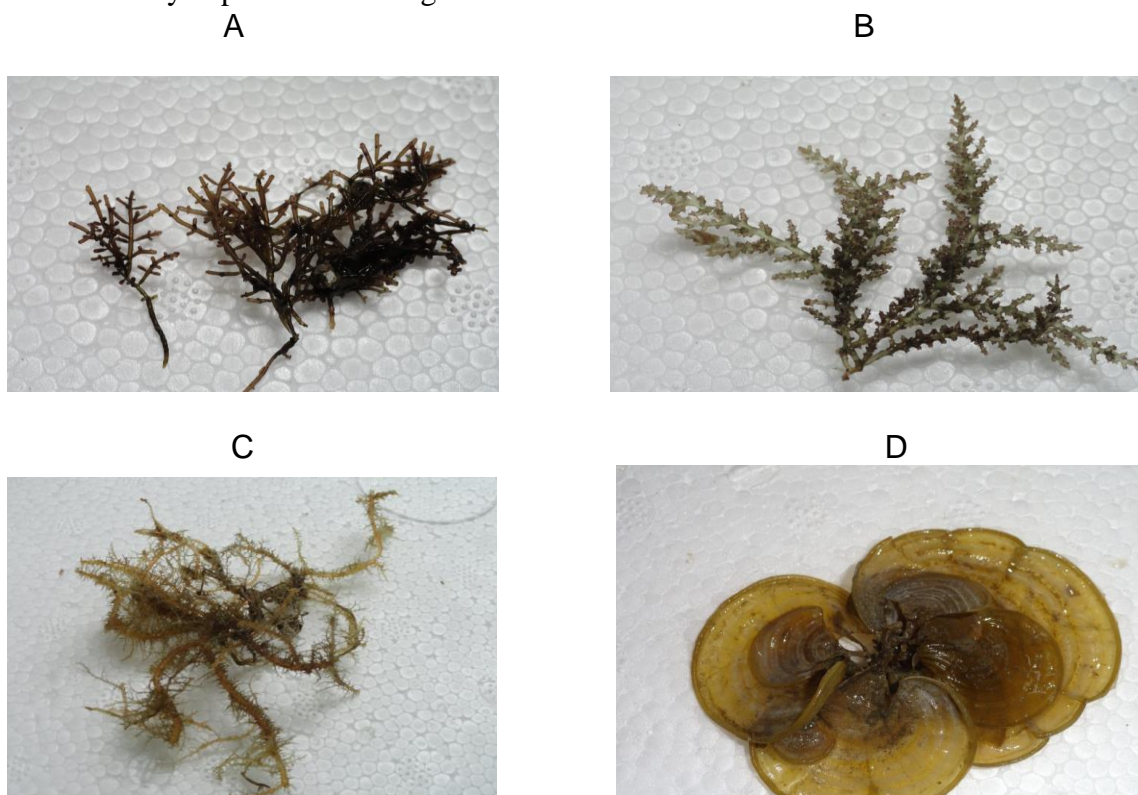


Fig. 1. Some commercially exploited macroalgae A) *Gracilaria dura*; B) *Acanthophora spicifera*; C) *Hypnea esperi*; D) *Padina pavonica*

The world production of macroalgae reached 8 million tons in 2003 (McHugh 2003). Many countries have now embarked on establishing large scale macroalgae

cultivation in their territories. Recent research (www.unbsj.ca/sase/biology/chopinlab) has shown the potential of macroalgae for large-scale culture in the Atlantic waters of Canada, France (Kaas 2006), Germany (Buck and Buchholz 2004), Ireland (Kraan *et al.* 2000), Isle of Man, UK (Kain *et al.* 1990), and Spain (Peteiro and Freire 2009). In Asian countries such as China, India, Philippines, South and North Korea, Indonesia, and Japan, macroalgae is being cultivated for various needs such as food, feed, chemicals, cosmetics, and pharmaceutical products (Carlsson *et al.* 2007).

Importance of Macroalgal Biomass

With substantial processing required for fossil fuels and the higher cost of vegetable oils, there has been a great deal of interest in the algal culture. Apart from that, algal biofuel production presents the following advantages:

1. Production of biofuel from the macroalgae cultivation in seawater is a new approach, since 70% of the earth's surface is covered by water. Macroalgae possess a unique life cycle. They are more productive in view of the fact that more than five harvests can be made in a year.

2. In addition, macroalgae can succeed in salty water with only sunlight and available nutrients from the seawater. They do not need any chemical fertilizer. Thus, large amounts of energy and money could be saved. These characteristic features favor the sustainability of the production of macroalgae-based bioethanol.

3. Production of bioethanol from terrestrial plants leaves a large impact on the environment in general and on human beings in particular due to eutrophication, acidification, and ecotoxicity. This is mostly caused by agricultural practices by the generation of waste water (Luo *et al.* 2009).

4. In general, macroalgae can live in a variety of environmental conditions. There is a wide range of organisms that grow along the coastal areas. With the advancement of genetic engineering, it is now possible to develop a suitable species of macroalgae for bioethanol production (Goh and Lee 2010). Genetically engineered macroalgae would need to be cultivated in enclosed bioreactors. These characters bring about high confidence for future improvement of macroalgae in renewable energy area such as bioethanol.

5. Converting the macroalgal biomass to ethanol rather than using terrestrial plant biomass have some important benefits, *i.e.*, no negative impact on the food security. The relatively high sugar content and lower lignin content than lignocelluloses facilitates high mass production (Adams *et al.* 2009; Wi *et al.* 2009)

6. Algal biomass can be cultivated in the unused vast ocean of the coastal area within the limited economic zone. In fact, utilization of sea water for the algal biomass production has great potential to relieve the water crisis. As for the ecology, macroalgae supplies oxygen to the sea and helps reduce the accumulation of carbon dioxide in the atmosphere (Goh and Lee 2010).

7. Several algal species are known for their ability to remove heavy metals from water, which can be useful to the environment (Aderhold *et al.* 1996). Certain algal species have the ability to produce high amounts of carbohydrates instead of lipids as preserved polymers. These are the ideal candidates for bioethanol production, as carbohydrates from algae can be extracted and then converted to fermentable sugars.

8. Apart from bioethanol production, algal biomass can be used for the production of an enormous variety of supplementary products *i.e.*, protein, pigments, plastics, *etc.* (Reith *et al.* 2005; Wijffels 2009). In addition to replacing fossil fuels,

thereby mitigating climate alteration, algal biomass can also serve in the recycling of heavy nutrients in the near and inshore waters (Kraan 2010).

9. Macroalgae provides a promising bioethanol feedstock owing to their high biomass yield with a superior production relative to various terrestrial crops (John *et al.* 2011).

In light of the considerations just mentioned, there is a need to develop large-sized culture areas in the open sea (off-shore) for the resources of biofuel production. In this context, the biofuel from the macro algae offers an excellent alternative to the currently used fossil fuels. Thus, the cultivation and engineering of the macro algae have drawn the world's attention in view of their value as a substitute for the conventional fossil fuels which are fast becoming depleted.

Composition and Processing of Macroalgal Biomass

Macroalgal biomass has a great potential both in quantity as well as in quality for the production of variety of specific bioenergy components. Previous studies (Reith *et al.* 2005) have shown that growing macroalgae can be efficient and feasible if the production processes of the bioenergy and bio-based products are combined. Certain products from the algal industry have long been used for the production of various products, *i.e.*, agars, alginates, and carrageenans (McHugh 2003). These polymers are storage materials located either in the cell walls or within the cells.

In general, chlorophyll *a* and *b* are the major pigments in green macroalgae. Starch is the photosynthetic product in green algae, the cell walls of which are primarily made up of pectin and cellulose (Trono Jr. and Ganzon-Fortes 1988). The r-phycoerythrin is a major pigment in red macroalgae, the cell walls of which have minimum amounts of cellulose, while the maximum is gelatinous or amorphous sulfated galactan polymers *i.e.*, funoran, agar, carrageenan, *etc.* Brown macroalgal colouration is related to the high amount of the xanthophyll pigments, particularly alginic acid and fucoxanthin (Ganzon-Fortes 1991), which are present along with cellulose and the other polysaccharides. The carbohydrates laminarin and mannitol are the food reserve materials that are particularly suitable for the production of ethanol (Davis *et al.* 2003). The content of carbohydrates in macroalgae varies widely among species and cultivar, and species selection can lead to evolution of strains having extremely high amounts of carbohydrate that can be utilized as an inventive bioethanol feedstock. The carbohydrate contents of some macroalgae are given in Table 1.

Some species of macroalgae gather a high amount of carbohydrates that are capable in the processes of microbial conversion as substrate, *i.e.*, production of biofuels or the other desirable and attractive chemicals with high product price (Kraan 2010). Recently, Maceiras *et al.* (2011) discovered that triglycerides from a number of macroalgae such as *Ascophyllum nodosum*, *Codium tomentosum*, *Enteromorpha intestinalis*, *Fucus spiralis*, *Saccorhiza polyschides*, *Sargassum muticum*, *Ulva rigida*, and *Pelvetia canaliculata*, *etc.* could be used to produce biodiesel by a transesterification process. Horn *et al.* (2000) and Ross *et al.* (2008) reported that the water content in the macroalgae is higher than that in the terrestrial plants (80 to 85%), making macroalgae more suited for microbial conversion than for the thermochemical conversion or direct combustion processes. Macroalgae such as *Sargassum* spp., *Gracilaria* spp., *Prymnesium parvum*, *Gelidium amansii*, and *Laminaria* spp. are promising candidates for bioethanol production (Wi *et al.* 2009; Adams *et al.* 2009). The red alga, *Gelidium* J.V. Lamouroux has been prepared for the production of paper in which the waste products have been

renewed into bioethanol (Seo *et al.* 2010). Also, green algae of *Ulva* spp., with a high-grade polysaccharide, Ulvan (Lahaye and Ray 1996) have been used in the production of ethanol and methane (Adams *et al.* 2009). Meinita *et al.* (2011) have recently reported bioethanol production from *Kappaphycus alvarezii*. Similarly, Karunakaran and Gurusamy (2011) also reported bioethanol production from *Eucheuma* and *Hypnea*. In mid-2008, studies were initiated for the production of bioethanol and biofertilizer from *K. alvarezii* on a laboratory scale by an integrated 2-product strategy (Mody *et al.* 2009). Benjamin (1993) in his US patent explained the utilization of genetically transformed marine green macro algal (*Enteromorpha*) cells for the bioethanol production. Uchida and Murata (2004) described lactic acid and ethanol fermentation using various types of green, brown, and red algae. In addition, Adams *et al.* (2009) reported the effect of enzymatic pretreatment for their bioethanol production in a brown alga, *Saccharina latissima* (*Laminaria saccharina*).

