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Algal Biomass and Biodiesel Production

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1. Introduction

Biodiesel has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources. The cost of biodiesel, however, is the main hurdle to commercialization of the product. The used cooking oil and algae are used as raw material, adaption of continuous transesterification process and recovery of high quality glycerol from biodiesel by-product (glycerol) are primary options to be considered to lower the cost of biodiesel. There are four primary ways to make biodiesel, direct use and blending, microemulsions, thermal cracking (pyrolysis) and transesterification. The most commonly used method is transesterification of vegetable oils and animal fats. The transesterification reaction is affected by molar ratio of glycerides to alcohol, catalysts, reaction temperature, reaction time and free fatty acids and water content of oils or fats. In the present chapter we will focus on how algae have high potentials in biodiesel production compared with other sources.

2. Algae as biological material

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. Examples of prokaryotic microorganisms are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae are for example green algae (Chlorophyta) and diatoms (Bacillariophyta) [Richmond, 2004]. A more in depth description of microalgae is presented by Richmond [Richmond, 2004]. Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed [Richmond, 2004]. Algae are aquatic plants that lack the leaves, stem, roots, vascular systems, and sexual organs of the higher plants. They range in size from microscopic phytoplankton to gain kelp 200 feet long. They live in temperatures ranging from hot spring to arctic snows, and they come in various colors mostly green, brown and red. There are about 25,000 species of algae compared to 250,000 species of land plants. Algae make up in quantity what they lack in diversity for the biomass of algae is immensely greater than that of terrestrial plants (Lowenstein, 1986). Phytoplankton comprises organisms such as diatome, dinoflagellates and macrophytes include: green, red and brown algae. As photosynthetic organisms, these groups play a key role in productivity of ocean and constitute the basis of marine food chain. On the other hand, the use of macroalgae as a potential source of high value chemicals and in therapeutic purpose has a long history.

Recently, macroalgae have been used as a noval food with potential nutritional benefits and in industry and medicine for various purposes.

Furthermore, macroalgae have shown to provide a rich source of natural bioactive compounds with antiviral, antifungal, antibacterial, antioxidant, anti-inflammatory, hypercholesterolemia, and hypolipidemic and antineoplasteic properties. Thus, there is a growing interest in the area of research on the positive effect of macroalgae on human health and other benefits. In Egypt, the macroalgae self grown on the craggy surface near to the seashore of the Mediterranean and Red Seas. Macroalgae have not used as healthy food, while in Japan and China the macroalgae are tradionally used in folk medicine and as a healthy food in addition to, biofuel production (Lee-Saung *et al.*, 2003). The present study was conducted to evaluate the potentialities of micro and macroalgae species for biodiesel production and study the effect of biotic and a biotic stress on biodiesel percentage and the difference between biodiesel production from vegetable sources and algae.

Algae were promising organisms for providing both novel biologically active substances and essential compounds for human nutrition (Mayer and Hamann, 2004**)**. Therefore, an increasing supply for algal extracts, fractions or pure compounds for the economical sector was needed (Dos Santos *et al*., 2005). In this regard, both secondary and primary metabolisms were studied as a prelude to future rational economic exploitation as show in Fig**.** 1.

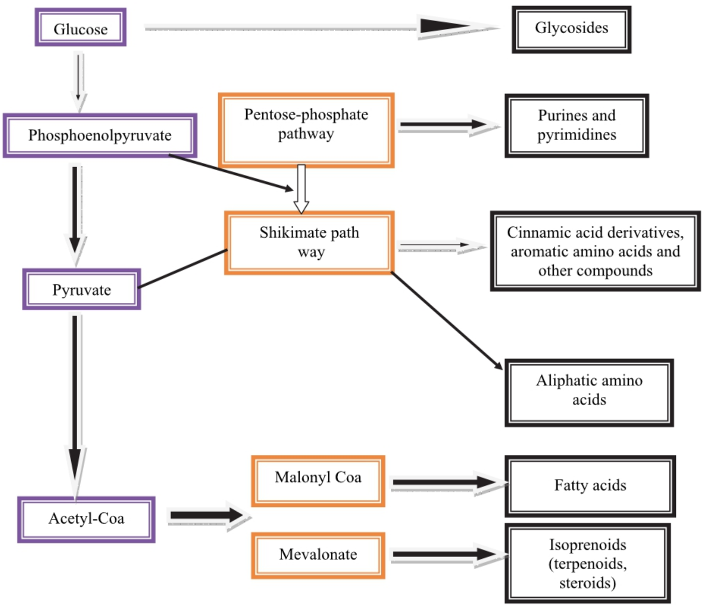

Fig. 1. Secondary and primary metabolites produced from algal cell

3. Diesel production problems

The transportation and energy sectors are the major anthropogenic sources, responsible in European Union (EU) for more than 20% and 60% of greenhouse gas (GHG) emissions, respectively [European Environmental Agency, 2004]. Agriculture is the third largest anthropogenic source, representing about 9% of GHG emissions, where the most important gases are nitrous oxide (N_2O) and methane (CH_4) [European Environmental Agency, 2007]. It is expected that with the development of new growing economies, such as India and China, the global consumption of energy will raise and lead to more environmental damage [International Energy Agency, 2007].

GHG contributes not only to global warming (GW) but also to other impacts on the environment and human life. Oceans absorb approximately one-third of the CO_2 emitted each year by human activities and as its levels increase in the atmosphere, the amount dissolved in oceans will also increase turning the water pH gradually to more acidic. This pH decrease may cause the quick loss of coral reefs and of marine ecosystem biodiversity with huge implications in ocean life and consequently in earth life [Ormerod *et al.*, 2002].

As GW is a problem affecting different aspects of human life and the global environment, not only a single but a host of solutions is needed to address it. One side of the problem concerns the reduction of crude oil reserves and difficulties in their extraction and processing, leading to an increase of its cost [Laherrere, 2005]. This situation is particularly acute in the transportation sector, where currently there are no relevant alternatives to fossil fuels. To find clean and renewable energy sources ranks as one of the most challenging problems facing mankind in the medium to long term. The associated issues are intimately connected with economic development and prosperity, quality of life, global stability, and require from all stakeholders tough decisions and long term strategies. For example, many countries and regions around the world established targets for CO_2 reduction in order to meet the sustainability goals agreed under the Kyoto Protocol. Presently many options are being studied and implemented in practice, with different degrees of success, and in different phases of study and implementation. Examples include solar energy, either thermal or photovoltaic, hydroelectric, geothermal, wind, biofuels, and carbon sequestration, among others [Dewulf *et al.*, 2006]. Each one has its own advantages and problems and, depending on the area of application.

