**Component-8**

# A Techno-Economic Assessment of Algae Bio-Fuel Production in Bangladesh


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# EXECUTIVE SUMMARY

Biofuel from algae can be considered to be one of the best possible ways to reduce green house gas emission as well as an alternative energy source to replace fossil fuels. The present study focuses on algae cultivation for biodiesel production and its prospect in Bangladesh. The goal of present research is to carry out an integrated assessment of the technical and economical aspects of algae-based biodiesel by analyzing local engineering design practices and knowledgebase and by availing local resources.

Our study focuses on determining a suitable system for algae cultivation in Bangladesh. This includes analysis of the open pond and closed system. At the same time, local algae strains were explored for cultivation. High lipid content and high growth rate were the preferred algal properties during the search. For growing algae, photobioreactors were constructed and problems arousing from the fabrication of the reactors were dealt with. Various parameters that affect algae growth and their lipid content were studied experimentally. Methodologies for proper harvesting and for analyzing growth of algae in the bioreactors were developed and implemented in the project. Finally, a cost analysis was performed to get a clear picture of current status of algae biodiesel production in Bangladesh and its sustainability.

From the study, the following findings can be summarized:

1. Our study shows that open pond system for algae cultivation may not be suitable for Bangladesh primarily because of its requirement of large land area and because of the risk of contamination of the local ecosystem.
2. Literature data on local algae availability is very limited and furthermore, knowledge of their lipid content is minimal. Also, their constant supply could not be ensured. Further exploration and studies of local algal strains is required.
3. Limitation of local resources for fabrication and construction of photobioreactors was a major problem faced during the project. Photobioreactor system made of glass of a moderate volume of 25 litres was fabricated following the design criteria. Although acrylic material was a preferred material for photobioreactor, fabrication limitation of local manufacturing facilities hindered its use.
4. Parameters controlling the growth of algae such as supply of carbon dioxide and air, nutrients, removal of oxygen, pH level, and temperature were carefully studied.
5. An oil extraction methodology consisting of a ball mill and a soxhlet extractor was implemented.
6. A technique employing a microscope and spectrophotometer was established to analyze the algal growth.
7. Through this project the high potentiality of algae based fuel to be used in Bangladesh replacing diesel for energy production in the future.

# CHAPTER 1
# INTRODUCTION

## 1.1 Energy Sources

### World Energy Scenario

Todays world is dependent on fossil fuels for its supply of energy. In 2008, total worldwide energy consumption was 474 exajoules with 80 to 90 percent derived from the combustible fossil fuels. Energy consumption in the last two decades consists of fifty percent of the total energy consumption since the industrial revolution. According to International Energy Association (IEA), Table 1.1 summarizes proven energy reserves. Real reserves may be up to four times higher. However, significant uncertainty exists for these numbers. The estimation of fossil fuel in the planet depends on a detailed understanding of earth crust. The understanding is still less than perfect.

Table 1.1: Estimated Remaining reserves of fossil fuels

| **Fuel** | **Proven Reserves ZJ (end of 2009)** |
|---|---|
| Coal | 19.8 |
| Oil | 8.1 |
| Gas | 8.1 |

[Source: Organization for Economic Co-Operation and Development]

However one should keep this at mind that these quantitative measures of amount of proven reserve of fossil fuel do not take into account several factors critical to the cost of extracting them from ground and critical to price the energy extracted from the fossil fuels. These factors include the accessibility of fossil deposits, the level of sulfur and other pollutants in the oil and the coal, transportation cost, risky location etc.

### Energy Scenario in Bangladesh

Bangladesh has one of the lowest rates of per capital energy consumption in the world. Bangladesh is not well endowed with conventional sources of energy. The country's energy sources are neither adequate nor varied. Non-conventional sources of energy include biomass fuels, agricultural residues, and animal dung. Conventional sources of energy in the country include fossil fuels, such as coal, oil, natural gas and hydropower. A brief accounting of these commercial sources of energy in Bangladesh has been provided in Fig 1.1. Total energy production in Bangladesh using different types of fuel is also shown in Fig 1.2.

