J. Agric. Mach. Bioresour. Eng. 5(1&2), 2009 : 59-68 ISSN 1993-4041

Feasibility study on the cultivation and oil production from algae in Bangladesh- A Review

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Abstract

Algae are known to all plant researchers for its importance to produce bio-fuel. Many works are being done in USA and other countries and they are producing bio-fuel which is equivalent to petroleum diesel fuel. These are plantlike organisms and abundantly grown in sweet and marine water. Types of algae, reproduction system, culturing technique, harvesting and oil extraction techniques are elaborately described in this paper. Algae contain at least 70 percent oil which is more than any other oil producing plants grown in the world. Bangladesh has wide scope to cultivate algae and to produce oil. The production method of oil from algae is not much complicated and industry for oil production can be established.

Key words : Algae, Algaculture, Reproduction system, Oil production

1. Introduction

Algae are plants or plantlike organisms that contain chlorophyll and other pigments that trap light from the Sun. This light energy is then converted into food molecules by the photosynthetic process. Most algae store energy as some form of carbohydrate (complex sugars). Algae have a nucleus enclosed within a membrane and chloroplasts bound in one or more membranes.[1] Algae constitute a paraphyletic and polyphyletic group,[1] as they do not all descend from a common algal ancestor, although their chloroplasts seem to have a single origin.[2] They are distinguished from protozoa in that they are photosynthetic. Many are photoautotrophic, although some groups contain members that are mixotrophic, deriving energy both from photosynthesis and uptake of organic carbon either by osmotrophy, myzotrophy, or phagotrophy. Some unicellular species rely entirely on external energy sources and have limited or no photosynthetic apparatus. They can occur in freshwater or salt water (most seaweeds are algae) or on the surfaces of moist soil or rocks.

With the increasing interest in biodiesel as an alternative to petro-diesel, many have looked at the possibility of growing more oilseed crops as a solution to the problem of peak oil. There are two problems with this approach: first, growing more oilseed crops would displace the food crops grown to feed mankind. Second, traditional oilseed crops are not the most productive or efficient source of vegetable oil. Micro-algae is, by a factor of 8 to 25 for palm oil, and a factor of 40 to 120 for rapeseed, the highest potential energy yield temperate vegetable oil crop. [28] Fig. 1 shows a common algae found in seawater.

1.1 Objective: The main objective of this review is to bring the available information together and to enhance knowledge to study the algae and to produce bio-diesel from them.

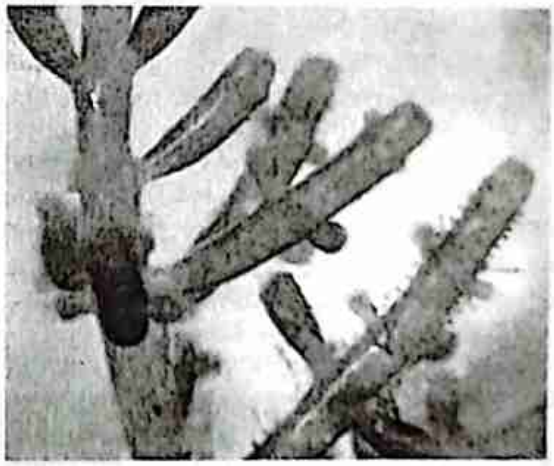

Fig. 1. *Laurencia*, a marine red alga

2. Types of algae

Although the term algae originally referred to aquatic plants, it is now broadly used to include a number of different groups of unrelated organisms. There are seven divisions of organisms that make up the algae. They are grouped according to the types of pigments they use for photosynthesis, the makeup of their cell walls, the types of carbohydrate compounds they store for energy, and the types of flagella (whiplike structures) they use for movement. The colors of the algae types

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are due to their particular mixtures of photosynthetic pigments, which typically include a combination of one or more of the green-colored chlorophylls as their primary pigments. [31]

2.1 Euglenoids (Euglenophyta): The euglenoids or Euglenophyta, are single-celled, protozoan-like algae, mostly occurring in freshwater. Unlike all other algae, they have no cell wall. Most euglenoids make their own food using light energy from the Sun but are capable of surviving in the dark if fed organic materials. Some species are heterotrophic, meaning they do not produce their own food but feed on organic matter suspended in the water.