4. Biodiesel instead of diesel

One important goal is to take measures for transportation emissions reduction, such as the gradual replacement of fossil fuels by renewable energy sources, where biofuels are seen as real contributors to reach those goals, particularly in the short term. Biofuels production is expected to offer new opportunities to diversify income and fuel supply sources, to promote employment in rural areas, to develop long term replacement of fossil fuels, and to reduce GHG emissions, boosting the decarbonisation of transportation fuels and increasing the security of energy supply. The most common biofuels are biodiesel and bio-ethanol, which can replace diesel and gasoline, respectively, in today cars with little or none modifications of vehicle engines. They are mainly produced from biomass or renewable energy sources and contribute to lower combustion emissions than fossil fuels per equivalent power output. They can be produced using existing technologies and be distributed through the available distribution system. For this reason biofuels are currently pursued as a fuel alternative that can be easily applied until other options harder to implement, such as hydrogen, are available.

Although biofuels are still more expensive than fossil fuels their production is increasing in countries around the world. Encouraged by policy measures and biofuels targets for transport, its global production is estimated to be over 35 billion liters [COM, 2006]. The main alternative to diesel fuel in EU is biodiesel, representing 82% of total biofuels production and is still growing in Europe, Brazil, and United States, based on political and economic objectives. Biodiesel is produced from vegetable oils (edible or non-edible) or animal fats. Since vegetable oils may also be used for human consumption, it can lead to an increase in price of food-grade oils, causing the cost of biodiesel to increase and preventing its usage, even if it has advantages comparing with diesel fuel.

The potential market for biodiesel far surpasses the availability of plant oils not designated for other markets. For example, to fulfill a 10% target in EU from domestic production, the actual feedstocks supply is not enough to meet the current demand and the land requirements for biofuels production, would be more than the potential available arable land for bio-energy crops [Scarlat *et al*., 2008]. The extensive plantation and pressure for land use change and increase of cultivated fields may lead to land competition and biodiversity loss, due to the cutting of existing forests and the utilization of ecological importance areas [Renewable Fuel Agency, 200]. Biodiesel may also be disadvantageous when replacing crops used for human consumption or if its feedstocks are cultivated in forests and other critical habitats with associated biological diversity. The negative impacts of global warming, now accepted as a serious problem by many people, have clearly been observed for past decade and seem to intensify every year. The release of the carbon oxides and related inorganic oxides are more than the amount that could be absorbed by the natural sinks in the world since 88% of the world energy demand is provided by carbon based non-renewable fuels (Baruch, 2008). It is vital to develop solutions to prevent and/or reduce the emission of greenhouse gases, such as carbon dioxide, to the atmosphere. Carbon dioxide neutral fuels like biodiesel could replace fossil fuels.

Biodiesel, an alternative diesel fuel, is made from renewable biological sources such as vegetable oils and animal fats. It is biodegradable and nontoxic, has low emission profiles and so is environmentally beneficial (Krawczyk, 1996). One hundred years ago, Rudolf Diesel tested vegetable oil as fuel for his engine (Shay, 1993). With the advent of cheap petroleum, appropriate crude oil fractions were refined to serve as fuel and diesel fuels and diesel engines evolved together. In the 1930s and 1940s vegetable oils were used as diesel fuels from time to time, but usually only in emergency situations. Recently, because of increases in crude oil prices, limited resources of fossil oil and environmental concerns there has been a renewed focus on vegetable oils and animal fats to make biodiesel fuels. Continued and increasing use of petroleum will intensify local air pollution and magnify the global warming problems caused by CO2 (Shay, 1993). In a particular case, such as the emission of pollutants in the closed environments of underground mines, biodiesel fuel has the potential to reduce the level of pollutants and the level of potential or probable carcinogens (Krawczyk, 1996). Edible vegetable oils such as canola, soybean, and corn have been used for biodiesel production and found to be a diesel substitute [Lang *et al*., 2002]. However, a major obstacle in the commercialization of biodiesel production from edible vegetable oil is its high production cost, which is due to the higher cost of edible oil. Waste cooking oil, which is much less expensive than edible vegetable oil, is a promising alternative to edible vegetable oil [Canakci *et al*., 2003]. Waste cooking oil and fats set forth significant disposal problems in many parts of the world. This environmental problem could be solved by proper utilization and management of waste cooking oil as a fuel. Many developed countries have set policies that penalize the disposal of waste cooking oil the waste drainage [Kulkarni *et al*., 2006]. The Energy Information Administration in the United States estimated that around 100 million gallons of waste cooking oil is produced per day in

USA, where the average per capita waste cooking oil was reported to be 9 pounds [Radich *et al.*, 2006]. The estimated amount of waste cooking oil collected in Europe is about 700,000–100,000 tons/year [Supple *et al.*, 2002]