Table 1.2: Share of total primary energy supply in Bangladesh in 2009

| Energy Source | Percentage |
|---|---|
| Natural Gas | 51.7 |
| Biofuels and Waste | 29.8 |
| Oil | 15.9 |
| Coal/Peat | 2.1 |
| Hydro | 0.5 |

[Source: Bangladesh Power Development Board]

**Natural Gas**

Natural Gas is the most important energy source for Bangladesh. From household uses to heavy industries, natural gas is used. Unplanned use of natural gas in earlier years and highly subsidized price of it has made it difficult for proper utilization of the full potential of total natural gas reserve in Bangladesh. Contrary to popular belief, Bangladesh does not have a large reserve of natural gas. According to the latest information found, the natural gas reserve situation of Bangladesh is given below:

Table 1.3: Natural Gas reserve in Bangladesh

| Natural Gas Reserve | Amount in Bcf |
|---|---|
| Reserve (Proven + Probable) | 28,619.70 |
| Reserve (Recoverable) | 20,631.45 |
| Cumulative Gas Production (Till Dec 2010) | 9,407.14 |
| Remaining Recoverable Reserve | 11,224.31 |
| Daily Gas Production in 2010-2011 (Till April 2011) | 2.19 |

[Source: PetroBangla, National natural gas reserve of Bangladesh]

So, from the information stated above, it is evident that Bangladesh has used up almost half the total reserve of natural gas. With the increasing rate of use of natural gases, Bangladesh will run out of Natural Gas reserve within 2025 if not sooner.

**Petroleum Oil**

Petroleum oil is the most important fossil fuel around the world. Due to its higher heating value and portability by carrying in fuel tanks, it has become the most prominent fuel for different uses especially transportation sector. Though presently a very large number of vehicles and industries are running in CNG in Bangladesh, petroleum oil is still a very big source of energy. Unlike the past Bangladesh is presently producing enough petrol to serve

its own needs. A healthy amount of petroleum products are being produced at different fields under Sylhet gas fields ltd.

### Coal

Although coal is a very important source of energy in worldwide, in Bangladesh, coal is not a popular source; coal has not been yet used in mass level. Presently, only one mine is on operation in Bangladesh: Barapukuria, Dinajpur. According to Centre for Energy Studies, BUET, the total amount of coal reserve in Bangladesh is 3.015 Billion MT of which 1.4 Billion MT is recoverable.

## 1.2 Renewable Energy

### Photovoltaic

In 1981 Bangladesh Power Development Board (BPDB) installed 55 solar powered warning lights on 11 towers of the East-west Power Interconnector in Aricha. It is reported that the solar panels have been operating without any problem since their installation in 1981.Grameen Shakti has sold PV systems to 1,506 users and installed 71.6 kW (Peak) capacities to the rural areas with a range of 13 W to 215 W according to needs. At present solar panels have been installed to around 300,000 households in the rural and peri-urban area of Bangladesh generating around 15MW power [34].

### Biogas technology

Biogas is obtained by anaerobic fermentation of cow dung and other organic matters. The gas consists of 55-65% methane and the rest mainly carbon dioxide. It can be used for cooking, lighting and other purposes. The potential of biogas technology is immense and this technology has far reaching benefits especially for the densely populated rural areas. According to official estimates, Bangladesh has a cattle population of 24 million and poultry population of 75 million. The dung supplied by the cattle, about 240 million kg/day, can produce 2.97 billion $m^3$ biogas, which is equivalent to 3.04 million tons of coal. Poultry litters can produce 0.525 million $m^3$/day of biogas. Besides, if each family of the country is linked to a biogas plant, the human excreta alone can produce 3.36 million $m^3$ gas daily and 1226.4 million $m^3$ annually [34].

### Micro-mini Hydropower

Micro and mini hydro-power, which is one of the most important branches of renewable energy sources, is a cheap and clean method of power generation. Unfortunately, the scope is very limited in Bangladesh by the country's topography. However, there are certain locations of the southeast and northeast hilly region of Bangladesh where micro or mini hydro power plants can be constructed to serve local needs of electricity.