2.2 Golden-brown algae (Chrysophyta): The Chrysophyta, or golden-brown algae and diatoms, are named for the yellow pigments they possess. These single-celled algae live both in freshwater and salt water. Their cell walls have no cellulose but are composed mostly of pectin, which is often filled with silica, a compound that makes the walls quite rigid. These algae store energy both as a carbohydrate and as large oil droplets. Diatoms have two glass shells made largely of silica that fit together like a pillbox and are exquisitely marked. Their species number from 40,000 to 100,000.

2.3 Fire algae (Pyrrophyta): Fire algae, or Pyrrophyta, are single-celled algae and include the dinoflagellates, which have two flagella used for locomotion. Most of these microscopic species live in salt water, with some occurring in freshwater.

2.4 Green algae (Chlorophyta): The green algae, or Chlorophyta, occur in freshwater, although some live in the sea. Most green algae are single-celled and microscopic (able to be seen only under a microscope), forming the silky green scum found in stagnant ponds. Others are larger and more complex, forming spherical (round) colonies composed of many cells or occurring as straight or branched filaments (long, thin series of cells).

2.5 Red algae (Rhodophyta): The red algae, or Rhodophyta, are marine plants that live mainly in shallow waters and deep tropical seas. A few also occur in freshwater. Their body forms range from single-celled to branched filaments. The larger species have filaments that are massed together and resemble the leaves and stems of plants. They have no flagella and typically grow attached to a hard surface or on other algae. Some species contain a red pigment; others range in color from green to red, purple, and greenish-black.

2.6 Brown algae (Phaeophyta): The brown algae, or Phaeophyta, are shiny brown seaweeds that are especially abundant along rocky coasts, although some float in the open ocean. Brown algae are large in size and include the giant kelps, which are located along the Pacific coast and form forests that provide habitat to a wide range of marine life. Brown algae contain an accessory brown-colored pigment that gives the plants their characteristic dark color. Other well-known brown algae are the common rockweed *Fucus* and *Sargassum.*

2.7 Yellow-green algae (Xanthophyta): The yellow-green algae, or Xanthophyta, primarily occur in freshwater. They can be either single celled or form colonies, their cell walls are made of cellulose and pectin compounds that sometime contain silica, they can have two or more flagella for locomotion, and they store their energy as carbohydrates. They derive their yellow-green color from the pigments carotenoids and xanthrophyll.

"In Bangladesh a large number of algal species occur in freshwater, brackish water and marine habitats. All these include both benthic and phytoplankton members of the Cyanophyceae, Chlorophyceae, Charophyceae, Euglenophyceae, Rhodophyceae, Bacillariophyceae, Chrysophyceae, Xanthophyceae and Chloromonadinae. These include, besides aquatic forms, terrestrial as well as sub aerial members.

Over 300 species and varieties of freshwater algae have been found in Bangladesh. The most common algae are freshwater blue-greens, euglenoids, chlorococcales, volvocales, zygnematales, oedogoniales, desmids, chaetophoralean algae, charalean members and red algae, freshwater diatoms, Xanthophyceae, Chrysophyceae, and Chloromonadinae. The water surface of many ponds all over Bangladesh become reddish in colour due to the heavy growth of some *Euglena* species. Roadside open drains in Bangladesh are invariably covered with a dark-greenish growth of *Oscillatoria*, *Lyngbya* or some other blue-green algae." (Banglapedia, 2008)

3. Chemical Composition of Algae

Algae are made up of eukaryotic cells with nuclei and organelles. All algae have plastids, the bodies with chlorophyll that carry out photosynthesis. All algae primarily comprise of the following, in varying proportions: Proteins, Carbohydrates, Fats and Nucleic Acids. While the percentages vary with the type of algae, there are algae types that are

comprised up to 40% of their overall mass by fatty acids. It is this fatty acid (oil) that can be extracted and converted into biodiesel. [31]

Algal-oil is very high in unsaturated fatty acids. The interest in algal oil is not new, though the widespread interest in making biodiesel from algal oil is more recent. Algal oil has been produced and used for the cosmetic industry, primarily from macroalgae (larger sized algae) such as oarleaf seaweed etc. Most current research on oil extraction from algae is however focused on microalgae.