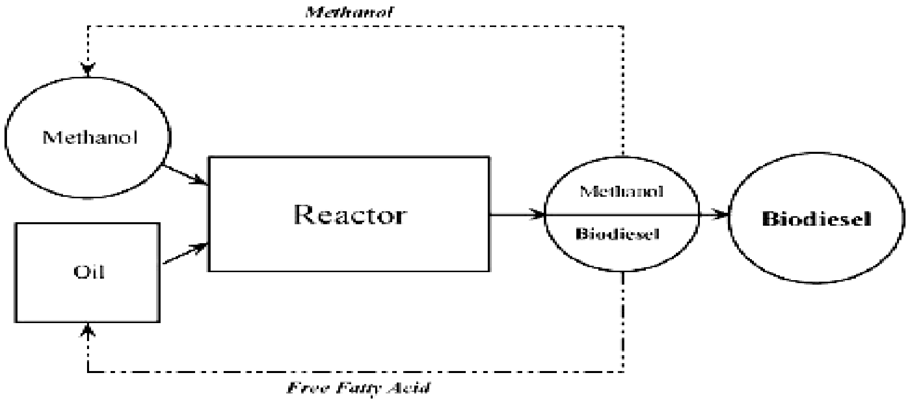

Fig. 2. Biodiesel production process

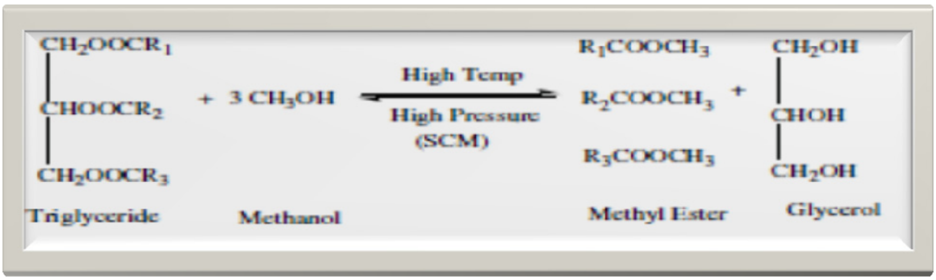

Fig. 3. Transesterification of triglycerides

Biodiesel is made from biomass oils, mostly from vegetable oils. Biodiesel appears to be an attractive energy resource for several reasons. First, biodiesel is a renewable resource of energy that could be sustainably supplied. It is understood that the petroleum reserves are to be depleted in less than 50 years at the present rate of consumption [Sheehan *et al.*, 1998]. Second, biodiesel appears to have several favorable environmental properties resulting in no net increased release of carbon dioxide and very low sulfur content [Antolin *et al.*, 2002]. The release of sulfur content and carbon monoxide would be cut down by 30% and 10%, respectively, by using biodiesel as energy source. Using biodiesel as energy source, the gas generated during combustion could be reduced, and the decrease in carbon monoxide is owing to the relatively high oxygen content in biodiesel. Moreover, biodiesel contains no aromatic compounds and other chemical substances which are harmful to the environment. Recent investigation has indicated that the use of biodiesel can decrease 90% of air toxicity and 95% of cancers compared to common diesel source. Third, biodiesel appears to have significant economic potential because as a non-renewable fuel that fossil fuel prices will increase inescapability further in the future. Finally, biodiesel is better than diesel fuel in terms of flash point and biodegradability [Ma *et al*., 1999].

5. Algae as potentials for biodiesel production

5.1 Separation of biodiesel from algae

5.1.1 Extraction of oil

Extraction of oil was carried out using two extraction solvent systems to compare the oil content in each case and select the most suitable solvent system for the highest biodiesel yield (Afify *et al*., 2010).

5.1.1.1 Chloroform /methanol (2:1, v/v) method

A known weight of each ground dried algal species (10 g dry weight) was mixed separately with the extraction solvent mixture; chloroform/methanol (100 ml, 2:1, v/v) for 20 min. using shaker, followed by the addition of mixture of chloroform/water (50 ml, 1:1, v/v) for 10 min. filter and the algal residue was extracted three times by 100 ml chloroform followed by filtration (Fig.1) according to Bligh and Dayer (1959).

5.1.1.2 Hexane/ether (1:1, v/v) method

A known weight of each ground dried algal species (10 g dry weight) was mixed with the extraction solvent mixture, hexane/ether (100 ml, 1:1, v/v), kept to settle for 24 hrs, followed by filtration (Fig. 1) according to Hossain and Salleh (2008).

5.1.2 Transesterification and biodiesel production

The extracted oil was evaporated under vaccum to release the solvent mixture solutions using rotary evaporator at 40- 45 °C. Then, the oil produced from each algal species was mixed with a mixture of catalyst (0.25g NaOH) and 24 ml methanol, a process called transesterification (Fig. 2, 3,4, 5 and Table 2), with stirring properly for 20 min. The Mixture was kept for 3hrs in electric shaker at 3000 rpm. (National Biodiesel Board, 2002). After shaking the solution was kept for 16 hrs to settle the biodiesel and the sediment layers clearly. The biodiesel layer was separated from sedimentation by flask separator carefully. Quantity of sediments (glycerin, pigments, etc) was measured. Biodiesel (Fig. 6) was washed by 5% water many times until it becomes clear then Biodiesel was dried by using dryer and finally kept under the running fan for 12 h. the produced biodiesel was measured (using measuring cylinder), pH was recorded and stored for analysis.

6. Biodiesel from algae

Sustainable production of renewable energy is being hotly debated globally since it is increasingly understood that first generation biofuels, primarily produced from food crops and mostly oil seeds are limited in their ability to achieve targets for biofuel production, climate changemitigation and economic growth. These concerns have increased the interest in developing second generation biofuels produced from non-food feedstocks such as microalgae, which potentially offer greatest opportunities in the longer term. This paper reviews the current status of microalgae use for biodiesel production, including their cultivation, harvesting, and processing. The microalgae species most used for biodiesel production are presented and their main advantages described in comparison with other available biodiesel feedstocks. The various aspects associated with the design of microalgae production units are described, giving an overview of the current state of development of algae cultivation systems (photo-bioreactors and open ponds). Other potential applications and products from microalgae are also presented such as for biological sequestration of CO_2, wastewater treatment, in human health, as food additive, and for aquaculture (Mata *et al.*, 2010).

Biodiesel seem to be a viable choice but its most significant drawback is the cost of crop oils, such as canola oil, that accounts for 80% of total operating cost, used to produce biodiesel (Demirbas, 2007). Besides, the availability of the oil crop for the biodiesel production is limited (Chisti, 2008). Therefore, it is necessary to find new feedstock suitable for biodiesel production, which does not drain on the edible vegetable oil supply. One alternative to oil crops is the algae because they contain lipids suitable for esterification/ transesterification. Among many types of algae, microalgae seem to be promising (Table 1) because:

1. They have high growth rates; e.g., doubling in 24 h (Rittmann, 2008).
2. Their lipid content could be adjusted through changing growth medium composition (Naik *et al*., 2006).
3. They could be harvested more than once in a year (Schenk *et al*., 2008).
4. Salty or waste water could be used (Schenk *et al*., 2008).
5. Atmospheric carbon dioxide is the carbon source for growth of microalgae (Schenk *et al*., 2008).
6. Biodiesel from algal lipid is non-toxic and highly biodegradable (Schenk *et al*., 2008).
7. Microalgae produce 15–300 times more oil for biodiesel production than traditional crops on an area basis (Chisti, 2007).