Table 1. Carbohydrate Contents of Macroalgae (Dhargalkar and Pereira 2005)

Species	Group (or phylum)	Carbohydrates (in percentage)
<i>Ulva</i>	Green algae	42.0
<i>Enteromorpha</i>	Green algae	64.9
<i>Monostroma</i>	Green algae	63.9
<i>Laminaria</i>	Brown algae	39.3
<i>Alaria</i>	Brown algae	39.8
<i>Sargassum</i>	Brown algae	33.0
<i>Padina</i>	Brown algae	31.6
<i>Porphyra</i>	Red algae	45.1
<i>Rhodomenia</i>	Red algae	44.6
<i>Gracilaria</i>	Red algae	61.75

In fact, only limited information has become available on the effectiveness of these processes with macroalgal carbohydrates (Horn *et al.* 2000), even though some breakthroughs have been newly made with respect to ethanol production from the brown macroalgae (Adams *et al.* 2009). Red algae produce high amounts of bioethanol-producing carbohydrates. Although macroalgae can look similar to the land plants, these organisms in fact, do not have the same lignin crosslinking molecules in their structures as cellulosic terrestrial plants because they grow in the water surroundings where they are able to grow erect despite their lack of lignin crosslinking (John *et al.* 2011). Although macroalgae have low amounts of lignin, they have significant amounts of sugars that could be used in the fermentation process for the production of bioethanol (Wi *et al.* 2009). Nevertheless, in certain algae such as marine red algae, the content of carbohydrate is influenced by the occurrence of agar, a polymer of galactose and galactopyranose. Recent research has sought to improve methods of saccharification to release galactose from the agar and glucose from cellulose to produce ethanol through fermentation (Wi *et al.* 2009). The potential of macroalgae for ethanol production can be estimated based on the following postulations: a content carbohydrate 60 % of dry weight and a 90 % of conversion levels to ethanol through fermentation of 1 g of sugar can yield 0.4 g of ethanol. It will ideally give up 0.22 kg or 0.27 L ethanol from 1 kg dry weight of

macroalgal biomass, equivalent to roughly 0.05 L ethanol per kg of wet weight (Kraan 2010).

Algae are also a potential source of commercial biogas products, such as biohydrogen and biomethane that can be used as gas fuels or for electricity generation (Mussgnug *et al.* 2010). Hydrogen produced by macroalgae is a popular attraction in the renewable energy scenario. Current research has revealed that *Laminaria japonica* (brown alga) and *Gelidium amansii* (red alga) are both potential biomass sources for the production of biohydrogen by anaerobic fermentation (Park *et al.* 2011). Macroalgae can produce biohydrogen under specific conditions. Ongoing discussions on the prospects of hydrogen production by algae have been well-documented (Prince and Kheshgi 2005; Rupprecht *et al.* 2006).

In a study of the feasibility of methane production from macroalgal biomass, case scenarios assumed yields of 11 dry t ha⁻¹ y⁻¹ based on data from commercial growers (Chynoweth 2002). Research to determine the technical and economic feasibility of biomethane production from marine biomass was conducted from 1968 until 1990 under the sponsorship of the U.S. Navy, the American Gas Association and Gas Research Institute, and the U.S. Department of Energy; such work was reviewed by Chynoweth (2002). The study compared the technical potential of different biomass sources (marine algae, wood and grass species, and municipal solid waste) to be used in the energy farms and concluded that the marine biomass offered the highest advantage. Marine algae, such as *Gracilaria* sp. and *Macrocystis* are excellent substrates for biomethane generation (Bird *et al.* 1990). The view expressed by many authors is that the best approach to biomethane production from macroalgae is the multipurpose use of algal biomass, for example gas evolution from the digestion of the residues from hydrocolloid extraction (Kerner *et al.* 1991). In this way, the coproduction of methane with the other products could bring down the production costs and could make biomethane production profitable. Methane, ethanol, and biohydrogen production from different macroalgal biomass sources are presented in Table 2.

Table 2. Different Strains of Macroalgae for Biofuel Production

Feedstock	Potential of biomass	Reference
<i>Laminaria</i> sp.	Methane production	Chynoweth <i>et al.</i> (1993)
<i>Gracilaria</i> sp.	Methane production	Bird <i>et al.</i> (1990)
<i>Sargassum</i> sp.	Methane production	Bird <i>et al.</i> (1990)
<i>Macrocystis</i> sp.	Methane production	Chynoweth <i>et al.</i> (1993)
<i>Ulva</i> sp.	Methane production	Adams <i>et al.</i> (2009)
<i>Gelidium</i> sp.	Ethanol production	Yung-Bum <i>et al.</i> (2010)
<i>Ulva</i> sp.	Ethanol production	Morand <i>et al.</i> (1991)
<i>Kappaphycus alvarezii</i>	Ethanol production	Khambhaty <i>et al.</i> (2012)
<i>Gelidium amansii</i>	Hydrogen production	Park <i>et al.</i> (2011)
<i>Laminaria japonica</i>	Hydrogen production	Shi <i>et al.</i> (2011)

From an economic point of view it is not viable to produce biofuels from macroalgae with the current technology, except if the production process is combined with another, such as pollutant removal or the production of bio-based products (Savage 2011; Pittman *et al.* 2011). It is estimated that macroalgae phycocolloids represent a world market of some US\$ 600 Mio y⁻¹ (Mc Hugh 2003). Using macroalgae as feedstock will add a new sector of this area for various commercial applications. However, production rates and costs are critical to the economic and commercial success of algal

products; these issues are less commonly studied. Thus, more efforts are required to understand these processes with a view to identifying the potential of such macroalgae components for its application.

Microalgae

In general, microalgae are photosynthetic microorganisms that are found in both marine and freshwater habitats. Microalgae have been classified based on various characteristics such as pigmentation, photosynthetic storage product, the arrangements of photosynthetic membranes, and other morphological features. At present, microalgae species are divided into four groups, namely diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue green algae (Cyanophyceae), and golden algae (Chrysophyceae) (Khan *et al.* 2009). The dominating species of microalgae in commercial production include *Isochrysis*, *Chaetoceros*, *Chlorella*, *Arthrospira* (*Spirulina*), and *Dunaliella* (Lee 1997). *Chlorella* species are capable of changing from phototrophic to heterotrophic modes of nutrition among microalgal species (Xiong *et al.* 2008; Xu *et al.* 2006). As heterotrophs, the algae rely on glucose or other utilizable carbon sources for carbon metabolism and energy. Some algae can also grow mixotrophically. The biomolecules such as carbohydrates, proteins, lipids, and nucleic acids are the common constituents in microalgae (Williams and Laurens 2010).

Importance of microalgal biomass

Research and commercial applications of microalgae have gained interest during the last few years. Owing to their rapid growth rate, *i.e.*, 100 times faster than the land-based plants which can double their biomass in less than 1 day, microalgae appear to be an attractive renewable energy source (Tredici 2010). This is mostly due to their simple cellular system and big surface to quantity ratio that gave them the facility to utilize more amounts of nutrients from the source of water and hence, supporting their algae growth rate (Khan *et al.* 2009). Many strains of microalgae are known to produce high quantities of lipids that can be converted into biodiesel. Biofuel production using microalgal farming offers the following advantages (Ahmad *et al.* 2011):

1. Increased efficiency or decrease in the cost. The sum of harvesting and transportation of microalgae costs can be relatively low compared to those of the other plant biomass resources. On the other hand, the production cycle does not directly affect the human food chain supply system, avoiding the food against fuel conflict.

2. Microalgae do not give any competition for land-based plants used for food production, fodder, and other value-added products (Huang *et al.* 2010).

3. Generally, microalgae can grow in fresh, brackish, or salt water environments or non-arable lands that are incompatible for growing other crops and conventional agriculture (Patil *et al.* 2008). In addition, they can be grown in photo-bioreactors (Janaun and Ellis 2010). For this reason – the nonselective growth – microalgae produce a greater yield per hectare with superior environmental attributes.

4. The most common microalgae contain oil ranges between 20 and 50% by dry weight of biomass, but superior productivities can be attained (Mata *et al.* 2010). Commonly, microalgae double their biomass within 24 h, but the exponential growth content can result in a doubling of their biomass in periods as short as 3.5 h (Chisti 2007).

5. Microalgae are able to produce various valuable supplementary products *i.e.*, carbohydrates, proteins, biopolymers, and residual biomass, and these can be used for

feed or fertilizer purposes. In addition, herbicides or pesticides are not required for the cultivation of microalgae (Rodolfi *et al.* 2008).

6. Consideration of microalgae as an efficient cellular system for harvesting solar energy for production of various organic compounds (Vonshak 1990).

7. Microalgae are able to fix carbon dioxide in the atmosphere, assisting the reduction of atmospheric carbon dioxide levels, which is recently considered a global crisis. In addition, production of microalgal biomass can affect the biofixation of waste carbon dioxide, reducing the releases of a major greenhouse gas (1 kg of dry microalgal biomass requires about 1.8 kg of carbon dioxide) (Chisti 2007; Rodolfi *et al.* 2008).

8. Microalgal lipids are typically neutral lipids. Owing to their high degree of saturation and fast accumulation in the cellular system at various stages of microalgal growth, lipids remain as a prospective replacement for diesel fuel (Danquah *et al.* 2009).

Besides lipid extraction, some microalgae (blue green) (which produce glycogen instead of starch) can produce biohydrogen under anaerobic conditions (Hankamer *et al.* 2007; Melis *et al.* 2000), and their fermentation can also be used for the production of methane. By extracting more than one type of microalgal biofuel as value-added products, the value of the biomass is increased while contributing additional offsets to the ecological impacts. As stated above, the combined biorefinery perception can be used to enhance ethanol substance from the algae (Danquah *et al.* 2009). This model can also be exploited in combination with the production of biohydrogen and biogas either by giving a valuable product before the techniques of fermentation or by using the products of gaseous fermentation to power the progress of producing those high value entities such as methane, biodiesel, and bio-hydrogen. Exploitation of micro-algae for combined applications of biofuels production is under research (Li *et al.* 2011; Chisti 2007).