Table 1. Chemical composition of algae expressed on a dry matter basis (%)

Strain	Protein	Carbohydrates	Lipids	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	22-38	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	-

Source: Becker, (1994)

4. Reproduction System

Algae reproduce in diverse ways. Some reproduce asexually, others use sexual reproduction, and many use both. In asexual reproduction an individual reproduces without combining its genetic material with that from another individual. The simplest form of asexual reproduction is binary fission, in which a unicellular organism simply divides into two new individuals. Some multicellular algae, including *Sargassum*, reproduce asexually through fragmentation, in which fragments of the parent develop into new individuals. In a similar process called budding, special buds detach from multicellular algae and develop into new individuals, commonly found in *Sphacelaria*. Many algae produce special cells called spores that are capable of growing into new individuals. If these spores move about using flagella, they are known as zoospores.

In sexual reproduction, genetic material from two individuals is combined. The simplest form of sexual reproduction in algae is conjugation, in which two similar organisms fuse, exchange genetic material, and then break apart. For example, in *Spirogyra*, which produces both asexually and sexually, two long, unbranched filaments join via conjugation tubes, through which genetic material is exchanged between cells. Most multicellular algae undergo a more complex form of sexual reproduction involving the union of special reproductive cells, called gametes, to form a single cell, known as a zygote.

Many algae incorporate both sexual and asexual modes of reproduction. This is well demonstrated in the life cycle of the alga *Chlamydomonas*. The mature alga is a single haploid cell—that is, it contains only one set of chromosomes. During asexual reproduction the cell undergoes mitosis, a type of cell division that produces genetically identical offspring. Four daughter cells are created

that emerge from the enclosing parent cell as spores. The spores develop into mature haploid cells that are genetically identical to the parent cell. [32]

5. Physical and Chemical Conditions for Algae Culture

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, turbulence, salinity and temperature. The most optimal parameters as well as the tolerated ranges are species specific and a broad generalization for the most important parameters is given in Table 2. Also, the various factors may be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for another.

Table 2. A generalized set of conditions for culturing micro-algae

Parameters	Range	Optima
Temperature (°C)	16-27	18-24
Salinity ($g.l^{-1}$)	12-40	20-24
Light intensity (flux)	1,000-10,000 (depends on volume and density)	2,500-5,000
Photoperiod (light: dark, hours)		16:8 (minimum) 24:0 (maximum)
pH	7-9	8.2-8.7

5.1 Culture medium/nutrients: Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients to make up for the deficiencies. Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate.

Silicate is specifically used for the growth of diatoms which utilize this compound for production of an external shell. Micronutrients consist of various trace metals and the vitamins thiamin (B_1), cyanocobalamin (B_{12}) and sometimes biotin. Two enrichment media that have been used extensively and are suitable for the growth of most algae. Commercially available nutrient solutions may reduce preparation labor. The complexity and cost of the above culture media often excludes their use for large-scale culture operations. Alternative enrichment media that are suitable for mass production of micro-algae in large-scale extensive systems contain only the most essential nutrients and are composed of agriculture-grade rather than laboratory-grade fertilizers.

5.2 Light: Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture (*e.g.* 1,000 lux is suitable for erlenmeyer flasks, 5,000-10,000 is required for larger volumes). Light may be natural or supplied by fluorescent tubes. Too high light intensity (*e.g.* direct sun light, small container close to artificial light) may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided. Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develop normally under constant illumination.