Strain	Protein	Carbohydrates	Lipid	Nucleic acid
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	–	1.9	–
Scenedesmus dimorphus	8–18	21–52	16–40	–
Chlamydomonas rheinhardii	48	17	21	–
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	–
Spirogyra sp.	6–20	33–64	11–21	–
Dunaliella bioculata	49	4	8	–
Dunaliella salina	57	32	6	–
Euglena gracilis	39–61	14–18	14–20	–
Prymnesium parvum	28–45	25–33	22–39	1–2
Tetraselmis maculata	52	15	3	–
Porphyridium cruentum	28–39	40–57	9–14	–
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina maxima	60–71	13–16	6–7	3–4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrica	43–56	25–30	4–7	–

Note: Algal-oil is very high in unsaturated fatty acids. Some UFA's found in different algal-species include: Arachidonic acid (AA), Eicospentaenoic acid (EPA), Docasahexaenoic acid (DHA), Gamma-linolenic acid (GLA) Linoleic acid (LA).

Table 1. Biochemical composition of algae expressed on a dry matter basis (Becker, 1994)

Algae are made up of eukaryotic cells. These are cellswith nuclei and organelles. All algae have plastids, the bodies with chlorophyll that carry out photosynthesis. But the various strains of algae have different combinations of chlorophyll molecules. Some have only Chlorophyll A, some A and B, while other strains, A and C [Benemann *et al*., 1978]. Algae biomass contains three main components: proteins, carbohydrates, and natural oil. The chemical compositions of various microalgae are shown in Table 1. While the percentages vary with the type of algae, there are algae types that are comprised of up to 40% of their overall mass by fatty acids [Becker, 1994]. It is this fatty acid (oil) that can be extracted and converted into biodiesel.

Type of transesterification	Advantage	Disadvantage
Chemical catalysis	1-reaction condition can be well controlled	1-reaction temperature is relative high and the process is complex
	2-Large scale production	2-The later disposal process is complex
	3-The cost of the production process is cheap	3-The process need much energy
	4-The methanol produced in the process can be recycled	4-Need a installation for methanol recycle
	5-high conversion of the production	5-the waste water pollute the environment
Enzymatic catalyst	1-Moderate reaction condition	1-Limitation of enzyme in the conversion of short chain fatty acids
	2-The small amount of methanol required in the reaction	2-Chemicals arise in the process of production are poisons to enzyme
	3-Have no pollution to natural environment	
Supercritical fluid techniques	1-Easy to be controlled	1-High temperature and high pressure in the reaction condition leads to high coast for production and waste energy
	2-It is safe and fast	
	3-friendly to environment	

Table. 2. Types of transesterification catalysts

Fig. 4. Biodiesel from algae

Fig. 5 shows a schematic representation of the algal biodiesel value chain stages, starting with the selection of microalgae species depending on local specific conditions and the design and implementation of cultivation system for microalgae growth. Then, it follows the biomass harvesting, processing and oil extraction to supply the biodiesel production unit.

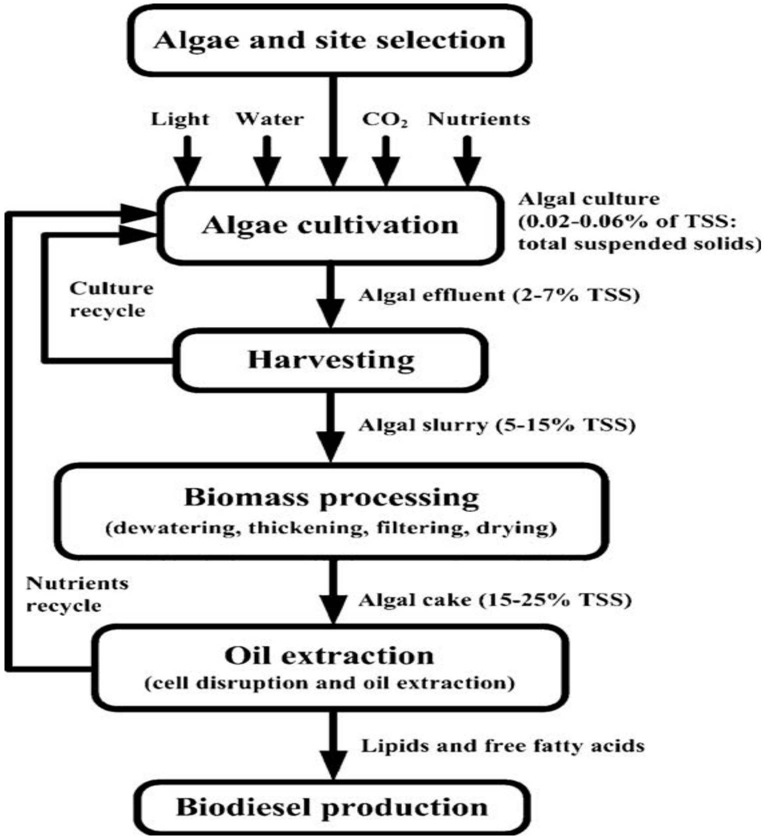

Fig. 5. Microalgae biodiesel value chain stages.

Algae's potential as a feedstock is dramatically growing in the biofuel market. Microalgae (to distinguish it from such macroalgae species as seaweed) have many desirable attributes as energy producers [Choe *et al.*, 2002]:

- Algae is the most promising non-food source of biofuels,
- Algae has a simple cellular structure,
- a lipid-rich composition (40–80% in dry weight),
- a rapid reproduction rate,
- Algae can grow in salt water and harsh conditions,
- Algae thrive on carbon dioxide from gas- and coal-fired power Plants,
- Algae biofuel contains no sulfur, is non-toxic and highly biodegradable.