### Wind

Another renewable energy technology appropriate for Bangladesh, especially the coastal areas, is wind energy. According to preliminary studies, wind energy will be viable in the coastal areas, offshore islands, rivers sides, and other inland open areas both for mechanical

power and electricity generation. A wind monitoring system was set up at St. Martin's Island. Collected wind data of one year was stored in an installed Data Logger at St. Martin' Island and analyzed with respect to various characteristics in the laboratory. Data monitoring is going on. Preparations are completed to set up two more wind monitoring systems; one at Teknaf or Chokoria and the other at Meghnaghat. Preparations are also complete to setup wind turbines: one 3 kW at St Martin's Island, one 1.2 kW either at Teknaf or at Chokoria, and one 0.5 kW and one wind pump at Meghnaghat. [34]

### 1.3 Algae Biodiesel

The crisis for energy is more acute in Bangladesh, as there is no petrofuel source but only natural gas, the reserve has also dropped down to an alarming level. Again the global warming is threatening Bangladesh to be climate change victim. So there is no alternative for Bangladesh rather than renewable energy sources. Biofuel from microalgae can be a solution of this problem. Oil from the algae lipid can be turned into biodegradable and carbon neutral Biodiesel. Use of this diesel can reduce air pollution at remarkable level. So here our study focuses on algae cultivation in Bangladesh. Oil extracted from algal biomass can be turned into biodegradable and carbon neutral biodiesel (Meher, 2006; Chisti, 2007) through a few simple processing steps as depicted in Fig 1.1.

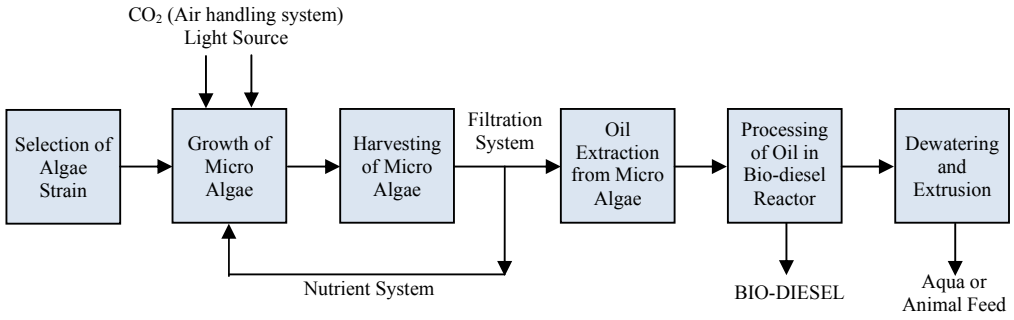


Fig 1.1: Production of biodiesel from algae.

### 1.4 Objectives of the Study

This study evaluates for the prospects for the large scale cultivation and harvesting of algae followed by its possible conversion into bio-fuel. The goal of the research is to carry out an integrated assessment of technological, engineering, environmental and economical aspects of algae based bio-fuels.

The work covers crucial issues in algae fuel technology and economics, like

1. Identification of locally available algal strains with substantial lipid content and high growth rate and selection of appropriate strains for biodiesel production.

2. Engineering design and fabrication of pilot scale bioreactor along with the design of control parameters.
3. Culture of algae on pilot / mass scale
4. Devising harvesting technology that is suitable to local condition
5. Harvesting algal biomass and extraction of oil from the biomass.
6. Processing alagal oil into biodiesel.
7. Cost analysis and feasibility study of algae biodiesel production in Bangladesh.

### 1.5 Outline of the Report

In this report, five important aspects of algal biodiesel production have been discussed: selection and collection process of algae strain, design and construction of photo bioreactor, selection and design of algae extraction process, identification of control parameters for algal growth and study of growth rate, and cost analysis of algal biodiesel production. These five topics have organized as five chapters of the report. Each of the issues can be further researched for detailing. Chapter two defines the algae and shows the importance of algae culture over different other bio fuel sources. It also reports a survey of different algae species cultivated in other photobioreactors (PBR). It contains the lipid content and algae growth rate of these species. However, most of them are foreign algae strains and may be harmful for local biodiversity. Local algae strains were searched for and few potential algae strains have been selected. Chapter three accounts for detail study of design and fabrication of closed photobioreactors. This chapter discusses the existing designs of PBR and parameters to be considered in designing a PBR. PBR design in context of Bangladesh is of main interest. The effects of availability of material, fabrication limitation, environmental condition on PBR design and fabrication are discussed in detail. Chapter four outlines the processes involving harvesting of algae and extraction of oil. Literature survey was conducted and reported briefly. A relatively easier technique for algae harvesting is selected. For oil extraction a ball mill set up is locally set up and a soxhlet apparatus is procured. Chapter five includes the study of algae growth using spectrophotometer and microscope. Chapter six analyze the feasibility of algae biodiesel project in agricultural sector of rural Bangladesh