Composition and processing of microalgal biomass to biofuel

Current research enterprises have shown that microalgal biomass appears to be one of the promising sources of renewable biodiesel, which is capable of facing the global demand. Oil content from the microalgal biomass can exceed 80% by dry weight (Rodolfi *et al.* 2008). Oil ranges of 20 to 50% are quite common (Chisti 2007) (Table 3).

Oil production yield can be defined as the mass of oil produced per unit volume of broth of microalgal culture per day based on the growth rate of microalgae and the oil content of the biomass. Several species of algae produce high content of lipids as storage materials, as high as 50 to 60% of their dry weight. These lipid systems are chemically analogous to other crop-derived oil-seed lipids, rendering algae a promising source of biodiesel production (Griffiths and Harrison 2009). Various techniques have been applied for more efficient lipid extraction from microalgae. Most common methods are expeller/oil press, ultrasound techniques, solvent extraction, and supercritical fluid extraction. The preferred characteristics of the extraction methods are that the process should be fast, non-damaging and effective to lipids extracted, and scaled up easily (Medina *et al.* 1998). Extraction of lipid by using a modified Bligh and Dyer (Bligh and Dyer 1959) method is widely used (Mutanda *et al.* 2011). Extraction of microalgal fatty acids by using direct esterification, simultaneous extraction, and transesterification can be performed on various types of biomass, making it a versatile method for biofuels production. This is a multistep process and it needs a mixture of solvent extraction, ultrasonication, heating at high pressure, filtration, density separation of solvent, and liquids and oil recovery by the process of evaporation to dryness (Belarbi *et al.* 2000). Table 4 shows the advantages and limitations of various lipid extraction methods for microalgae oil.

Table 3. Oil Content of Microalgae by Chisti (2007) and Rodolfi *et al.* (2008)

Feedstock	Oil content (% dry wt)
<i>Botryococcus braunii</i>	25-75
<i>Chlorella</i> sp.	28-32
<i>Chlorella vulgaris</i> CCAP211/11b	19.2
<i>Chlorococcum</i> sp. UMACC112	19.3
<i>Chaetoceros muelleri</i> F&M	33.6
<i>Chaetoceros calcitrans</i> CS178	39.8
<i>Cryptocodinium cohnii</i>	20
<i>Cylindrotheca</i> sp.	16-37
<i>Dunaliella primolecta</i>	23
<i>Isochrysis</i> sp.	25-33
<i>Monallanthus salina</i>	>20
<i>Nannochloris</i> sp.	20-35
<i>Nannochloropsis</i> sp.	31-68
<i>Neochloris oleoabundans</i>	35-54
<i>Nitzschia</i> sp.	45-47
<i>Phaeodactylum tricornutum</i>	20-30
<i>Pavlova lutheri</i> CS182	30.9
<i>Schizochytrium</i> sp.	50-77
<i>Scenedesmus</i> sp. F&M-M19	19.6
<i>Scenedesmus</i> sp. DM	21.1
<i>Skeletonema</i> sp. CS252	31.8
<i>Tetraselmis suecica</i>	15-23

Table 4. Advantages and Limitations of Various Lipid Extraction Methods for Microalgae Oil (Harun *et al.* 2010c).

Methods	Advantages	Limitations	References
Oil press	Easy to use, no solvent involved	Large amount of sample required, slow process	Popoola and Yangomodou 2006
Solvent extraction	Solvent used are relatively inexpensive; reproducible	Most organic solvents are highly flammable and/or toxic; solvent recovery is expensive and energy intensive; large volume of solvent needed	Herrero <i>et al.</i> 2004; Galloway <i>et al.</i> 2004
Supercritical fluid extraction	Non-toxicity (absence of organic solvent in residue or extracts), 'green solvent' used; non-flammable, and simple in operation	Often fails in quantitative extraction of polar analytes from solid matrices; insufficient interaction between supercritical CO ₂ and the samples	Macias-Sanchez <i>et al.</i> 2005
Ultrasound	Reduced extraction time; reduced solvent consumption; greater penetration of solvent into cellular materials; improves the release of cell contents into the bulk medium	High power consumption; difficult to scale-up	Luque-Garcia and Luque De Castro 2003

These extraction methods are still on a laboratory scale and none of them has been demonstrated to be practical and economical for commercial production (Chen *et al.* 2009). Currently, most of the lipid extraction methods are facing many problems with high costs coupled with water removal and difficulties with disrupting the algal cellular system to make lipids efficiently accessible.

In spite of the high productivity, biodiesel from microalgae still has not yet become economical; algal biodiesel has been priced at US \$1.25/lb, whereas petroleum-based diesel has been priced at US \$0.43/lb (Li *et al.* 2011). The expenditure for the algae-derived biodiesel is proportional to the algal species-specific efficiency to carbon dioxide sequestration as lipids. Hence, microalgal prospecting would greatly impact the upcoming efficiencies and thus help reduce the production cost of algal biodiesel (Griffiths and Harrison 2009). Bioprospectors look for potential strains that are not only large lipid producers, but also show abundant growth and harvesting uniqueness (Mutanda *et al.* 2011). Several diatoms also have been examined for their lipid production, including *Amphora* (De la Pena 2007), *Chaetoceros calcitrans* (Rodolfi *et al.* 2008), *Cyclotella cryptica* (Sheehan *et al.* 1998), some species of *Nitzschia* (Griffiths and Harrison 2009), *Phaeodactylum tricornutum* (Ceron Garcia *et al.* 2000), *Thalassiosira pseudonana* (Rodolfi *et al.* 2008), *Chaetoceros gracilis*, and *Tetraselmis tetrathele* (Araujo *et al.* 2011). Microalgae also have been studied for bioethanol production. Table 5 presents the bioethanol yield from various strains of microalgae.

Table 5. Bioethanol Production from Various Strains of Microalgae

Feedstock	Ethanol yield (g ethanol/g substrate)	Reference
<i>Chlorococcum humicola</i>	0.52	Harun and Danquah (2011)
<i>Chlorococcum infusionum</i>	0.26	Harun <i>et al.</i> (2010b)
<i>Chlamydomonas reinhardtii</i>	0.24	Choi <i>et al.</i> (2010)
<i>Spirogyra</i> sp.	-	Eshaq <i>et al.</i> (2011)

The green algae *Chlorococum* spp. and *Spirogyra* spp. have been revealed to accumulate high contents of polysaccharides together in their complex cell walls and as starch. This accumulation of starch can be used in the bioethanol production (Harun *et al.* 2010a; Eshaq *et al.* 2011). Harun *et al.* (2010a) have stated that the green algae *Chlorococum* sp. produces 60% higher ethanol from samples that are pre-extracted for lipids against those that stay as desiccated undamaged cells. This implies that microalgae can be utilized for the production of both lipid biofuels and for ethanol biofuels from the similar microalgal biomass as a way to boost their overall economic value. Bioethanol has the prospect of being an alternative fuel, but it is highly important to ensure that the expansion of this fuel is not hindered by the raw material constraints (Harun *et al.* 2010b). In this context, the harvesting cycle of microalgae cells has a very short period (1 to 10 days) compared with the other feedstock (harvesting time once or twice per year), and thus can provide enough supplies to meet demands for the ethanol production (Schenk *et al.* 2008). Additionally, algae have the photon conversion ability and can synthesize and accumulate large amounts of carbohydrate biomass for the production of bioethanol from the cheapest source of raw materials (Subhadra and Edwards 2010). Hon-Nami (2006) has described the fermentation of *Chlamydomonas perigranulata* to

produce ethanol, butanediol, acetic acid, and CO₂, thus showing the multiutility of the algal biomass. Interestingly, Harun *et al.* (2010a) showed that the lipid-extracted microalgae yielded 60% higher ethanol than that from the dried/intact microalgae, thus implying the significance of using the spent biomass for ethanol production.

Anaerobic fermentation

In recent times, microalgae have also become a subject of interest in the biogas production by the process of anaerobic fermentation. Biogas production is considered to lead to a net reduction for the emissions of greenhouse gas; this is because methane would otherwise be released into the atmosphere (Fredriksson *et al.* 2006). For this purpose, anaerobic digestion is an initial process that can solve the waste biomass issue as well as the energetic and economical balance of such a successful technology for algal biofuel production (Sialve *et al.* 2009). Chisti (2008) mentioned the energy recovery from the microalgal residues after biodiesel production, highlighting its importance to meet the current energy demands of the preceding processes. He also theoretically calculated that an average heating value of 9360 MJ/metric tons of microalgal residues was recoverable as methane. Furthermore, co-digesting the microalgal residues with a glycerol co-product obtained during the transesterification of algal oils in quantities equivalent to those produced was observed to increase the CH₄ yields by 5 to 8% when compared to the digestion of the residues alone (Ehimen *et al.* 2008). Blue-green algae are also able to produce biohydrogen via an anaerobic process involving the oxidation of ferredoxin by the hydrogenase enzyme activity (Yacoby *et al.* 2011). Nevertheless, hydrogenases are directly involved with other metabolic processes for the detachment of electrons, and not all functions of hydrogenases activities alike. Thus, a significant volume of the recent research on microalgal photobiohydrogen production has been aimed at identifying the vigorous hydrogenase activities, accepting their interaction with ferredoxin and the other metabolic functions, and genetically changing these interactions to enhance the effectiveness for the production of biohydrogen (Yacoby *et al.* 2011; Wecker *et al.* 2011). Although hydrogen production from the algae is still a long way from its commercial viability, continued progress in this area indicates its ultimate potential.