- The utilization of microalgae for biofuels production can also serve other purposes. Some possibilities currently being considered are listed below.
- Removal of CO_2 from industrial flue gases by algae bio-fixation [Wang *et al*., 2008], reducing the GHG emissions of a company or process while producing biodiesel. Wastewater treatment by removal of NH^{+}_4, NO^{-}_3, PO^{-3}_4, making algae to grow using these water contaminants as nutrients [Wang *et al*., 2008].
- After oil extraction the resulting algae biomass can be processed into ethanol, methane, livestock feed, used as organic fertilizer due to its high N:P ratio, or simply burned for energy cogeneration (electricity and heat) [Wang *et al*., 2008];
- Combined with their ability to grow under harsher conditions, and their reduced needs for nutrients, they can be grown in areas unsuitable for agricultural purposes independently of the seasonal weather changes, thus not competing for arable land use, and can use wastewaters as the culture medium, not requiring the use of freshwater.
- Depending on the microalgae species other compounds may also be extracted, with valuable applications in different industrial sectors, including a large range of fine chemicals and bulk products, such as fats, polyunsaturated fatty acids, oil, natural dyes, sugars, pigments, antioxidants, high-value bioactive compounds, and other fine chemicals and biomass [Raja *et al*., 2008].
- Because of this variety of high-value biological derivatives, with many possible commercial applications, microalgae can potentially revolutionize a large number of biotechnology areas including biofuels, cosmetics, pharmaceuticals, nutrition and food additives, aquaculture, and pollution prevention [Raja *et al*., 2008].

7. Environmental advantages of algal biofuels

In order to be a viable alternative energy source, a biofuel should provide a net energy gain, have environmental benefits, be economically competitive and be producible in large quantities without reducing food supplies [Hill, 2006]. In the subsections below we illustrate how the use of microalgae as feedstocks for biodiesel production can provide significant environmental benefits by reducing the land, pollutant and water footprints of biofuel production.

7.1 Advantages of biodiesel from algae oil (Table 3)

- Rapid growth rates
- Grows practically anywhere
- A high per-acre yield (7–31 times greater than the next best crop – palm oil)-
- No need to use crops such as palms to produce oil
- A certain species of algae can be harvested daily
- Algae biofuel contains no sulfur
- Algae biofuel is non-toxic
- Algae biofuel is highly bio-degradable
- Algae oil extracts can be used as livestock feed and even processed into ethanol
- High levels of polyunsaturates in algae biodiesel is suitable for cold weather climates
- Can reduce carbon emissions based on where it's grown

7.2 Disadvantages of biodiesel from algae oil

- Produces unstable biodiesel with many polyunsaturates
- Biodiesel performs poorly compared to it's mainstream alternative
- Relatively new technology

Type of organism	Advantage	Disadvantage
Microalgal oil	1-Fatty acid profile similar to vegetable oil	1-Most algal lipid have lower fuel value than diesel fuel
	2-Under certain condition it may be as high as 85% of the dry weight	2-The cost of cultivation is higher compared to common crop oil currently
	3-Short-time growth cycle	
	4-Composition is relative single in microalgae	
Bacteria oil	1-Fast growth rate	1-Most of bacteria can not yield lipids but complicated lipoid
Oleaginous yeast and mildews	1-Resource are abundant in the nature	1-Filteration and cultivation of yeasts and mildews with high-content are required
	2-High oil content in some species	2-Process of oils extracted is complex and new technology
	3-Short time growth cycle	3-The cost of cultivation is also higher compared to common crops currently
	4-Strong capability of growth in different cultivation on conditions	
Waste oils	1-The waste oil is cheap compared to crop oils	1-Conataing a lot of saturated fatty acids which is hard to converted to biodiesel by catalyst

Table 3. Advantage and disadvantage of algae as biodiesel source compared with bacteria, yeast and waste oils

8. Comparison between biodiesel production from algae and vegetables

Quantifying the land use changes associated with intensive biofuel feedstock production relies upon many assumptions [Chisti,. 2007], but it is clear that the accelerated cultivation of terrestrial plant biomass for biofuels will have an exceptionally large land footprint (Table 4). For example, the United States has the fourth largest absolute biodiesel potential of the 119 countries studied by Johnston and Holloway [Johnston, M. and Holloway, 2007]. However, recent work has suggested that the projected year 2016 demand for corn ethanol alone would require 43% of all U.S. land used for corn production in 2004 [Chisti,. 2007]. A related study concluded that the annual corn production needed to satisfy one half of all U.S. transportation fuel needs would require an area equivalent to more than eight times the U.S. land area that is presently used for crop production [Chisti,. 2007]. Other land-based crops would require less cropland, based on their oil content: oil palm (24% of current cropland area), coconut (54%), jatropha (77%), canola (122%) and soybean (326%) [Chisti,. 2007]. Moreover, recent work indicates that the ability of countries to grow terrestrial crops explicitly for the production of biofuels such as ethanol and biodiesel is significantly overestimated [Johnston, M. and Holloway, 2007], contributing to concerns that these biofuels are not feasible options for providing a significant fraction of global fuel demand.

Biodiesel feedstock	Area needed to meet global oil demand (10^6 hectans)	Area required as a percent of total global land	Area required as a percent of total arable global land
Cotton	15000	101	757
Soybean	10900	73	552
Mastard seed	8500	57	430
Sunflower	5100	34	258
Rapeseed/Canola	4100	27	207
Jatropha	2600	17	130[b]
Oil palm	820	5.5	41
Microalgae (10 g/m³/day, 30%TAG)	410	2.7	21[c]
Microalgae (50 g/m³/day, 50%TAG)	49	0.3	25[c]

[b] Jatropha is mainly grown on marginal land
[c]Assuring that microalgal ponds and bioreactors are located on non-arable land

Table 4. Comparison of estimated biodiesel production efficiencies from vascular plants and microalgae

9. The physical and chemical properties of biodiesel produced from algal cell

Analysis of the produced biodiesel from the promising alga *Dictyochloropsis splendida* (Table 5). showed that the unsaturated fatty acids percentage was increased in alga cultivated in nitrogen free media (0.0g/l N) two times more than normal conditions (13.67, 4.81% respectively). However, the composition of fatty acids was different in these algae depending on its growth condition as showed in table 3. These results were in agreements with those reported by Wood (1974) relative to *Chlorophycean* species. Furthermore Ramos *et al*. (2009) reported that monounsaturated, polyunsaturated and saturated methyl esters were built in order to predict the critical parameters of European standard for any biodiesel, composition. The extent of unsaturation of microalgae oil and its content of fatty acids with more than four double bonds can be reduced easily by partial catalytic hydrogenation of the oil (Jang *et al*., 2005, Dijkstra, 2006). Concerning the fatty acids contents of the produced biodiesel from microalgae, Chisti (2007) reported in his review that, microalgal oils differ from vegetable oils in being quite rich in polyunsaturated fatty acids with four or more double bands (Belarbi *et al*., 2000) as eicosapentanoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) which occurred commonly in algal oils. The author added that, fatty acids and fatty acid methyl esters with four and more double bands are susceptible to oxidation during storage and this reduces their acceptability for use in biodiesel especially for vehicle use (European standard EN 14214 limits to 12%) while no such limitation exists for biodiesel intended for use as healing oil. In addition to the content of unsaturated fatty acids in the biodiesel also its iodine value (represented total unsaturation) must be taken in consideration (not exceeded 120 g iodine/100g biodiesel according to the European standard.