5.3 pH: The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. The latter is accomplished by aerating the culture. In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of up to pH 9 during algal growth.

5.4 Aeration/mixing: Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (*e.g.* in outdoor cultures) and to improve gas exchange between the culture medium and the air. The latter is of primary importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. For very dense cultures, the CO_2 originating from the air (containing 0.03% CO_2) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply (*e.g.* at a rate of 1% of the volume of air). CO_2

addition furthermore buffers the water against pH changes as a result of the CO_2/HCO_3^- balance. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test tubes, Erlenmeyer), aerating (bags, tanks), or using paddle wheels and jet pumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing.

5.5 Temperature: The optimal temperature for phytoplankton cultures is generally between 20 and 24°C, although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of micro-algae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are lethal for a number of species. If necessary, algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air - conditioning units.

5.6 Salinity: Marine phytoplankton are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water. Salinities of 20-24 $g.l^{-1}$ have been found to be optimal.

6. Algal Culture Technique

6.1 Preliminary shake culture: Watanabe, 1959c. [29] has transferred the alga from agar slants to round flasks, which were contently shaken at 32° C. The cultures were illuminated by incandescent lamps set in the inner wall of the thermostat. Two weeks old cultures were used as inocula for the tank cultures.

6.2 Tank culture: The culture tank was made of pyrex glass cylinder of 30 litres capacity and housed in a water bath thermostated at 32°C. The water bath was provided with three windows for illuminating the culture from outside. The tank was provided with a stirrer and a pipe line for aeration. The carbon dioxide enriched air (3 percent) was bubbled at a rate of 350 ml/min.into the culture solution. To detach the attaching alga on the walls of the glass cylinder, a rubber tipped wiper was also provided. The light intensity was control by adjusting the position of the light source. Prior to inoculation, sterilization of the chamber and the culture medium was effected by steam obtained from a boiler for 15 minutes at 0.1 kg/mm^2. When the culture attained a density of about 2g (dry weight)/l, about half of the culture was withdrawn and used as the inoculums for outdoor cultures. The tank was then filled with fresh medium to the initial volume. The average growth rate obtained with the tank culture was 0.2g (dry wt)/l/day (Watanabe, 1959c). Two types of tank cultures, rectangular and a circular type were developed initially. The maximum growth rate obtained in these tanks with tolypothrix tenuis was 0.18g/l/day. [29]

6.3 Closed circulation culture: For outdoor culture the culture chamber was made of long polyvinyl flat bag (.8x6x5.3m; 300 litre capacity), the two inch of which were connected with each other through a 3 cm broad and 8m long tube. The chamber was directly connected two the tank to facilitate aseptic transfers from the culture tank to the outdoor growth chamber. The culture was constantly stirred by a whirl pump at a rate of 20 L/min and 5 percent carbon dioxide enriched air was bubbled into the culture. Cooling of the culture was effected by pouring tap water over the culture bag and the intensity of incident light was controlled by setting a bamboo blind. Before starting the cultures, sterilization of the entire system was done with one percent solution of H_2O_2, which was later removed by streaming in sterilized water the maximum yield obtained with this outdoor device was 7.9gm (dry wt)/m^2/day.(Watanabe, 1959c). [29]

6.4 Gravel culture: Taking advantage of the preparation of the Japanese Koji (a product of steamed rice grains on the surface of which *Aspergillus oryzae* is grown), Watsnbe (1959b) employed a special volcanic gravel, commercially known as Kanuma-Tsuchi, for growing *Tolypothrix tenuis*. Sieved gravels were washed several times with distilled water, soaked in nitrogen-free medium and sterilized with heat at 15lb for 15 minutes. The gravels were then mixed with an equal amount of concentrated algal suspension and the inoculated gravels were placed in polyvinyl tube under weak illumination. After about 4 weeks incubation the gravel cultures were transferred to vinyl bags or glass bottles and kept at room temperature. These were used for field inoculation. Various devices are being examined by the author and his group to prepare low cost algal fertilizer. [29]