The production of biogas efficiency has been revealed to be species-dependent and is based on the relative efficacy of cell deprivation and on the absence or presence of molecules that might prohibit the methanogenic archaea (Mussgnug *et al.* 2010). Production of biogas from algae may also involve an important function in phycoremediation, as harmful algal blooms in ponds, lakes, or oceans can result in the release of poisonous secondary metabolites that can cause deleterious effects on these environments; clearing these algae for the production of biogas can minimize these impacts (Yuan *et al.* 2011). Currently, the biogas production from algae is still incomplete, owing to the need to heat the digesters and the necessity for more land area and infrastructure to generate the same content of energy that can be obtained from the algal biodiesel (Collet *et al.* 2011). The value-added product can comprise biohydrogen produced anaerobically just before the process of anaerobic digestion for the production of biogas (Mussgnug *et al.* 2010). In addition, generation of electricity from biogas can be used to offset the requirements of energy for the microalgal anaerobic digestion during biogas production; agriculturally originated biogas can be used to give a CO₂ stream for algal growth and the production of coproduct and biogas can be used to power the cultivation and lipid extraction methods for the biodiesel (Collet *et al.* 2011; Douskova *et al.* 2010; Harun *et al.* 2011).

Catalytic cracking and liquefaction for thermochemical conversion

Preliminary studies made by several researchers on *Scenedesmus* and *Spirulina* indicate that biomass with ~20% oil content is suitable for conversion into biogasoline through the catalytic cracking process, as the product is rich in hydrocarbons (Ueda *et al.* 1996; Biller and Ross 2011). Similarly, all forms of carbon present in this biomass can be converted into biocrude oil through a thermochemical liquefaction process. Thermochemical conversion technologies of biomass are certainly not the most important opportunity in recent times; combustion is responsible for over 97% production of the world's bio-energy (Balat 2009). The primary thermochemical conversion processes are pyrolysis, gasification, and liquefaction. Biorenewable feedstocks can be converted into liquid or gaseous forms for the generation of electricity, heat, chemical, and gaseous or liquid fuels (Demirbas 2008). Although microbial or enzymatic transformations have more cost-reduction potentials in view of the recent developments and the constant efforts for their optimization, these routes are less prone to commercialization in the immediate future, thereby rendering the thermochemical conversion processes commercially viable, as these rely on processes that have been thoroughly studied over the years (Sims *et al.* 2010).

BIOFUEL APPROACHES

Algal Culture Systems

In general, the algal biomass grown with the industrial wastewaters can also be converted into biocrude oil using a thermochemical liquefaction process. Hence, growing algae in wastewaters for biofuel and bioenergy production seems a viable and eco-friendly option for the future. Two main culture systems are available for algal production. An open system generally combines waste treatment with algal production. This system employs the use of ponds, which range from the oxidation ponds to the high-rated algae ponds. An oxidation pond recycles nutrients through a bacteria-algae symbiotic process. The pond is one to two meters deep and unmixed. The algal yield in such a pond is thus low. In contrast, the high-rate algae pond (HRAP), which consists of an open raceway mixed by paddle-wheels, is very shallow and is capable of producing very high yields. High-rate algae ponds are suitable for the generation of algal biomass for high-quality animal feed and extraction of useful compounds such as protein and pigments. Research on the combined algal production and waste-treatment systems has been done in Israel (Shelef *et al.* 1980), India (Venkataraman *et al.* 1980), Thailand (Tanticharoen *et al.* 1990), the United States (Christenson and Sims 2012; Ellis *et al.* 2012; Rahman *et al.* 2012; Christenson and Sims 2011; Lincoln and Hill 1980), and some other countries.

As fresh water sources are scarce, utilization of poor quality wastewaters such as treated municipal sewage wastewater as low-cost nutrient growth medium for mass cultivation of biofuel algae appears a viable option for the future. In recent times, research into microalgal cultivation has gained importance because of application of this resource in the production of biofuels. Cultivation of microalgae in the open pond systems has been used since the 1950s (Borowitzka 1999), and raceway ponds are the most commonly used artificial systems. Open ponds provide a very efficient method of cultivating algae, but they become contaminated with the algal species very easily (Khan *et al.* 2009).

The major advantage of the open ponds is that they are very easy to construct and operate; in comparison to most closed systems, they are easy to clean up after cultivation and are ideal for mass cultivation of microalgae (Ugwu *et al.* 2008). This should be given consideration in view of the escalating equipment costs, particularly the use of the reactor-style systems that lack a reliable scale-up method. While considering the economic and the environmental aspects, a raceway pond coupled with a low cost harvesting technique would be a preferable choice to produce biodiesel.

While the demand for the production of biofuel is in part driven by ecological concerns, there is no doubt that constructing and operating an HRAP dedicated to producing algal biomass for biofuels can have an ecological impact. For example, resources of fresh water are consumed through evaporation, thus contributing to a water footprint (Park *et al.* 2011). Indeed, Clarens *et al.* (2010) summarized that production of algal biomass using freshwater and fertilizers would consume high energy, result in more greenhouse gas emissions, and use a lot of water compared to biofuel production from land-based crops, *i.e.*, canola, switch grass, and corn. Production of algal biomass using wastewater HRAPs, by contrast, offers a far more interesting proposition from an ecological point of view. The impacts of the HRAP construction and operation are a necessity of providing the treatment of wastewaters and hence, the subsequent algal production represents a biofuel feedstock free of this ecological issue (Park *et al.* 2011).

Among the various cultivation systems involved in producing algal biomass, the aspect of harvesting biomass is an important economic issue. It was estimated that harvesting algae biomass can account for 20 to 30% of the total production cost (Gudin and Thepenier 1986). When, the algae grow phototrophically, their concentration is about 0.5 to 1.0 g L⁻¹ for open ponds and around 5 to 10 g L⁻¹ biomass concentration for closed systems (Chisti 2007). For the production of 1 g L⁻¹ algal biomass, 1000 kg of water must be used to capture 1 kg of biomass.

Methods of algal biomass harvesting, such as filtration, centrifugation, sedimentation and flocculation, and floatation are being practiced either individually or in any combination. Several literature reviews have provided for the algae harvesting techniques (Mutanda *et al.* 2011; Grima *et al.* 2003; Chen *et al.* 2009; Harun *et al.* 2011). Among the various methods, centrifugation is a possible method suitable for higher-value products but is very expensive in an integrated system producing lower-value products, such as algal oils (<http://www.ecs.umass.edu/biofuels>). In the case of algal-derived biofuels, the low-cost promising method is gravity settling enhanced by flocculation, without benefit of chemical flocculants (Molina Grima *et al.* 2003). Other mechanisms exist, including the autoflocculation process, and it depends on the coprecipitation of calcium carbonate with microalgal cells and other precipitates that form in hard waters subject to high pH. Apart from settling, in some cases the biomass will float, either due to high oil content or by using a dissolved air flotation (DAF) process. Employing minor amounts of flocculants to assist in such a process could be cost effective, depending on the amount used. In general, the harvesting method of choice depends on algal species, the cultivation conditions, and the application of the product. For biofuels applications, low-cost algal harvesting techniques have not yet become established (Darzins *et al.* 2010). If new algal harvesting techniques have been developed, they have not yet been assessed publicly and, therefore, are not documented in this review. Significant research effort will be needed to develop the cost-effective techniques.

Integrated palm oil mill effluent treatment and biofuel production

Algae are a substantial component of ecosystems ranging from marine and fresh water ecosystems to desert sands and from hot springs to ice. In recent years, microalgae have been identified using morphological and molecular tools for their several purposes reported by many authors (Jayappriyan *et al.* 2010, 2011). Worldwide, algae contain thousands of diverse strains. When combined with the recent advances in bioremediation, these strains can provide a good initial point for further improvement of microalgal production methods based on treatment of wastewaters. Being a developing country, Malaysia releases a significant amount of pollutants into water bodies. Modern industries, animal and agriculture husbandry, agrobased industries, and the activities of urbanization have all contributed to the devastation of the natural environment (Goh and Lee 2010). Algae-based solutions have offered certain benefits to the various countries. Malaysia is basically an agricultural country, its main revenue earners being palm oil and rubber. Major pollution problems have arisen from the agroindustries based on these two major crops as well as on the increased waste from farm animals. The combined wastewater discharges from the oil palm and rubber industries contribute an organic load of 0.5 million kg of Biochemical Oxygen Demand (BOD) per day (Phang 1987). Various treatment methods, including biological, chemical, and mechanical, have been developed over the last two decades, with some being successfully implemented (Phang 1987). Researchers at the University of Malaya and the other government institutions have demonstrated the feasibility of using microalgae to treat agroindustrial wastewaters. Significantly, combining microalgal production and wastewater treatment offers an advantage over the conventional treatment systems.

Realizing the vast potential of microalgae cultivation towards sustainable energy development, researchers in this area have noted a few key points in up-scaling the overall process. One of the difficulties in up-scaling microalgae culture to an industrial scale is the source of food nutrients in the culture medium. The requirement of high nitrogen content and the other related chemical fertilizers to cultivate microalgae on a large scale has moved the process towards a loss of the environment. Also, cultivation of microalgae can basically play an important role as a self-purification process of the wastewaters in a natural condition (Soeder 1980). Municipal wastewater using conventional treatment that involves primary and secondary bio-treatment helped to remove only a portion of the nitrogen and phosphorus contained in the wastewater (Orpez *et al.* 2009). Therefore, culturing of microalgae in the wastewaters offers an inexpensive alternative to the conventional forms of tertiary treatment of wastewaters and at the same time consumes the nitrogen and the phosphorus contents in the wastewater to generate the microalgal biomass for the production of biofuel.

Recently, the concept of utilizing POME (Palm Oil Mill Effluent) as a nutrient source for the culture of microalgae in Malaysia has caught the attention of researchers. Because of its practical low cost and high value, most palm oil millers desire the culture of microalgae as a tertiary treatment before POME is released. Therefore, most of the nutrients such as nitrate and orthophosphate that are not eliminated during anaerobic digestion are further subjected to bioconversion in a microalgae treatment pond. After secondary treatment, the total nitrogen content of POME is still high and does not meet the discharge standard limit for wastewater which is 200 mg/L. In the meantime, the source of nitrogen, commonly existing in the nitrate form, plays an important function in promoting microalgal growth. In order to grow microalgae effectively, the basic nitrate concentration required should be in the range of 200 to 400 mg/ L (Li *et al.* 2008). Other

minerals that are essential for microalgal growth, *i.e.* K, Ca, Mg, Fe, Zn, and P, are also present in POME (Habib *et al.* 1998). Hence, POME has emerged as an alternative choice for the nutrient removal to grow microalgae for the production of biomass and concurrently acts as a part of the wastewater treatment process and is used for biofuel production.