# CHAPTER 2
# SELECTION OF ALGAE STRAIN

## 2.1 What is Algae?

Algae is a large group of primitive, mostly aquatic, photosynthetic chlorophyll-bearing plants, lacking specialized tissues and organs namely roots, stems, leaves, flowers etc. Algae can be broadly categorized into two groups: microalgae and macroalgae. Microalgae are very small (+/- 1 to 50 ìm) while macroalgae can reach sizes up to 60 m in length. Algae are usually found in damp places or bodies of water and thus are common in terrestrial as well as aquatic environments. Like plants, most algae require primarily three components to grow: sunlight, carbon-dioxide and water.

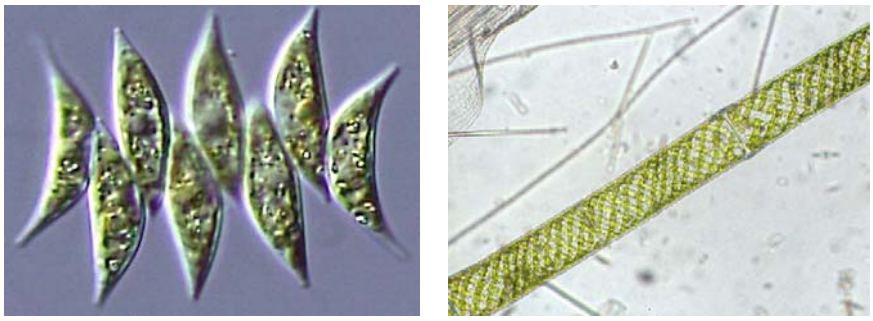

Fig 2.1: Microscopic view of algal strains: (a) S. dimorphus (b) Spirogyra

### Composition of Algae

Algae are made up of prokaryotic as well as eukaryotic cells. These are cells with nuclei and organelles. All algae have plastids, the bodies with chlorophyll that carry out photosynthesis. But the various lines of algae have different combinations of chlorophyll molecules. Some have only Chlorophyll A, some A and B, while other lines, A and C. All algae primary comprise of the following, in varying proportions: Proteins, Carbohydrates, Fats and Nucleic Acids. While the percentages vary with the type of algae, from 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.

### Microalgae lipid content and productivity

Cultivation of microalgae is gaining popularity because of its high lipid content and high growth rate. Average lipid content of microalgae varies between 1 and 70% but in certain conditions some species can reach higher values. Table 2.1 shows lipid content and lipid and biomass productivities of different feed stocks including microalgae. Productivity of microalgae per acre estimation shows that other food grain cannot compete with microalgae for biofuel production. Globally biofuel research is thereby shifting towards non food grain type biofuel sources.

Table 2.1: Lipid content and productivity of different source [Kunjapur 2010]

| Plant source | Seed oil content (% oil by wt in biomass) | Oil yield (L oil/year) | Land use (m2 year/kg biodiesel) | Biodiesel productivity (kg biodiesel/ year) |
|---|---|---|---|---|
| Corn | 44 | 172 | 66 | 152 |
| Soybean | 18 | 636 | 18 | 562 |
| Jatropha | 28 | 741 | 15 | 636 |
| Camelina | 42 | 915 | 12 | 809 |
| Sunflower | 41 | 1,070 | 11 | 946 |
| Palm oil | 40 | 5,366 | 2 | 4,747 |
| Microalgae(low oil content) | 30 | 58,700 | 0.2 | 51,927 |
| Microalgae(medium oil content) | 50 | 97,800 | 0.1 | 80,515 |
| Microalgae(High oil content) | 70 | 136,900 | 0.1 | 121,104 |

Algae experts state *Chlorella* seems a good option for biodiesel production. Though many other species may seem as efficient and productive as this one, the selection should also depend upon other factors such as nutrients available in the environment and conditions and not just lipid content. Composition of fatty acids of different microalgae species is also very important as they have significant effects on the characteristics of bio-diesel produced. Different nutritional and environment factors and cultivation conditions may affect the fatty acid composition. The microalgae oil yield is strain dependent. It is generally much greater than other vegetable crops. Table shows that although the oil contents are similar between seed plants and microalgae there are significant variation in the overall biomass productivity with a clear advantage for microalgae.