6.5 Dry sand culture: Sieved quartz sand was washed well and boiled in distilled water for about 2 hours and dried at 120°C. The dried sand was soaked in nitrogen free medium and sterilized at 20lb for 30 minutes. The sand was then mixed with

a concentrated algal suspension and gradually sun dried. The dried sand could be preserved in tubes or paper bags and stored. However the 'sand culture' has the disadvantage in that the sand particles, being heavier, sink into the mud, thus hampering the rapid growth of the adhering alga. [29]

6.6 Foam culture: Synthetic sponge is cut into rectangular cubes of 2-4 cm boiled in water, soaked in nitrogen free medium under suction and sterilized. The sponge pieces are than spared on metallic boats, layered with vinyl sheeting. The top of the boat is covered by a thick vinyl sheet, supported by a number of arch shaped metallic wire attached to the sides of the boat. The sponge pieces are either mixed with algal suspension before spreading them on the boats or the algal is sprayed over them. The boats carrying the inoculated sponge pieces are incubated under weak illumination. Within 3 to 4 days, the sponge pieces are completely covered with the algal growth and after a suitable period of incubation they are removed and stored in plastic bags. [29]

6.7 Algal productions in outdoor ponds: Large outdoor ponds either with a natural bottom or lined with cement, polyethylene or PVC sheets have been used successfully for algal production. The nutrient medium for outdoor cultures is based on that used indoors, but agricultural-grade fertilizers are used instead of laboratory-grade reagents. Culture depths are typically 0.25-1 m. Cultures from indoor production may serve as inoculums for monospecific cultures. Alternatively, a phytoplankton bloom may be induced in seawater from which all zooplankton has been removed by sand filtration. Algal production in outdoor ponds is relatively inexpensive, but is only suitable for a few, fast-growing species due to problems with contamination by predators, parasites and "weed" species of algae. Furthermore, outdoor production is often characterized by a poor batch to batch consistency and unpredictable culture crashes caused by changes in weather, sunlight or water quality. Mass algal cultures in outdoor ponds are commonly applied in Taiwanese shrimp hatcheries where Skeletonema costatum is produced successfully in rectangular outdoor concrete ponds of 10-40 tons of water volume and a water depth of 1.5-2 m.

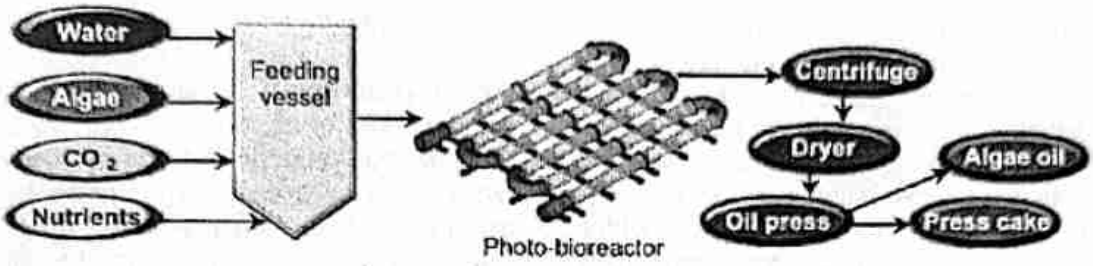
6.8 Photobioreactors: Algae can also be grown in a photobioreactor (PBR). A PBR is a bioreactor which incorporates some type of light source. Virtually any translucent container could be called a PBR, however the term is more commonly used to define a closed system, as opposed to an open tank or pond. Because these systems are closed, all essential nutrients must be introduced into the system to allow algae to grow and be cultivated. Essential nutrients include carbon dioxide, water, minerals and light (the basic reaction is carbon dioxide + water + light energy = glucose + oxygen + water [16]). A pond covered with a greenhouse could be considered a PBR. A PBR can be operated in "batch mode", but it is also possible to introduce a continuous stream of sterilized water containing nutrients, air, and carbon dioxide. As the algae grows, excess culture overflows and is harvested. If sufficient care is not taken, continuous bioreactors often collapse very quickly, however once they are successfully started, they can continue operating for long periods. An advantage of this type of algae culture is that algae in the "log phase" is produced which is generally of higher nutrient content than old "senescent" algae. It can be shown that the maximum productivity for a bioreactor occurs when the "exchange rate" (time to exchange one volume of liquid) is equal to the "doubling time" (in mass or volume) of the algae.