The University Kebangsaan Malaysia (UKM) has been involved in developing algae-based technology to treat the palm oil industry effluents with the collaboration of Simerdarby (R & D), Malaysia since 2011. Various algal cultures were obtained from the Algaetech Company, Malaysia and maintained under laboratory conditions. They were allowed to grow in 2000 L of the POME as medium. Among them, *Spirulina platensis* showed higher growth rates and nutrient removal efficiency. A maximum growth rate of 1.1825 g/L was achieved by *Spirulina platensis* in anaerobically digested Palm Oil Mill Effluent on the 12th day in the culture flasks (Zainal *et al.* 2012). A pilot plant facility with raceway ponds is to be established at UKM for developing a continuous cultivation technology for algae in the treated (POME) wastewater for biofuel applications. The study also aims to evaluate the possibility of using the CO₂-rich flue gas emissions from the biogas generator for growing the alga. Algal technology for treating effluents is to be tried in some Malaysian industries in the future. In Malaysia, various industries and research institutes have demonstrated mass cultivation and biomass production of various micro algae in different industrial waste waters. Recently, Algaetech Industry has demonstrated mass cultivation of microalgae in the open raceway pond and the possibility of conversion of the total lipid into biodiesel.

Global Status of Biofuel

Fossil fuels continue to be exploited extensively by developing countries despite objection from several ecological activist organizations. Development and the economic growth of a country depend considerably on how the demand for the biofuel is best met. The currently available feedstock involved in the biofuel production includes vegetable oils derived from the oilseed crops, *e.g.*, sunflower, soybean, jatropha, oil palm or rapeseed, waste cooking oil and the animal fat, *e.g.*, beef tallow and pork lard (Moser Bryan 2009). Currently, exponential research growth, development of technology, and demonstration enterprises have kick-started the investigation of algal biomass as an alternative resource for the biofuel industries. One such renewable energy is algal biofuel, which has shown a huge potential to serve as a replacement for petroleum-based diesel. Recently, a significant number of companies proposing to use algae for producing biofuels and abating the climate change through CO₂ mitigation have emerged. Recently, two Canadian companies announced the formation of a new company with a proposal to convert CO₂ to algal biomass. For example, researchers from the Tokyo University of Marine Sciences and Technology released the details of a proposal for large scale bioethanol-fuel production from macroalgae (Carlsson *et al.* 2007). Yamazaki (2007) accounted the initiative of Japanese companies on launching bioethanol fuel to the marketplace with the expectations of reducing CO₂ emissions considerably.

Aizawa *et al.* (2007) reported that the "Oceans 2007" project has proposed to produce bioethanol by farming and harvesting *Sargassum horneri*. Stroazzo of Bio Fuel-System stated that production of biofuel from algae is a promising and ecofriendly approach. Radulovich (2008) also specified the use of macroalgae as a resource of fuel, apart from food, animal feed, and fertilizer. Kraan (2010) observed that Ireland is likely to become an important player in the future generation for the biofuel production with its

wealthy, sustainable macroalgal sources. Mullins (2009) stated that a group at the Korea Institute of Technology in South Korea has developed a method to use macroalgae to produce bioethanol and avoid taking up land together. Seambiotic (2009), in collaboration with the Inventure Chemicals, successfully demonstrated the bioethanol production via fermentation of polysaccharides in algal biomass. In their plant, algae were cultivated in the fossil fuel power plants to eliminate CO₂ emitted as a source of inorganic carbon.

The production of ethanol in 2009 attained 73.9 billion liters, which illustrates a more than 400% increase compared with that in 2000 of 17 billion liters in the world. While it has been predicted that the global ethanol production would continue to rise until 2017, reaching a level twice that of 2007, it also predicted that the United States and Brazil would remain as the biggest ethanol producers through 2017 followed by China, India, and Thailand. In addition, the FAO analysis shows that with the exclusion of bioethanol from sugar cane in Brazil, biofuels are commonly not economically viable relative to the fossil fuels in the absence of subsidies. Also, as for global biodiesel production, the report projects a slightly higher growth rate than for bioethanol to achieve 24 billion liters by 2017 and that the EU, comprising over 50% of the global production, will continue to be the leading biodiesel producer in 2017 and will be followed by Brazil, Indonesia, the United States, and Malaysia, respectively (Renewable fuels association, 2010).

The production of algal biomass integrated with remediation is the greater option, since it will not influence the agricultural land and water (Christenson and Sims 2011, 2012). Cultivation of algae in waste waters will make the whole process system cheaper and more economically viable. Among these, many microalgal genera and species are remarkably rich in oils that can be converted into biodiesel with the available technology.

It is believed that investigations on identifying novel algal resources, both micro and macro types, would herald a remarkable energy revolution in the years to come. To our knowledge, large-scale commercial operations are yet to be established to date. It is recommended that the three main stakeholders such as the government, the researchers, and the industry could join in a consortium that will be able to constitute a closer framework so as to ensure a sustainable development.

RECOMMENDATIONS

Apart from the export potential, job opportunities to be created, and the environmental security, there is potential for securing a prestigious status of a global leader in the production of biodiesel production on a path toward achieving the status of a developed country. This could also serve to demonstrate the country's capabilities in the international arena and thus, could help lift up the country's self esteem. In the long run, this would surely lead to enhanced motivation or spur more interest in other countries to follow suit with similar objectives or goals and thus could help transform the country into a developed nation.

Therefore, given the intrinsic advantages of the microalgae, and now existing restraints of using other oil crops, microalgae growing in wastewater would facilitate a cost-effective production of the biodiesel. Moreover, the current highly sophisticated R&D centers are conducting extensive research on various aspects of biofuel production, and particularly biodiesel. During the last decade, in view of the importance placed on

bioprospecting for better resources, especially in the agriculture sector, many well-equipped labs have been established in various countries, the world over.

This makes research organizations capable candidates to achieve projects on microalgal genetic engineering in order to address the rising biodiesel demand in the country. From the point of view of cultivation, it is imperative to note that apart from the free and unlimited access to salt water and plentiful sunshine, Malaysia and other tropical countries are known for their rich biodiversity of microalgae. Generally, technology development for large scale cultivation of macroalgae on the coastal regions for the production of bioenergy does not compete with that of food sources and does not alter the environment. The viability of algae mass cultivation can be judged from Malaysia's wide seashore surrounded by several islands, which can offer the ideal habitats for the algae propagation. Additionally, it has been shown by various reports that countries with abundance of under-utilized rice land are suitable for cultivating algae. Those marginal lands are infertile because of salty water diffusion, and farmers are looking for other options. Cultivation of algae can provide one such alternative as the marine algae naturally grow well in the salt waters.

Research and Development on Gene Sequence for Biofuel Production in Algae

Conversion of polysaccharides from macroalgae to fermentable sugar and thus to ethanol and biogas has not yet been studied in detail (Goh and Lee 2010). Although there is a wide availability of macroalgae the world over, there are still no viable suggestions for the production of bioethanol from macroalgae. Successful mapping of the oil-algae genome could help recognize the genetic traits responsible for the production of oil, and it could find strains susceptible to contamination by the basal bacteria or fungi which could cause the algal biomass to deteriorate in the open raceway ponds. Moreover, the genetic information can also be applied to develop the harvesting regimes of the oil algae so that the biomass could be harvested at the appropriate period. Such a technological understanding is imperative to ensure a constant progress in the world's biodiesel industry, as the land suitable for this purpose is so limited.

The successful decoding of the oil-algae genome is believed to have paved the way for a manipulation of the oil-algae genome for use in the future. The genetic manipulation in oil-bearing algae could provide the potential to produce high quantity of superior oil. With advanced biochemical and molecular techniques such as DNA recombination and site-directed mutagenesis, more groundbreaking outcomes could be expected to be employed in the near future (Eathington *et al.* 2007). These areas of research have laid a foundation for an understanding of the macroalgal biology. Despite the development and progress made in the functional genomics in terrestrial plants, macroalgae have received little attention worldwide and have not been incorporated in the approach to elucidate gene functions. It was not until the 1990s that studies on the genetic engineering of macroalgae were started. These studies have led the way with the improvement of the genetic transformation techniques on macroalgae (Cheney and Kurtzman 1992) and the characterization of genes involved in the synthesis of carbohydrates (Zhou and Ragan 1995). Macroalgal research has not received due interest and funding in the past, and the availability of the macroalgal genomic information is still meager in comparison to that of terrestrial plants. Given the fact that macroalgae share little similarity with the other organisms, the limited amount of genomic

information available makes the mission of exposing the role of unknown genes from the macroalgae a difficult proposition.

CONCLUSIONS

Biofuel production and environmental effects have been issues of some concern worldwide. In this review, the authors have described the advantages of biofuel, and the kind of materials that can serve as a source for this purpose. This was followed by the argument that under the present global scenario, algae appear to be the most effective raw material for biofuel production. Furthermore, the importance of sustainable energy sources such as bioethanol, biodiesel, and biohydrogen were also discussed. Among these, biodiesel production from algae biomass still would be the major component. Nevertheless, diversified biofuels production from algae biomass is very important to improve overall energy balance. For example, higher net value could be achieved by using a combined operation in which algae-produced lipids are converted to diesel fuel and the cellulosic part of the algal biomass (after lipid extraction) is enzymatically converted to glucose, which is fermented to produce bioethanol and other byproducts. Apart from that, biofuel contributes to energy security and helps reduce CO₂ emissions. A thorough understanding of the past may serve to overcome the past lapses toward building a better future. These recent biofuel discussions demonstrate two issues. First, they show the wide potential utility of these organisms that are capable of producing multiple products ranging from energy, chemicals, and materials to exploitation in the sequestration of carbon and remediation of wastewater. Second, they show the need for energetic support based on factual information to confirm decisions for the strategic improvement of algae and to counter those declarations made on a solely tentative basis to promote commercial investment.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support of the various grants (UKM-YSD endowment; LRGS-TD-2011-UMP-PG-04; AP-2012-008; INDUSTRI-2012-040).