Fatty acids	*RT	Fatty acids percentage	
		Algae cultivated under normal conditions	Algae cultivated under free nitrogen media
C10:0 (Capric acid)	1.223	0.0	1.26
C14:0 (Myristic acid)	2.437	13.04	13.88
C16:0 (Palmitic acid)	2.860	81.14	69.59
C17:0 (Margeric acid)	3.240	1.01	1.21
C18:0 (stearic acid)	4.335	0.0	0.38
C18:1 (Oleic acid)	4.667	0.26	1.11
C18:2 (Linoleic acid)	5.333	4.39	12.14
C18:3 (linolenic acid)	6.948	0.15	0.42
Total saturated fatty acids		95.19	86.33
Total unsaturated fatty acids		4.81	13.67
TU/TS		0.05	0.16

*Retention time; TU/TS: total unsaturated/ total saturated fatty acids ratio.

Table 5. Analysis of fatty acids of the obtained biodiesel from the promising green microalgae *Dictyochloropsis* sp

10. Enhancement the biodiesel production from algae

Lipid productivity, the mass of lipid that can be produced per day, is dependent upon plant biomass production as well as the lipid content of this biomass. Algal biodiesel production will therefore be limited not only by the standing crop of microalgae, but also by its lipid content, which can vary from <1% to >50% dry weight [Shifrin, N.S. and Chisholm, 1980]. Given that a strong and predictable response of microalgal biomass to phosphorus enrichment has consistently been exhibited by freshwater ecosystems worldwide (Box 2), it can be expected that the volumetric lipid content (in mg L–1) of water contained in algal bioreactors should also in general increase with an increase in the total phosphorus content of the system, as has been reported for lakes by Berglund *et al.* [Berglund, 2001]. However, both the quantity and the quality of lipids produced will vary with the identity of the algal species that are present in the water, as well as with site-specific growth conditions. This variability probably reflects modifications in the properties of cellular membranes, and alterations in the relative rates of production and utilization of storage lipids [Roessler, 1990]. In the presence of moderate temperatures and sufficient light, many dozens of studies during the past several decades have revealed that algal lipid content is particularly sensitive to conditions of nutrient limitation . For example, silicon-starved diatoms can contain almost 90% more lipids than silicon-sufficient cells [Shifrin, N.S. and Chisholm, 1980]. However, silicon will be a growth-limiting nutrient only for the limited subset of microalgal species that have an absolute requirement of this element for their cellular growth. A stronger stimulation of lipid production occurs in response to conditions of nitrogen limitation, which potentially can occur in all known microalgae. Nitrogen-starved cells can contain as much as four times the lipid content of Nsufficient cells [Shifrin, N.S. and Chisholm, 1980], and maximizing the lipid production of pond bioreactors should therefore depend on their operators' ability to reliably and consistently induce N-limitation in the resident algal cells. Resource-ratio theory and the principles of ecological stoichiometry, provide additional new insights into the control of algal biomass and lipid production in pond bioreactors. the nutrient limitation status of microalgae can be directly controlled by regulating the ratio of nitrogen and phosphorus (N:P) supplied in the incoming nutrient feed: nitrogen limitation occurs at N:P supply ratios that lie below the optimal N:P ratio for microalgal growth, whereas phosphorus limitation occurs at ratios that exceed this ratio. A transition between N- and P-limitation of phytoplankton growth typically occurs in the range of N:P supply ratios between ca. 20:1 to ca. 50:1 by moles . Such shifts between N- and P-limitation have extremely important implications for algal biofuel production because diverse species of microalgae grown under nitrogen-limited conditions (i.e. low N:P supply ratios) can exhibit as much as three times the lipid content of cells grown under conditions of phosphorus limitation (high N:P supply ratios) . Both the total phosphorus concentration as well as the total nitrogen concentration in the nutrient feeds to pond bioreactors should therefore impact algal biodiesel production, because the N:P ratio of incoming nutrients will strongly influence algal biomass production as well as the cellular lipid content. Given the inverse relationship observed between N:P and cellular lipids , and the positive, hyperbolic relationship observed between N:P and microalgal biomass , we conclude that optimal lipid yields (in terms of mass of lipid produced per unit bioreactor volume per day) should occur at intermediate values of the N:P supply ratio. From the strong apparent interactions between the effects of nitrogen and carbon dioxide availability on microalgal lipids, we also conclude that the effects of N:P supply ratios on volumetric lipid production might be even greater if the bioreactors are simultaneously provided with supplemental CO2 (cf. Figure 2).

Algal species	Chloroform/methanol (2:1, v/v)	Hexane/ether (1:1, v/v)
Jania rubens	4.4±0.12	2.8±0.04
Galaxaura oblongata	2.5±0.09	2.4±0.01
Gelidium latifolium	3.0±0.0	3.1±0.02
Asporagopsis taxiformis	4.1±0.08	3.4±0.05
Ulva lactuca	4.2±0.1	3.5±0.1
Colpomenia sinuosa	3.5±0.05	2.3±0.03
Dictyochloropsis splendida	12.5±0.23	2.4±0.14
Spirulina platensis	9.2±0.25	3.0±0.10
LSD	0.3261	0.3261

Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.05 according to Duncan's multiple range test.

Table 6. Comparison between lipid percentage (%) produced by eight algal species using two different extraction system.