6.9 Batch culture: The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase and the larger culture volumes are then brought to a maximum density and harvested. The following consecutive stages might be utilized: test tubes, 2 l flasks, 5 and 20 l carboys, 160 l cylinders, 500 l indoor tanks, 5,000 l to 25,000 l outdoor tanks.

According to the algal concentration, the volume of the inoculums which generally corresponds with the volume of the preceding stage in the up scaling process amounts to 2-10% of the final culture volume. Where small amounts of algae are required, one of the simplest types of indoor culture employs 10 to 20 l glass or plastic carboys, which may be kept on shelves backlit with fluorescent tubes.

Batch culture systems are widely applied because of their simplicity and flexibility, allowing to change species and to remedy defects in the system rapidly. Although often considered as the most reliable method, batch culture is not necessarily the

most efficient method. Batch cultures are harvested just prior to the initiation of the stationary phase and must thus always be maintained for a substantial period of time past the maximum specific growth rate. Also, the quality of the harvested cells may be less predictable than that in continuous systems and for example vary with the timing of the harvest (time of the day, exact growth phase).



Another disadvantage is the need to prevent contamination during the initial inoculation and early growth period. Because the density of the desired phytoplankton is low and the concentration of nutrients is high, any contaminant with a faster growth rate is capable of outgrowing the culture. Batch cultures also require a lot of labor to harvest, clean, sterilize, refill, and inoculate the containers.

6.10 Continuous culture: The continuous culture method, (i.e. a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out), permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished:

- Turbidostat culture, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.
- Chemostat culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (*e.g.* nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant.

Laing (1991) described the construction and operation of a 40 l continuous system suitable for the culture of flagellates, *e.g. Tetraselmis suecica* and *Isochrysis galbana*. The culture vessels consist of internally-illuminated polyethylene tubing supported by a metal framework. This turbidostat system produces 30-40 l per day at cell densities giving optimal yield for each flagellate species.

The disadvantages of the continuous system are its relatively high cost and complexity. The requirements for constant illumination and temperature mostly restrict continuous systems to indoors and this is only feasible for relatively small production scales. However, continuous cultures have the advantage of producing algae of more predictable quality. Furthermore, they are amenable to technological control and automation, which in turn increases the reliability of the system and reduces the need for labor.

6.11 Harvesting of algae: Algae can be harvested using microscreens, by centrifugation, or by flocculation.[17] Froth flotation is another method to harvest algae whereby the water and algae are aerated into a froth, the algae then removed from the water.[18] Alum and ferric chloride are chemical flocculants used to harvest algae. A commercial product called "Chitosin", commonly used for water purification, can also be used as a flocculant. The shells of crustaceans are ground into powder and processed to acquire chitin, a polysaccharide found in the shells, from which chitosin is derived. Water that is more brackish, or saline requires additional chemical flocculant to induce flocculation. Harvesting by chemical flocculation is a method that is often too expensive for large operations. Interrupting the carbon dioxide supply to an algal system can cause algae to flocculate on its own, which is called "autoflocculation". Ultrasound based methods of algae harvesting are currently under development, and other, additional methods are currently being developed.[19][20]

7. Oil extraction method

Algae oils have a variety of commercial and industrial uses, and are extracted through a wide variety of methods.

7.1 Mechanical crushing: The simplest method is mechanical crushing. Since different strains of algae vary widely in their physical attributes, various press configurations (screw, expeller, piston, etc) work better for specific algae types.