**Where do algae grow?**

Algae are some of the most robust organisms on earth, able to grow in a wide range of conditions. Algae are usually found in damp places or bodies of water and thus are common in terrestrial as well as aquatic environments. However, terrestrial algae are usually rather inconspicuous and far more common in moist, tropical regions than dry ones, because algae lack vascular tissues and other adoptions to live on land  As mentioned above, algae grow in almost every habitat in every part of the world.

**Why Algae**?

1. From a practical point of view they are easy to cultivate and can grow with little or no attention.
2. They can be grown using water unsuitable for human consumption and have the ability to easily obtain nutrient from the environment. They can be produced even using ocean and waste water.
3. Algae has a fast growth, and all what they need to grow are water, sunlight and carbon dioxide (CO2). They have the ability to reproduce themselves using photosynthesis by converting sun energy into chemical energy completing an entire growth cycle every few days.
4. Different algae species can be adapted to grow in different environmental conditions. So it is possible to find best suitable local environments for different species. But this still has not been possible with other feed stocks such as soybean, sunflower, palm oil etc.
5. The per unit area yield of oil from algae is estimated to be from between 5,000 to 20,000 US gallons per acre per year, and this is 7 to 30 times greater than the next best crop, Chinese tallow. So we can see that the growth rate to land ratio for algae is much higher than other agricultural crops and biodiesel feedstock.
6. Algal biodiesel's advantage over other bio fuels such as corn bio fuel is that it does not compete with food demand, algae grow on marginal land, and it produces more oil per hector area of crop cultivation.
7. Algae can be used for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol etc. It contains sulfur and performs as well as petroleum diesel.
8. We know that carbon dioxide is the greenhouse gas mostly responsible for climate change problem that is released in the atmosphere by fossil fuels burning. Some latest studies have shown that the production of each gallon of oil from algae consumes 13 to 14 kilograms of the carbon dioxide.
9. Also algae can be used reduce the environmental impact of algae is to draw municipal wastewater into algae plantations, as a source of nitrogen and phosphorus. This could reduce the amount of fertilizer required.

## 2.2 Algae Strains

Microalgae have many different species with widely varying compositions and live as single cells or colonies without any specialization. Although this makes their cultivation easier and more controllable, their small size makes subsequent harvesting more complicated. Macro algae are less versatile, there are far fewer options of species to cultivate and there is only one main viable technology for producing renewable energy: anaerobic digestion to produce biogas. Both groups will be considered, but there is more research, practical experience, more fuel options from microalgae, for this it take a bigger share in most research (GBEP, 2009). Biologists have categorized, microalgae in a variety of classes, mainly distinguished by their pigmentation, lifecycle and basic cellular structure, but the most important four are diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue-green algae (Cyanophyceae), golden

algae (Chrysophyceae). A comparison between microalgae and macroalgae is shown in Fig 2.2.

Table 2.2: Distinction between macro and micro algae

| **Macro algae** | **Microalgae** |
|---|---|
| Commonly called seaweeds | Too small to see with naked eye |
| Properly called sea plants | Best grown in slurry systems |
| Big enough to tie on ropes | Some grown in open system |
| Many can be chopped down to mini size, like:<br><br>1-Green algae<br><br>2-Brown algae (Kelp)<br><br>3-Red algae | Must be enclosed for pure cultures, like:<br><br>a-Blue green algae (usually benthic)<br><br>b-Diatoms (major phytoplankton group, can be benthic)<br><br>c-Din flagellates (major phytoplankton group)<br><br>d-Others, including raphidophytes |

There are more than (30,000) to (100,000) kind of strain of algae, each kind includes many species (Nichols, J), but researches focused on microalgae for mass-production of oil, the preference toward microalgae is due to its less complex structure, fast growth rate, and high oil content. Table 2.3 summarizes the percentage lipid content by weight for some microalgae strains.