REFERENCES CITED

- Adams, J. M., Gallagher, J. A., and Donnison, I. S. (2009). "Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments," *J. Appl. Phycol.* 21(5), 569-574.
- Aderhold, D., Williams, C. J., and Edyvean, R. G. J. (1996). "The removal of heavy metal ions by seaweeds and their derivatives," *Bioresour. Technol.* 58(1), 1-6.
- Ahmad, A. L., Mat Yasin, N. H., Derek, C. J. C., and Lim, J. K. (2011). "Microalgae as a sustainable energy source for biodiesel production: A review," *Renew. Sust. Energy Rev.* 15, 584-593.
- Aizawa, M., Asaoka, K., Atsumi, M., and Sakao, T. (2007). "Seaweed bioethanol production in Japan the Ocean Sunrise Project," In: *Oceans 2007*. Vancouver, Can.
- Antoni, D., Zverlov, V. V., and Schwarz, W. H. (2007). "Biofuels from microbes,"

- Appl Microbiol Biotechnol.* 77, 23-35.
- Araujo, G. S., Matos, L. J. B. L., Goncalves, L. R. B., Fernandes, F. A. N., and Farias, W. R. L. "Bioprospecting for oil producing microalgal strains: Evaluation of oil and biomass production for ten microalgal strains," *Bioresour. Technol.* 102, 5248-5250.
- Aresta, M., Dibenedetto, A., and Barberio, G. (2005). "Utilization of macro-algae for enhanced CO₂ fixation and biofuels production: development of a computing software for an LCA study," *Fuel Processing Technology* 86, 1679-1693.
- Balat, M. (2009). "New biofuel production technologies," *Energy Educ. Sci. Technol. Part A.* 22, 147-161.
- Belarbi, E. H., Molina, E., and Chisti, Y. (2000). "A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil," *Enzyme Microb. Technol.* 26, 516-529.
- Benjamin, M. (1993). "Methods and compositions for producing metabolic products for algae," US Patent No. 5,270,175.
- Biller, P., and Ross, A. B. (2011). "Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content", *Bioresour. Technol.* 102, 215-225.
- Bird K. T., Chynoweth, D. P., and Jerger, D. E. (1990). "Effects of marine algal proximate composition on methane yields," *J. Appl. Phycol.* 2, 207-213.
- Bligh, E. G., and Dyer, W. J. (1959). "A rapid method for total lipid extraction and purification," *Can. J. Biochem. Physiol.* 37, 911-917.
- Borowitzka, M. A. (1999). "Commercial production of microalgae: Ponds, tanks, tubes and fermenters," *J. Biotech.* 70(1-3), 313-321.
- Buck, B. C., and Buchholz, C. M. (2004). "The offshore ring: A new system design for the open ocean aquaculture of macroalgae," *J. Appl. Phycol.* 16, 355-69.
- Carlsson, A. S., Van beilen, J. B., Moller, R., and Clayton, D. (2007). *Micro and Macro Algae: Utility for Industrial Applications*, Bowles, D. (ed.), Cpl Press, Newbury, UK.
- Ceron Garcia, M. C., Fernandez Sevilla, J. M., Acien Fernandez, F. G., Molina Grima, E., and Garcia Camcho, F. (2000). "Mixotrophic growth of *Phaeodactylum tricor- nutum* on glycerol: growth rate and fatty acid profile," *J. Appl. Phycol.* 12, 239-248.
- Chan, C. X., Ho, C. L., and Phang, S. M. (2006). "Trends in seaweed research," *Trends in Plant Science* 11, 165-166.
- Cheney, D., and Kurtzman, A. (1992). "Progress in protoplast fusion and gene transfer in red algae," *Proc. Int. Seaweed Symp.* 16-21.
- Chen, P., Min, M., Chen, Y., Wang, L., Li, Y., Chen, Q., Wang, C., Wan, Y., Wang, X., Cheng, Y., Deng, S., Hennessy, K., Lin, X., Liu, Y., Wang, Y., Martinez, B., and Ruan, R. (2009). "Review of the biological and engineering aspects of algae to fuels approach", *Int J Agric & Biol Eng.* 2 (4), 1-30.
- Chisti, Y. (2007). "Biodiesel from microalgae," *Biotechnol. Adv.* 25, 294-306.
- Chisti, Y. (2008). "Biodiesel from microalgae beats bioethanol," *Trends Biotechnol.* 26, 126-131.
- Choi, S. P., Nguyen, M. T., and Sim, S. J. (2010). "Enzymatic pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production," *Bioresour. Technol.* 101, 5330-5336.
- Christenson, L., and Sims, R. (2011). "Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts," *Biotechnol. Adv.* 29, 686-702.

- Christenson, L. B., and Sims, R. C. (2012). "Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels by-products," *Biotechnology and Bioeng.* 109, 1674-1684.
- Chynoweth, D. P. (2002). "Review of biomethane from marine biomass," Review of history, results and conclusions of the "US Marine Biomass Energy Program" (1968-1990), 194.
- Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E., and Peck, M. W. (1993). "Biochemical methane potential of biomass and waste feedstocks," *Biomass & Bioenergy* 5, 95-111.
- Clarens, A. F., Resurreccion, E. P., White, M. A., and Colosi, L. M. (2010). "Environmental life cycle comparison of algae to other bioenergy feedstocks," *Environ. Sci. Technol.* 44, 1813-1819.
- Collet, P., He lias, A., Lardon, L., Ras, M., Goy, R. A., and Steyer, J. P. (2011). "Life-cycle assessment of microalgae culture coupled to biogas production," *Bioresour. Technol.* 102, 207-214.
- Danquah, M. K., Gladman, B., Moheimani, N., and Forde, G. M. (2009). "Microalgal growth characteristics and subsequent influence on dewatering efficiency," *Chem. Eng. J.* 151, 73-78.
- Darzins, A., Pienkos, P., and Edye, L. (2010). "Current status and potential for algal biofuels production," *A Report to IEA Bioenergy Task 39*, 1-131.
- Davis, T. A., Volesky, B., and Mucci, A. (2003). "A review of the biochemistry of heavy metal biosorption by brown algae," *Water Research* 37, 4311-4330.
- De la Pena, M. R. (2007). "Cell growth and nutritive value of the tropical benthic diatom, *Amphora* sp., at varying levels of nutrients and light intensity and different culture locations," *J. Appl. Phycol.* 19, 647-655.
- Demirbas, A. (2008). "Liquefaction of biomass using glycerol," *Energ. Sources Part A*, 30, 1120-1126.
- Dhargalkar, V. K., and Pereira, N. (2005). "Seaweed: Promising plant of the millennium," *Science and Culture* 71 (3-4), 60-66.
- Douskova, I., Kastanek, F., Maleterova, Y., Kastanek, P., Doucha, J., and Zachleder, V. (2010). "Utilization of distillery stillage for energy generation and concurrent production of valuable microalgal biomass in the sequence: Biogas cogeneration microalgae products," *Energ. Convers. Manage.* 51, 606-611.
- Eathington, S. R., Crosbie, T. M., Edwards, M. D., Reiter, R. S., and Bull, J. K. (2007). "Molecular markers in a commercial breeding program," *Crop Science* 47S3, S154-S163.
- Ehimen, E. A., Connaughton, S., Sun, Z., and Carrington, C. G. (2008). "Energy recovery from lipid extracted transesterified and glycerol co-digested microalgae biomass," *GCB Bioenergy*.1, 371-381.
- Ellis, J. T., Hengge, N. N., Sims, R. C., and Miller, C. D. (2012). "Acetone, butanol and ethanol production from wastewater algae," *Bioresour. Technol.* 111, 491-495.
- Eshaq, F. S., Ali, M. N., and Mohd, M. K. (2011). "Production of bioethanol from next generation feed stock alga *Spirogyra* species," *Int. J. Eng. Sci. Technol.* 3, 1749-1755.
- Fredriksson, H., Baky, A., Bernesson, S., Nordberg, A., Noren, O., and Hansson, P. A. (2006). "Use of on-farm produced biofuels on organic farms evaluation of energy balances and environmental loads for three possible fuels," *Agric. Syst.* 89, 184-203.

- Galloway, J. A., Koester, K. J., Paasch, B. J., and Macosko, C. W. (2004). "Effect of sample size on solvent extraction for detecting cocontinuity in polymer blends," *Polymer* 45, 423-428.
- Ganzon-Fortes, E. T. (1991). "Characteristics and economic importance of seaweeds," In: Proceedings of the Seaweed Research Training and Workshop for Project Leaders, Philippine Council for Aquatic and Marine Research and Development.
- Gavrilescu, M., and Chisti, Y. (2005). "Biotechnology – A sustainable alternative for chemical industry," *Biotechnol. Adv.* 23, 471-499.
- Goh, C. S., and Lee, K. T. (2010). "A visionary and conceptual macroalgae-based third generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development," *Renew. Sust. Energy Rev.* 14, 842-848.
- Griffiths, M. J., and Harrison, S. T. L. (2009). "Lipid productivity as a key characteristic for choosing algal species for biodiesel production," In: *3rd Congress of the International Society for Appl Phycol.*
- Grima, E. M., Belarbi, E. H., Fernandez, F. G. A., Medina, A. R., and Chisti, Y. (2003). "Recovery of microalgal biomass and metabolites: Process options and economics," *Biotechnol. Adv.* 20, 491-515.
- Gudin, C., and Thepenier, C. (1986). "Bioconversion of solar energy into organic chemicals by microalgae," *Adv. Biotechnol. Process*, 6, 73-110.
- Habib, M. A. B., Yusoff, F. M., Phang, S. M., Kamarudin, M. S., and Mohamad, S. (1998). "Chemical characteristics and essential nutrients of agroindustrial effluents in Malaysia," *Asian Fish J.* 11, 279-286.
- Hankamer, B., Lehr, F., Rupprecht, J., Mussnug, J. H., Posten, C., and Kruse, O. (2007). "Photosynthetic biomass and H₂ production by green algae: From bioengineering to bioreactor scale up," *Physiol. Plantarum* 131, 10-21.
- Harun, R., and Danquah, M. K. (2011). "Influence of acid pre-treatment on microalgal biomass for bioethanol production," *Proc. Biochem.* 46, 304-309.
- Harun, R., Davidson, M., Doyle, M., Gopiraj, R., Danquah, M., and Forde, G. (2011). "Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility," *Biomass Bioenerg.* 35, 741-747.
- Harun, R., Danquah, M. K., and Forde, G. M. (2010a). "Microalgal biomass as a fermentation feedstock for bioethanol production," *J. Chem. Technol. Biotech.* 85, 199-203.
- Harun, R., Jason, W. S. Y., Cherrington, T., and Danquah, M. K. (2010b). "Exploring alkaline pretreatment of microalgal biomass for bioethanol production," *Appl. Energy*. 88, 3464-3467.
- Harun, R., Singh, M., Forde Gareth, M., and Danquah Michael, K. (2010c). "Bioprocess engineering of microalgae to produce a variety of consumer products," *Renew. Sust. Ener. Rev.* 14, 1037-1047.
- Herrero, M., Ibanez, E., Senorans, J., and Cifuentes, A. (2004). "Pressurized liquid extracts from *Spirulina platensis* microalga: Determination of their antioxidant activity and preliminary analysis by micellar electrokinetic chromatography," *Journal of Chromatography A* 1047, 195-203.
- Hon-Nami, K. (2006). "A unique feature of hydrogen recovery in endogenous starch to alcohol fermentation of the marine microalga, *Chlamydomonas perigranulata*," *Appl. Biochem. Biotechnol.* 131, 808-828.
- Horn, S. J., Aasen, I. M., and Ostgaard, K. (2000). "Ethanol production from seaweed extract," *J. Ind. Microbiol. Biotechnol.* 25, 249-254.