Often, mechanical crushing is used in conjunction with other extraction methods. Estimates of the cost to extract oil from microalgae vary, but are likely to be around $1.80/kg (compared to $0.50/kg for palm oil).[21]

7.2 Chemical solvents: Algal oil can be extracted using chemicals. Benzene and ether have been used, oil can also be separated by hexane extraction, which is widely used in the food industry and is relatively inexpensive. The downside to using solvents for oil extraction are the dangers involved in working with the chemicals. Soxhlet extraction is an extraction method that uses chemical solvents. Oils from the algae are extracted through repeated washing, or percolation, with an organic solvent such as hexane or petroleum ether, under reflux in a special glassware.[22]

7.3 Enzymatic extraction: Enzymatic extraction uses enzymes to degrade the cell walls with water acting as the solvent, this makes fractionation of the oil much easier. The costs of this extraction process are estimated to be much greater than hexane extraction.[16] The enzymatic extraction can be supported by ultrasonication. The combination "sonoenzymatic treatment" causes faster extraction and higher oil yields. [23]

7.4 Expression/Expeller press: When algae is dried it retains its oil content, which then can be "pressed" out with an oil press. Many commercial manufacturers of vegetable oil use a combination of mechanical pressing and chemical solvents in extracting oil.

7.5 Osmotic shock: Osmotic shock is a sudden reduction in osmotic pressure, this can cause cells in a solution to rupture. Osmotic shock is sometimes used to release cellular components, such as oil.

7.6 Supercritical fluid: In supercritical fluid/CO_2 extraction, CO_2 is liquefied under pressure and heated to the point that it has the properties of both a liquid and a gas; this liquified fluid then acts as the solvent in extracting the oil.[24][25]

7.7 Ultrasonic-assisted extraction: Ultrasonic extraction, a branch of sonochemistry, can greatly accelerate extraction processes. Using an ultrasonic reactor, ultrasonic waves are used to create cavitation bubbles in a solvent material, when these bubbles collapse near the cell walls, it creates shock waves and liquid jets that causes those cells walls to break and release their contents into the solvent.[26]

Other methods are still being developed, including ones to extract specific types of oils, such as those with a high production of long-chain highly unsaturated fatty acids.[19][20]

7.8 Oil production per unit area: Currently most research into efficient algal-oil production is being done in the private sector, but predictions from small scale production experiments bear out that using algae to produce biodiesel may be the only viable method by which to produce enough automotive fuel to replace current world diesel usage.[33]

Microalgae have much faster growth-rates than terrestrial crops. The per unit area yield of oil from algae is estimated to be from between 5,000 to 20,000 gallons per acre, per year (4.6 to 18.4 l/m^2 per year); this is 7 to 30 times greater than the next best crop, Chinese tallow (699 gallons).[27]

Michael Briggs at the Univ. of N. Hampshire Biodiesel group estimates that using open, outdoor, racetrack ponds, only 15,000 square miles could produce enough algae to meet all of the USA's ground transportation needs. Under optimum growing conditions micro-algae will produce up to 4 lbs./sq. ft./year or 15,000 gallons of oil/acre/year. Micro-algae are the fastest growing photosynthesizing organisms. They can complete an entire growing cycle every few days. One quad (1015 BTU or 7.5 billion gal.) of biodiesel could be produced on 200,000 ha of desert land (equivalent to 772 sq. mi., roughly 500,000 acres). (To produce one quad from a rapeseed crop would require 58 million acres or 90,000 sq. mi.)[28] The comparative oil yield from different sources shown in table.