Table2.3: Lipid Content of some Microalgae (Chisti, Y.2007)

| **Microalgae** | **Lipid Content (%dry wt)** |
|---|---|
| *Botryococcus braunii* | 25-75 |
| *Chlorella sp* | 28-32 |
| *Crypthecodinum cohnii* | 20 |

| | |
|---|---|
| *Cylindrotheca sp* | 16-37 |
| *Dunaliella primolecta* | 23 |
| *Isochrysis sp* | 25-33 |
| *Monallanthus salina* | >20 |
| *Nannochloris sp* | 20-35 |
| *Nannochloropsis sp* | 31-68 |
| *Neochloris oleoabundans* | 35-54 |
| *Nitzschia sp* | 45-47 |
| *Phaeodactylum tricornutum* | 20-30 |
| *Schizochytrium sp* | 50-77 |
| *Tetraselmis sueica* | 15-23 |

**2.3 Selection of** Algal **Strain**

From the discussion above we can understand, while such a large number might indicate exceptional potential for containing lipid, carbon capture and sequestration from algae, in reality, only a small percentage will actually possess the expected potential to sequester carbon as well as yield oil that can make the process economically sustainable and profitable.

Ideally, algal strains are selected based on the following criteria

1. Ability to capture large quantities of carbon dioxide
2. Lipid content/capability to produce high levels of algal oil
3. Resistance to contamination
4. Adaptability to temperature extremes
5. Specificity to the type of industry, source of CO2 and to local water conditions in growth ponds.

Theoretically biodiesel produced from algae appears to be the only feasible solution today for replacing petro-diesel completely. No other feedstock has the oil yield high enough for it to be in a position to produce such large volumes of oil. But every algae strain do not contain same amount of oil of their dry weight. For small country like Bangladesh, there is shortage

of land available in the country be dedicated to biodiesel crop production as we also needed to cultivate crops for food. So we have to select best algae with high lipid content that will save our cultivable land.

In Bangladesh there are thousands of algae strains. All are not preferable for biodiesel production. Literature survey gave us the some potential algae strains:

1. *Oedogonium*
2. *Spirogyra*
3. *Sirogonium*
4. *Clostrium*
5. *Spirulina*
6. *Gloeocapsa*
7. *Navicola*
8. *Cholorococcum sp*
9. *Oscillatoria*
10. *Pithophora*

*Oedogonium:* Its availability is common and can be collected from nearby ponds or other still water sources. It can suit well at Bangladeshi atmosphere. Also its growth rate is also good. It contains 29-49% of lipid of its dry weight from which the biodiesel can be extracted.

Fig 2.2: Oedogonium

*Spirogyra:* It is one of the common and available algae found in Bangladesh. Its growth rate is very good in Bangladeshi atmosphere. But its lipid content is lower than the Oedogonium. It contains around 18-22% lipid of its dry weight.

*Clostrium:* Clostridium is a single cell algae found around. But its problem is to isolate the cell. Again the lipid content is low.

*Sirogonium:* This is an algae type found rarely in Bangladesh but has a high growth rate. Also its lipid content is low.

*Gloeocapsa:* It is also single cell algae. But the maintenance is tough and life time is quite short. Also another challenge is to separate it for weight measurement.

*Navicola:* It is also a single cell alga. It is not so available but found at some areas. Its Lipid content is higher around 25-40% of its dry weight.

Other strains are available but lipid content is very low. So we have selected two strains for our study *Spirulina* and *Cholorococcum sp* for their high lipid content and availability.

**2.4 Challenges**

In Bangladesh research on algae has been very limited and there are almost no documents or papers supporting which kind of algae strains have high lipid contents and should be used for oil extraction. Not having enough resources in this particular area, different institutes of the country have been communicated with. Collection of a single strain of algae for single uncontaminated production seemed very difficult. Differentiating strains through a microscope by their cellular traits needed strong expertise and experience in this particular field of Biology.

A link with a group from the Department of Botany at Rajshahi University working on production and growth rates of different algae strains at laboratory scale became useful. Several lot of cultured algae strains of different species have been brought from there for cultivation. Researchers at BCSIR working with algae also have been communicated for supply of algae strains. Apart from these two sources, algae strains have been collected from many other indigenous sources.