Eight algal species (4 *Rhodo*, 1 *chloro* and 1 *phaeophycean* macroalgae, 1 *cyanobacterium* and 1 green microalga) were used for the production of biodiesel using two extraction solvent systems (Hexane/ether (1:1, v/v)) and (Chloroform/ methanol (2:1, v/v)) Table 6. Biochemical evaluations of algal species were carried out by estimating biomass, lipid, biodiesel and sediment (glycerin and pigments) percentages. Hexane/ ether (1:1, v/v) extraction solvent system resulted in low lipid recoveries (2.3-3.5% dry weight) while; chloroform/methanol (2: 1, v/v) extraction solvent system was proved to be more efficient for lipid and biodiesel extraction (2.5 – 12.5% dry weight) depending on algae species (Table 7). The green microalga *Dictyochloropsis splendida* extract produced the highest lipid and biodiesel yield (12.5 and 8.75% respectively) followed by the cyanobacterium *Spirulina maxima* (9.2 and 7.5 % respectively). On the other hand, the macroalga (red, brown and green) produced the lowest biodieselyield. The fatty acids of *Dictyochloropsis splendida* Geitler biodiesel were determined using gas liquid chromatography. Lipids, biodiesel and glycerol production of *Dictyochloropsis splendida* Geitler (the promising alga) were markedly enhanced by either increasing salt concentration or by nitrogen deficiency (Table 8) with maximum production of (26.8, 18.9 and 7.9 % respectively) at nitrogen starvation condition. (Afify *et al*., 2010)

Algal sp.	Lipid %	Biodiesel%	Sediment %	Biodiesel color
Jania rubens	4.4±0.12	0.25±0.01	4.2^{a} ± 0.05	Light brown
Galaxaura oblongata	2.5±0.09	2.06±0.02	0.08±0.0	Light green
Gelidium latifolium	3.0±0.0	1.3±0.0	1.6±0.01	yellow
Asporagopsis taxiformis	4.1±0.08	3.64^{c} ± 0.10	0.40±0.01	Dark green
Ulva lactuca	4.2±0.1	3.8±0.12	0.44±0.0	Light green
Colpomenia sinuosa	3.5±0.05	3.1±0.05	0.31±0.05	yellow
Dictyochloropsis splendida	12.5±0.23	8.75±0.24	3.75±0.08	colorless
Spirulina platensis	9.2±0.25	7.5±0.30	1.66±0.06	Light green
LSD	0.3261	0.3314	0.1786	

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range test.

Table 7. Total lipid, biodiesel, sediment percentage and biodiesel color of eight algal species

Natural biotic communities in outdoor bioreactors require the external provision of potentially growth-limiting resources (e.g. light, carbon dioxide and the essential mineral nutrients N and P). These resources act as ''bottom-up'' regulators of the potential microalgal biomass that can be produced. Once harvested, the cellular lipids in this microalgal biomass can be extracted and processed to create biodiesel fuels. The lipid content of microalgal biomass is not constant, however, and can be influenced by many factors, including nitrogen:phosphorus supply ratios, light, CO_2 and the hydraulic residence time of the bioreactor. Moreover, natural assemblages of microalgae are taxonomically diverse: some species are small and can easily be consumed by herbivorous zooplankton. Undesirable grazing losses of edible microalgae (and their cellular lipids) to large-bodied zooplankton can be reduced by adding zooplanktivorous fish, which can greatly restrict large-bodied zooplankton growth via sizeselective predation (''top-down'' regulation).

Sample culture conditions	Lipid content (%)	Biodiesel content (%)	Glycerol+ pigments content (%)	Biodiesel color
Control (2.5 g/l NaCl and 25g/l $NaNO_3$)	12.50±0.36	8.75±0.25	3.75±0.12	Colorless
NaCl stress				
5 g/l	14.50±1.2	8.90±0.62	5.60±0.18	Colorless
7.5 g/l	17.00±0.53	11.94±0.98	5.06±0.22	Light green
10 g/l	17.50±0.36	11.38±0.80	5.11±0.24	Light green
Nitrogen stress				
12.5 g/l	15.40±2.10	8.90±0.36	6.50±0.30	Yellowish green
6.25g/l	16.20±1.8	10.01±1.0	6.19±0.12	Light Yellow
0.0g/l	26.80± 2.12	18.90±1.2	7.9±0.50	Yellow
LSD	0.3643	0.1681	0.1431	

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range test.

Table 8. Total lipid, biodiesel, sediment percentage and biodiesel color of Dictyochloropsis sp cultivated under stress

11. Wastewater nitrogen and phosphorous as microalgae nutrients

There is a unique opportunity to both treat wastewater and provide nutrients to algae using nutrient-rich effluent streams. By cultivating microalgae, which consume polluting nutrients in municipal wastewater, and abstracting and processing this resource, then the goals of sustainable fuel production and wastewater treatment can be combined (Andersen, 2005). Treated wastewater is rich in nitrogen and phosphorus, which if left to flow into waterways, can spawn unwanted algae blooms and result in eutrophication (Sebnem Aslan, 2006). These nutrients can instead be utilized by algae, which provide the co-benefit of producing biofuels and removing nitrogen and phosphorus as well as organic carbon (Mostafa and Ali, 2009). Wastewater treatment using algae has many advantages. It offers the feasibility to recycle these nutrients into algae biomass as a fertilizer and thus can offset treatment cost. Oxygen rich effluent is released into water bodies after wastewater treatment using algae (Becker, 2004).

Cyanobacteria strains (Anabaena flos aquae, Anabaena oryzae, Nostoc humifusum, Nostoc muscorum, Oscillatoria sp., Spirulina platensis, Phormedium fragile and Wollea saccata) and the green alga strain Chlorella vulgaris were obtained from the Microbiology Department, Soils, Water and Environment Res. Inst. (SWERI), Agric. Res., Center (ARC). Cyanobacteria strains were maintained in BG11 medium (Rippka *et al.*, 1979) except Spirulina platensis which was cultivated in Zarrouk medium (Zarrouk, 1966). While, Bold medium (Nichols and Bold, 1965) was used for the green alga Chlorella vulgaris. Cultures were incubated in a growth chamber under continuous shaking (150 rpm) and illumination (2000 lux) at 25 ± 1 ˚C for 30 days. Shalaby *et al*. (2011). The effluent of the secondary treated sewage wastewater from Zenien Waste Water Treatment Plant (ZWWTP), Giza

Governorate, Egypt was used after filtered using glass microfiber filter to remove large particles and indigenous bacteria for the experiment and the chemical and physical parameters were analysis as reported by APHA (1998) Table (2). The supplementation of $NaNO_3$, K_2HPO_4 and $FeSO_4.7H_2O$ in amounts equal to those of the standard BG11, Bold and Zarrouk were used as basal media. The algal strains were grown in 500 ml Erlenmeyer flasks containing 200 ml of 100% effluent supplemented with basal nutrients and 100% effluent without basal nutrients with/without sterilization and the synthetic media (BG11, Bold and Zarrouk) were used as control. Two per cent algal inoculums were added to each flask. The experiment was conducted in triplicates and cultures were incubated at 25 °C ±1°C, under continuous shaking (150 rpm) and illumination (2000 lux) for 15 days. This work aimed to evaluate the laboratory cultivation of nine algal strains belonging to Nostocales and Chlorellales in secondary treated municipal domestic wastewater for biomass and biodiesel production as shown in Table (9 and 10).