Table 3. Gallons of Oil per Acre per Year obtained from different sources. [28]

Source	Gallons of oil per acre per year
Corn	18
Soybeans	48
Safflower	83
Sunflower	102
Rapeseed	127
Oil Palm	635
Micro Algae	5000-15000

8. Use of algae

Fertilizer : For centuries seaweed has been used as a fertilizers, soil conditioners and are a source of livestock feed.[3] Because many species are aquatic and microscopic, they are cultured in clear tanks or ponds and either harvested or used to treat effluents pumped through the ponds. Maerl is commonly used as a soil conditioner, it is dredged from the sea floor and crushed to form a powder. Chemical analysis of maerl showed that it contained 32.1% $CaCO_3$ and 3.1% $MgCO_3$ (dry weight). [3]

8.1 Energy source

- Algae can be used to make biodiesel, bioethanol and biobutanol and by some estimates can produce vastly superior amounts of vegetable oil, compared to terrestrial crops grown for the same purpose.
- Algae can be grown to produce biohydrogen. *Chlamydomonas moeweesi* is a good strain for the production of hydrogen.[4]
- Algae can be grown to produce biomass, which can be burned to produce heat and electricity. [5]
- Algae can be used in oil production which could replace the petrol and other gas products in the near future.

8.2 Pollution control

- Algae are used in wastewater treatment facilities, reducing the need for greater amounts of toxic chemicals that are already used.
- Algae can be used to capture fertilizers in runoff from farms. When subsequently harvested, the enriched algae itself can be used as fertilizer.
- Algae Bioreactors are used by some powerplants to reduce CO_2 emissions. [6] The CO_2 can be pumped into a pond, or some kind of tank, on which the algae feed. Alternatively, the bioreactor can be installed directly on top of a smokestack. This technology has been pioneered by Massachusetts-based Green Fuel Technologies.[7]

8.3 Nutrition: Seaweeds are an important source of food, especially in Asia; they are excellent sources of many vitamins including: A, B1, B2, B6, niacin and C. They are rich in iodine, potassium, iron, magnesium and calcium.[9]

Algae is commercially cultivated as a nutritional supplement. One of the most popular microalgal species is Spirulina *(Arthrospira platensis)*, which is a Cyanobacteria (known as blue-green algae), and has been hailed by some as a superfood.[10] Other algal species cultivated for their nutritional value include; Chlorella (a green algae), and Dunaliella (*Dunaliella salina*), which is high in beta-carotene and is used in vitamin C supplements.

In China at least 70 species of algae are eaten as is the Chinese "vegetable" known as *fat choy* (which is actually a cyanobacterium). Roughly 20 species of algae are used in everyday cooking in Japan.[9]

The oil from some algae have high levels of unsaturated fatty acids. Arachidonic acid (a polyunsaturated fatty acid), is very high in *Parietochloris incisa*, (a green alga) where it reaches up to 47% of the triglyceride pool (Bigogno C et al. Phytochemistry 2002, 60, 497). [11] [12]

8.4 Other uses

- There are also commercial uses of algae as agar.[8]
- The natural pigments produced by algae can be used as an alternative to chemical dyes and coloring agents.[13]
- Algae can be used to make pharmaceuticals[14]Sewage can be treated with algae as well[15] Some cosmetics can come from microalgae as well.

9. Conclusion

Following conclusions are drawn on the basis of information given above:

1. Bangladesh has both fresh and sea water in its territory. As the land area is limited in Bangladesh, water bodies can be utilized for the cultivation of Algae for production of bio-diesel.
2. Mass scale cultuivation of algae is possible in Bangladesh.
3. Most of the algae contain Lipids which is responsible to produce bio-ethanol or methanol. This bio-fuel can be blended to produce appropriate fuel grade.
4. In the artificial culture, sufficient amount of light, CO_2 need to be supplied for the efficient production of algae.
5. Extraction of oil from algae is easy. Chemical extraction of oil from algae using hexane yields more than the extraction of oil by pressing mill.
6. Biodiesel produced from algae can be a better alternative to other biofuel and natural petrolium in terms of price, quality and availability. Algae can be harvested once in a

week which is not possible from other plants like Jatropha, Karanj, Verenda, Mohringa etc. Ponds, *Beel, Haor* etc can be utilized for the cultivation of appropriate type algae.

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