# CHAPTER 3
# DESIGN AND FABRICATION OF PHOTOBIOREACTORS

## 3.1 Cultivation of Algae

Like plants, algae use the sunlight for the process of photosynthesis. Photosynthesis is an important biochemical process in which plants, algae, and some bacteria convert the energy of sunlight to chemical energy. Algae capture light energy through photosynthesis and convert organic substances into simple sugars using the captured energy. There are two main methods of cultivation

1. Open Pond
2. Photobioreactors (PBR)

### Open Pond Cultivation System

Open cultivation system uses ponds or lakes with added mechanical equipment to grow microalgae. Open ponds were the first cultivation technology for mass cultivation of microalgae. In this system water levels are kept no less than 15 cm, and algae are cultured under conditions identical to their natural environment. The pond is designed in a raceway structure, as shown in Fig 3.1, in which a paddlewheel circulates and mixes the algal cells and nutrients.

The raceways are typically made from poured concrete or they are simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around the bends in order to minimize space. The system is often operated in a continuous mode, where the fresh feed (containing nutrients including nitrogen phosphorus and inorganic salts) is added in front of the paddlewheel, and algal broth is harvested behind the paddlewheel after it has circulated through the loop. Depending on the nutrients required by algal species, several sources of wastewater can be used for algal culture. For some marine-type microalgae, seawater or water with high salinity can be used.

Fig 3.1: Open pond for algae cultivation [1]

Although open ponds cost less to build and operate than closed systems using Photobioreactors, this culture system has its disadvantages. The ponds can be built on any type of land but need large land areas for considerable biomass yield. Because they are in the open air, the water levels are affected from evaporation and rainfall. Natural $CO_2$ levels in the atmosphere (0.03%-0.06%) are not enough for continuous mass growth of microalgae. Biomass productivity is also limited by contamination with unwanted algal species,

organisms that feed on algae or other poisonous particles. Only few species can be grown in normal conditions. Other types of construction use: 1) circular ponds where circulation is provided by rotating arms; 2) inclined systems where mixing is achieved through pumping and gravity flow.

**Photobioreactor (PBR)**

Photobioreactor is a closed system which provides a controlled environment and enables high productivity of algae. All growth requirements of algae are introduced into the system and controlled according to the requirements. Fig 3.2 shows a PBR system that facilitates better control of culture environment such as carbon dioxide supply, makeup water supply, optimal temperature, efficient exposure to light, culture density, pH levels, gas supply rate, mixing regime, etc. From the feeding vessel, the flow progresses to the diaphragm pump which moderates the flow of the algae into the actual tube. PBR is used to promote biological growth by controlling environmental parameters including light. The tubes are made of acrylic/glass and are designed to have light and dark intervals to enhance the growth rate. PBR should have a cleaning system that cleans the inner sides of tubes without stopping the production. After the algae have completed the flow through PBR, it passes back to the feeding vessel. As it progresses through the hoses, the oxygen sensors determine how much oxygen has built up in the plant and this oxygen is released in the feeding vessel itself. It is also at this stage that the optical cell density sensor determines the harvesting rate.

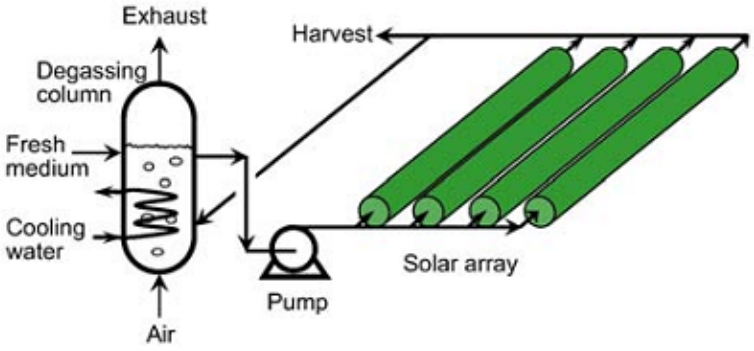


Fig 3.2: Photobioreactor system [1]

When the algae are ready for harvesting, they pass through the connected filtering system. This filter collects the algae that are ready for processing, while the remaining algae passes back to the feeding vessel. The flow continues.