- Huang, G., Chen, F., Wei, D., Zhang, X., and Chen, G. (2010). "Biodiesel production by microalgal biotechnology," *Appl. Energy*, 87, 38-46.
- IEA Report. (1994). "Carbon dioxide utilization: evaluation of specific biological processes which have the capability of directly utilizing high concentrations of carbon dioxide as found in the flue gas streams from power generation plant," *Chemical Society of Japan Publishers*.
- Janaun, J., and Ellis, N. (2010). "Perspectives on biodiesel as a sustainable fuel," *Renew. Sust. Energy Rev.* 14, 1312-1320.
- Jayappriyan, K. R., Rajkumar, R., and Rengasamy, R. (2011). "Unusual occurrence of non carotenogenic strains of *Dunaliella bardawil* and *Dunaliella parva* in India," *J. Basic Microbiol.* 51, 473-483.
- Jayappriyan, K. R., Rajkumar, R., Sheeja, L., Nagaraj, S., Divya, S., and Rengasamy, R. (2010). "Discrimination between the morphological and molecular identification in the genus *Dunaliella*," *Int. J. Cur. Res.* 8, 73-78.
- John, R. P., Anisha, G. S., Nampoothiri, K. M., and Pandey, A. (2011). "Micro and macroalgal biomass: a renewable source for bioethanol," *Bioresour. Technol.* 102, 186-193.
- Kaas, R. (2006). "The seaweed resources of France," In: A. T. Critchly, D. B. Ohno M, Largo (eds.), *The Seaweed Resources of the World*, Interactive CD ROM, ETI, The Netherlands.
- Kain, J. M., Holt, T. J., and Dawes, C. P. (1990). "European laminariales and their cultivation," In: C. Yarish, C. A. Penniman, and P. Van Patten (eds.), *Economically Important Marine Plants of the Atlantic: Their Biology and Cultivation*, Connecticut Sea Grant College Program, Groton, 95-111.
- Kapdan, I. K., and Kargi, F. (2006). "Bio-hydrogen production from waste materials," *Enzyme Microb. Technol.* 38, 569-582.
- Karunakaran, S., and Gurusamy, R. (2011). "Bioethanol production as renewable biofuel from rhodophytes feedstock," *Int. J. Biol. Biotechnol.* 2(2), 94-99.
- Kerner, K. N., Hanssen, J. F., and Pedersen, T. A. (1991). "Anaerobic digestion of waste sludges from the alginate extraction process," *Bioresour. Technol.* 37, 17-24.
- Khambhaty, Y., Mody, K., Gandhi, M. R., Thampy, S., Maiti, P., and Brahmabhatt, H. (2012). "*Kappaphycus alvarezii* as a source of bioethanol," *Bioresour. Technol.* 103, 180-185.
- Khan, S. A., Rashmi., Hussain M. Z., Prasad, S., and Banerje, U. C. (2009). "Prospects of biodiesel production from microalgae in India," *Renew. Sust. Energy Rev.* 13, 2361-2372.
- Kraan, S. (2010). "Mass cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production," *Mitig. Adapt. Strateg. Glob Change* (DOI: 10.1007/s11027-010-9275-5).
- Kraan, S., Verges Tramullas, A., and Guiry, M. D. (2000). "The edible brown seaweed *Alaria esculenta* (Phaeophyceae, Laminariales): Hybridisation, growth and genetic comparisons of six Irish populations," *J. Appl. Phycol.* 12, 577-583.
- Lahaye, M., and Ray, B. (1996). "Cell wall polysaccharides from the marine green alga *Ulva rigida* (Ulvales, Chlorophyta)-NMR analysis of ulvan oligosaccharides," *Carbohydr. Res.* 283, 161-173.
- Lee, Y. K. (1997). "Commercial production of microalgae in the Asia-Pacific rim," *J. Appl. Phycol.* 9, 403-411.

- Li, P., Miao, X., Li, R., and Zhong, J. (2011). "In situ biodiesel production from fast-growing and high oil content *Chlorella pyrenoidosa* in rice straw hydrolysate," *J. Biomed. Biotechnol.* (DOI: 10.1155/2011/141207).
- Li, Y., Horsman, M., Wu, N., Lan, C. Q., and Dubois-Calero, N. (2008). "Biofuels from microalgae," *Biotechnol. prog.* 24, 815-820.
- Lincoln, E. P., and Hill, D. T. (1980). "An integrated microalgae system," In: *Algae Biomass*, G. Shelef and C. J. Soeder (eds.), Elsevier/North Holland, Biomedical Press, The Netherlands, pp. 229-243.
- Luning, K., and Pang, S. J. (2003). "Mass cultivation of seaweeds: Current aspects and approaches," *J. Appl. Phycol.* 15, 115-119.
- Luo, L., Voet, E. V. D., and Huppel, G. (2009). "An energy analysis of ethanol from cellulosic feedstock corn stover," *Renew. Sust. Energy Rev.* 13(8), 2003-2011.
- Luque-Garcia, J. L., and Luque De Castro, M. D. (2003). "Ultrasound: A powerful tool for leaching," *TrAC-Trends in Analytical Chemistry* 22, 41-47.
- Maceiras, R., Rodriguez, M., Cancela, A., Urrejola, S., and Sanchez, A. (2011). "Macroalgae: Raw material for biodiesel production," *Appl. Ener.* 88, 3318-3323.
- Macias-Sanchez, M. D., Mantell, C., Rodriguez, M., Martinez De La Ossa, E., Lubian, L. M., and Montero, O. (2005). "Supercritical fluid extraction of carotenoids and chlorophyll a from *Nannochloropsis gaditana*," *J. Food Engineering* 66, 245-251.
- Mata, T. M., Martins, A. A., and Caetano, N. S. (2010). "Microalgae for biodiesel production and other applications: A review," *Renew. Sust. En. Rev.* 14(1), 217-232.
- McHugh, D. J. (2003). "A guide to the seaweed industry," FAO Fisheries Technical Paper No 441, 105.
- Medina, A. R., Grima, E. M., Gimenez, A. G., and Gonzalez, M. J. I. (1998). "Downstream processing of algal polyunsaturated fatty acids," *Biotechnol. Adv.* 3, 517-580.
- Meinita, M. D. N., Kang, J. Y., Jeong, G. T., Koo, H. M., Park, S. M., and Hong Y. K. (2011). "Bioethanol production from the acid hydrolysate of the carrageenophyte *Kappaphycus alvarezii* (cottonii)," *J. Appl. Phycol.* (DOI 10.1007/s10811-011-9705-0).
- Melis, A., Zhang, L. P., Forestier, M., Ghirardi, M. L., and Seibert, M. (2000). "Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*," *Plant. Physiol.* 122, 127-136.
- Mody, K. H., Ghosh, P. K., Sana, B., Gopalasamy, G., Shukla, A. D., Eswaran, K., Brahmabhatt, H. R., Shah, B. G., Thampy, S., and Jha, B. (2009). "A process for integrated production of ethanol and seaweed sap from *Kappaphycus alvarezii*," Patent Filed Indian Application No. 1839/ DEL/ 2009 dated 07/09/09; WO 2011/027360A1 dated 10.03.11.
- Molina Grima, E., Belarbi, E. H., Acien Fernandez, F. G., Robles Medina, A., and Chisti, Y. (2003). "Recovery of microalgal biomass and metabolites: process options and economics," *Biotechnol. Adv.* 20, 491-515.
- Morand, P., Carpentier, B., Charlier, R. H., Maze, J., Orlandini, M., Plunkett, B. A., and de Waart, J. (1991). "Bioconversion of seaweeds," In: *Seaweed Resources in Europe, Uses and Potential*, Guiry, M. D., and Blunden, G. (eds.), Wiley, Chichester, 432.
- Moser Bryan, R. (2009). "Biodiesel production, properties and feedstocks," *In Vitro Cellular and Developmental Biology- Plant* 45, 229-266.