Algal species	**Total lipids**	**Biodiesel content**	**Glycerin + pigments**	**Color**	**pH**
Nostoc muscorum	16.80±3.62	12.52±1.74	4.28±1.74	Brown	7.4±0.33
Anabaena flous aquae	5.50±0.58	4.00±0.41	1.50±0.41	Red	6.9±0.95
Chlorella vulgaris	12.50±1.20	8.8±0.16	3.70±0.16	Green	8.1±1.0
Oscillatoria sp	8.00±0.58	4.30±0.32	3.70±0.32	Yellow	7.5±0.85
Spirulina platensis	10.0±0.11	7.80±0.17	2.20±0.17	Light green	8.0±0.32
Anabaena oryzae	7.40±0.90	4.50±0.10	2.90±0.10	Orange	7.3±0.96
Wollea sp	6.30±1.31	3.90±0.60	2.40±0.60	Yellow	7.8±0.35
Nostoc humifusum	14.80±2.40	10.20±1.30	4.6±1.30	Yellowish brown	7.5±0.50
Phormedium sp	12.20±1.66	10.10±1.50	2.10±1.50	Dark brown	7.1±0.0
LSD	0.159	0.151	0.151		1.659

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range tests.
T1: waste water without treatment; T2: waste water after sterilization; T3: waste water+ nutrients with sterilization T4: waste water+ nutrients without sterilization

Table 9. Total lipids, biodiesel, glycerine+pigments percentage and color, pH of biodiesel from different microalgae species cultivated in different waste water

Algal species	Optimal waste water treatment	Total lipids	Biodiesel	Glycerin + pigments
Nostoc muscorum	T3	12.50±2.65	7.40±0.74	5.10±0.74
Anabaena flous aquae	T3	7.40±0.95	5.00±0.61	2.40±0.61
Chlorella vulgaris	T3	13.20±1.87	8.50±1.74	4.70±1.74
Oscillatoria sp	T2	6.80±0.65	3.80±0.32	3.00±0.32
Spirulina platensis	T3	7.30±0.44	5.00±0.51	2.30±0.51
Anabaena oryzae	T4	8.00±0.16	4.70±0.12	3.30±0.12
Wollea sp	T3	7.20±1.32	4.00±0.22	3.23±0.22
Nostoc humifusum	T1	15.50±1.65	11.80±1.52	3.70±1.52
Phormedium sp	T2	11.60±0.88	8.40±0.65	3.20±0.65
LSD		0.159	0.159	0.152

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range tests.
T1: waste water without treatment; T2: waste water after sterilization; T3: waste water+ nutrients with sterilization T4: waste water+ nutrients without sterilization

Table 10. Total lipids, biodiesel, glycerine+pigments percentage and color, pH of biodiesel from different microalgae species cultivated in different waste water

12. Economic importance

Compared to biofuels from agricultural crops, the amount of land required would be minimal. Trials in ideal conditions show that fast-growing micro-algae can yield 1800–2000 gallons/(acre - year) of oil—compare this with 50 gallons for soyabeans, 130 gallons for rapeseed and _650 gallons for palm oil. It can grow on fresh or brackish water on marginal land so that it does not compete with areas for agricultural cultivation. As Sean Milmo points out in his article in Oils and Fats International [Milmo, 2008]; oil from algae on 20–40 M acres of marginal land would replace the entire US supply of imported oil, leaving 450 M acres of fertile soil in the country entirely for food production. Biomass can also be harvested from marine algae blooms and algae can even be cultivated in sewage and water treatment plants. However, most estimates of algal fuel productivity estimate that with current production technologies algal diesel can be manufactured for, at best, $4.54 per gallon using high density photobioreactors. In order to compete economically with petroleum diesel costs – and not accounting for any potential subsidy scheme, which is a likely possibility – requires the reduction of these costs to near $1.81 per gallon relative to

2006 fuel prices. These cost reduction figures take into account the fact that materials input and refining of fuels (in this case the algae vegetable oil) account for roughly 71% of total at pump fuel cost [Chisti, 2007]. Algal biodiesel becomes even more plausible given the potential for GHG regulation in the near future. Since for every ton of algal biomass produced, approximately 1.83 tons of carbon dioxide is fixed while petroleum diesel carries a massive negat balance, the competitiveness of algae diesel increases as GHG externalities are taken into account. Given certain research objectives these cost reductions are achievable in the near future. The National Renewable Energy Laboratory (NREL) outlines many such research objects including: increasing photosynthetic efficiency of algae species for high lipid production, control of mechanisms of algae biofocculation, understanding the effects of non-steady-state operating conditions, and methods of species selection and control [Sheehan *et al*., 1998].

13. The problems related with algae

Most problems with marine microalgae cultures are related to predation by various types of protozoans (e.g. zooflagellates, ciliates, and rhizopods). Other problem is the blooming of unwanted or toxic species such as the blue-green algae or dinoflagellates (red tides) that can result in high toxicity for consumers and even for humans. Examples are the massive development of green chlorococcalean algae, such as Synechocystis in freshwater, and also the development of Phaeodactylum in seawater that is undesirable for bivalve molluscs. [De Pauw *et al*., 1984].

14. Other application of algae

Algae have mainly been used in west countries as raw material to extract alginates (from brown algae) and agar and carragenates (from red algae). Moreover, algae also contain multitude of bioactive compounds (phenolic compounds, alkaloids, plant acids, terpenoids and glycosides) that might have antioxidant, antibacterial, antiviral, anticarcinogenic, etc. properties. (Plaza, *et al*., 2008).

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