- Mullins, J. (2009). "Invention: Biofuel from the oceans," (<http://www.newscientist.com>).
- Mussgnug, J. H., Klassen, V., Schluter, A., and Kruse, O. (2010). "Microalgae as substrates for fermentative biogas production in a combined biorefinery concept," *J. Biotechnol.* 150, 51-56.
- Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A., and Bux, F. (2011). "Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production," *Bioresour. Technol.* 102, 50-57.
- Naika, S. N., Goud, V. V., Rout, P. K., and Dalai, A. K. (2010). "Production of first and second generation biofuels: A comprehensive review," *Renewable and Sustainable Energy Reviews* 14, 578-597.
- National Science Foundation, American Chemical Society, and Department of Energy. (2007). "Breaking the chemical and engineering barriers to lignocellulosic biofuels: Next generation hydrocarbon biorefineries" (<http://www.ecs.umass.edu/biofuels/Images/RoadmapFinal.pdf>).
- Notoya, M. (2010). "Production of biofuel by macroalgae with preservation of marine resources and environment," *Seaweeds and their Role in Globally Changing Environments, Cellular Origin, Life in Extreme Habitats and Astrobiology*, Israel et al. (eds.), Springer Science+Business Media B.V. 15, 217-228 (DOI 10.1007/978-90-481-8569-6-13).
- Orpez, R., Martinez, M. E., Hodaifa, G., El Yousfi, F., Jbari, N., and Sanchez, S. (2009). "Growth of the microalga *Botryococcus braunii* in secondarily treated sewage," *Desalination.* 246, 625-630.
- Park, J. H., Yoon, J. J., Park, H. D., Kim, Y. J., Lim, D. J., and Kim, S. H. (2011). "Feasibility of biohydrogen production from *Gelidium amansii*," *Int. J. Hydrogen Energy* 36, 13997-14003.
- Patil, V., Tran, K. Q., and Gislerod, H. R. (2008). "Towards sustainable production of biofuels from microalgae," *Int. J. Mol. Sci.* 9, 1188-1195.
- Peteiro, C., and Freire, O. (2009). "Effect of outplanting time on commercial cultivation of kelp *Laminaria saccharina* at the southern limit in the Atlantic coast, N.W. Spain Chinese," *J. Oceanology and Limnology* 27(1), 54-60.
- Phang, S. M. (1987). "Agro-industrial wastewater reclamation in peninsular Malaysia," *Arch. Hydrobiol. Beih.* 28, 77-94.
- Pittman, J. K., Dean, A. P., and Osundeko, O. (2011). "The potential of sustainable algal biofuel production using wastewater resources," *Bioresour. Technol.* 102 (1), 17-25.
- Popoola, T. O. S., and Yangomodu, O. D. (2006). "Extraction, properties and utilization potentials of cassava seed oil," *Biotechnology* 5, 38-41.
- Prince, R. C., and Kheshgi, H. S. (2005). "The photobiological production of hydrogen: Potential efficiency and effectiveness as a renewable fuel," *Critical Reviews in Microbiol.* 31, 19-31.
- Radulovich, R. (2008). "Let's use seaweed as fuel," (<http://www.cosmosmagazine.com>).
- Rahman, A., Ellis, J. T., and Miller, C. D. (2012). "Bioremediation of domestic wastewater and production of bioproducts from microalgae using waste stabilization ponds," *J. Bioremed. Biodeg.* 3, 113. (DOI:10.4172/2155-6199.1000e113).
- Reith, J. H., Deurwaarder, E. P., Hemmes, K., Curvers, A. P. W. M., Kamermans, P., and Brandenburg, W., and Zeeman, G. (2005). "Bio-offshore, Scale cultivation of seaweeds in combination with offshore wind farms in the North Sea," ECN-C--05-008 ECN project number 8.20300,137 pp.
- Renewable fuels association. 2010. (<http://www.ethanolrfa.org>).

- Rodolfi, L., Zittelli, G. C., Bassi, N., Padovani, G., Biondi, N., and Bonini, G. (2008). "Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low cost photobioreactor," *Biotechnol. Bioeng.* 102(1), 100-112.
- Ross, A., Jones, J. M., Kubacki, M. L., and Bridgeman, T. G. (2008). "Classification of macroalgae as fuel and its thermochemical behavior," *Bioresour. Technol.* 99, 6494-6504.
- Rupprecht, J., Hankamer, B., Mussgnug, J. H., Ananyev, G., Dismukes, G. C., and Kruse, O. (2006). "Perspectives and advances of biological H₂ production in microorganisms," *Applied Microbiol. and Biotechnol.* 72, 442-449.
- Savage, N. (2011). "Algae: The scum solution," *Nature* 474 (7352), S15-S16.
- Sawayama, S., Inoue, S., Dote, Y., and Yokoyama, S. Y. (1995). "CO₂ fixation and oil production through microalga," *Energy Convers. Manage.* 36, 729-731.
- Schenk, P. M., Thomas-Hall, S. R., Stephens, E., Marx, U. C., Mussgnug, J. H., Posten, C., Kruse, O., and Hankamer, B. (2008). "Second generation biofuels: High-efficiency microalgae for biodiesel production," *Bioener. Res.* 1, 20-43.
- Seambiotic company Ltd., 2009. (<http://www.seambiotic.com>).
- SeaWeb aquaculture news. (2008). "Mussels and kelps help salmon farmers reduce pollution," (<http://www.unbsj.ca/sase/biology/chopinlab>).
- Seo, Y. B., Lee, Y. W., Lee, C. H., and You, H. C. (2010). "Red algae and their use in paper making," *Bioresour. Technol.* 101, 2549-2553.
- Sheehan, J., Dunabay, T., Benemann, J., and Roessler, P. (1998). "A look back at the US Department of Energy Aquatic species program: Biodiesel from algae," *Nat Renew Energy Lab*, 326.
- Shelef, G., Azov, Y., Moraine, R., and Oron, G. (1980). "Algal reclamation as an integral part of wastewater treatment and reclamation system," In: *Algae Biomass*, G. Shelef, and C. J. Soeder (eds.), Elsevier/North Holland, Biomedical Press, The Netherlands, 163-190.
- Shi, X., Jung, K. W., Kim, D. H., Ahn, Y. T., and Shin, H. S. (2011). "Direct fermentation of *Laminaria japonica* for biohydrogen production by anaerobic mixed cultures," *Int. J. Hydrogen Energ.* 36, 5857-5864.
- Sialve, B., Bernet, N., and Bernard, O. (2009). "Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable," *Biotechnol. Adv.* 27, 409-416.
- Sims, R. E. H., Mabee, W., Saddler, J. N., and Taylor, M. (2010). "An overview of second generation biofuel technologies," *Bioresour. Technol.* 101, 1570-1580.
- Soeder, C. J. (1980). "Massive cultivation of microalgae: results and prospects," *Hydrobiologia.* 72, 197-209.
- Spolaore, P., Joannis-Cassan, C., Duran, E., and Isambert, A. (2006). "Commercial applications of microalgae," *J. Biosci. Bioeng.* 101, 87-96.
- Subhadra, B., and Edwards, M. (2010). "An integrated renewable energy park approach for algal biofuel production in United States," *Energy Policy* (DOI:10.1016/j.enpol.2010.04.036).
- Tanticharoen, M., Bhumiratana, S., Jeyashoke, N., Bunnag, B., Reungjitchawaly, M., Chithumaub, P., Wantawin, C., and Lertriluck, S. (1990). "Cultivation of *Spirulina* using tapioca starch wastewater," Regional Seminar on Management and Utilization of Agricultural and Industrial Wastes, Kuala Lumpur, Malaysia.

- Thomas, F. R. (2006). "Algae for liquid fuel production Oakhaven Permaculture Center," Retrieved on 2006-12-18," *Permaculture Activist* 59, 1-2.
- Tredici, M. R. (2010). "Photobiology of microalgae mass cultures: Understanding the tools for the next green revolution," *Biofuels* 1, 143-162.
- Trono Jr, G. C., and Ganzon-Fortes, E. (1988). *Philippine Seaweeds*, National Bookstore Inc., The Philippines.
- Uchida, M., and Murata, M. (2004). "Isolation of a lactic acid bacterium and yeast consortium from a fermented material of *Ulva* spp. (Chlorophyta)," *J. Appl. Microbiol.* 97, 1297-1310.
- Ueda, R., Hirayama, S., Sugata, K., and Nakayama, H. (1996). "Process for the production of ethanol from microalgae," US patent 5578, 472.
- Ugwu, C. U., Aoyagi, H., and Uchiyama, H. (2008). "Photobioreactors for mass cultivation of algae," *Bioresour. Technol.* 99, 4021-4028.
- Venkataraman, L. V., Nigam, B. P., and Ramanathan, P. K. (1980). "Rural oriented freshwater cultivation and production of algae in India," In: *Algae Biomass*, G. Shelef and C. J. Soeder (eds.), Elsevier/North Holland, Biomedical Press, The Netherlands, 81-96.
- Vonshak, A. (1990). "Recent advances in microalgal biotechnology," *Biotechnol. Adv.* 8, 709-727.
- Wecker, M. S. A., Meuser, J. E., Posewitz, M. C., and Ghirardi, M. L. (2011). "Design of a new biosensor for algal H₂ production based on the H₂-sensing system of *Rhodobacter capsulatus*," *Int. J. Hydrogen Energy.* 36, 11229-11237.
- Wi, S. G, Kim, H. J, Mahadevan, S. A, Yang, D., and Bae, H. (2009). "The potential value of the seaweed Ceylon moss (*Gelidium amansii*) as an alternative bioenergy resource," *Bioresour. Technol.* 100, 6658-6660.
- Wijffels, R. (2009). "Microalgae for production of bulk chemicals and biofuels," The 3rd Congress of Tsukuba 3E Forum, Tsukuba International Conference Centre, Tsukuba, Japan.
- Williams, P. J. L. B., and Laurens, L. M. L. (2010). "Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics," *Energy Environ. Sci.* 3, 554-590.
- Xiong, W., Li, X., Xiang, J., and Wu, Q. (2008). "High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbiodiesel production", *Appl. Microbiol. Biotechnol.* 78, 29-36.
- Xu, H., Miao, X., and Wu, Q. (2006). "High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters," *J. Biotechnol.* 126, 499-507.
- Yacoby, I., Pochekailov, S., Topotrik, H., Ghirardi, M. L., King, P. W., and Zhang, S. (2011). "Photosynthetic electron partitioning between (FeFe)-hydrogenase and ferredoxin: NADP⁺-oxidoreductase (FNR) enzymes in vitro," *Proc. Natl. Acad. Sci.* 108, 9396-9401.
- Yamazaki, H. (2007). "Japan experiments with new biofuels," (<http://checkbiotech.org>).
- Yuan, X., Shi, X., Zhang, D., Qiu, Y., Guo, R., and Wang, L. (2011). "Biogas production and microcystin biodegradation in anaerobic digestion of blue green algae," *Energy Environ. Sci.* 4, 1511-1515.

Zainal, A., Yaakob, Z., Takriff, M. S., Rajkumar, R., and Ghani J. A. (2012).

“Phycoremediation in anaerobically digested palm oil mill effluent using cyanobacterium, *Spirulina platensis*,” *Int. J. Biobased Mat. Bioener.* 6, 1-6.

Zhou, Y. H., and Ragan, M. A. (1995). “Cloning and characterization of the nuclear gene and cDNAs for triosephosphate isomerase of the marine red alga *Gracilaria verrucosa*,” *Curr. Genet.* 28, 317-323.

Article submitted: April 1, 2013; Peer review completed: May 27, 2013; Revised version received: November 11, 2013; Accepted: November 12, 2013; Published: December 2, 2013.