Fig 3.3: Algae production in a photobioreactor system [12]

Table 3.1: Comparison between open pond and photobioreactors

| **Culture systems for microalgae** | **Closed system (PBRs)** | **Open system (Ponds)** |
|---|---|---|
| Contamination control | Easy | Difficult |
| Contamination risk | Reduced | High |
| Sterility | Available | None |
| Process control | Easy | Difficult |
| Species control | Easy | Difficult |
| Mixing | Uniform | Very poor |
| Operation regime | Batch or semi continuous | Same |
| Space required | A matter of productivity | Same as PBRs |
| Area/Volume ratio | High (20-200 m-1) | Low (5-10 m-1) |
| Algal cell density | High | Low |
| Investment | High | Low |
| Operation cost | High | Low |
| Light utilization efficiency | High | Poor |
| Temperature control | More uniform temperature | Difficult |
| Productivity | 3-5 more productive | Difficult |

| Culture systems for microalgae | Closed system (PBRs) | Open system (Ponds) |
|---|---|---|
| Water losses | Depend upon cooling design | Not specific |
| Hydrodynamic stress on algae | Low-High | Very low |
| Evaporation of growth medium | Low | High |
| Gas transfer control | High | Low |
| C02 losses | Depend on pH, alkalinity etc | Same as PBRs |
| 02 inhibition | Greater problem in PBRs | PBRs>Ponds |
| Biomass concentration | 3-5 times in PBRs | PBRs>Ponds |
| Scale up | Difficult | Difficult |

### 3.2 Photobioreactor Design Considerations

Numerous aspects influence the growth rate and lipid content of algae. The reaction driving the initial conversion of sunlight into stored energy is photosynthesis. Therefore, all of the components involved in photosynthesis contribute to growth. The factors discussed here are lighting, mixing, $CO_2$, $O_2$ removal, nutrient supply, temperature, pH and water. It is important to note that in each category the precise conditions for optimal growth depend on the strain of algae selected for cultivation.

The primary factors that affect algae growth are-

1. Lighting
2. Mixing
3. $CO_2$ Consumption
4. $O_2$ Removal
5. Temperature
6. Nutrient Supply
7. Water consumption

### Design Parameters

Although there is only a limited number of algae production plants in operation employing closed reactors, a direct evaluation is hardly possible. One problem in comparing different designs of photo-bioreactors is the use of different measures depending on the purpose of a reactor and even depending on the research discipline.

*Volume of Reactor:* Total working volume of the reactor includes liquid and gas phase; the volumes of the pure liquid are usually not given as it would be necessary for mass balancing.

*Area of Reactor*: Total surface area of the transparent part of the reactor determines the amount of light which could eventually enter the reactor; detailed analysis is necessary, to calculate how much light can really hit the surface. The surface area makes a serious contribution to reactor cost.

*Aperture Area*: The aperture (ground) area of the reactor measures the area from which light energy is collected. For multiple installations the area between two reactors has to be included on in the areal calculation to facilitate accurate scale. This can give cause for concern in cases of single but high fences which have a small footprint but nevertheless large tailed area. To distinguish clearly between the footprint of a single reactor and the area requirements of an arrangement of reactors including space between them, the term ‘‘overall areal productivity’’ (OAP) has been introduced.

*Productivity of Reactor*: The volumetric productivity measures product formation per reactor volume and time span. Lab scale experiments are often given on this volumetric basis. This is an important value for high value applications like production of pharmaceuticals and for assessment of process intensification. Volume contributes to the overall cost.

*Areal Productivity of Reactor*: The areal productivity PG is the most important parameter to assess larger photo-bioreactor plants. It allows for balancing in terms of energy efficiency between incident light as the main energy source and biomass or product formation on an areal basis and is the determining performance criterion. This is especially true for conversion of solar energy to chemical energy e.g. biodiesel produced by microalgae. Although the areal productivity is quite informative for comparison of designs, the value depends to a great extent on the irradiation during the measurement period.

*Irradiance* (at the surface): It is given as photon flux density (PFD). PPFD is the photosynthetic photon flux density. This unit accounts for the fact that photons can only be used from the photosynthetic active radiation range (PAR 400nm–700 nm). For the calculation of large areas e.g. for feasibility studies of bio-energy production, the irradiance is given as power density in [W/m2]. Specification whether it is for PAR or the whole solar spectrum including UV and IR should be given. While for macroscopic considerations the radiation is measured in normal direction to earth, for kinetic studies it is measured in normal direction to reactor surface. The index 0 stands for the value at the surface.

*Photoconversion Efficiency*: Photoconversion efficiency measures the fraction of the solar energy that is converted to chemical energy in a photo-bioprocess. The maximum theoretical value has been estimated to be 9% [74] for full sunlight. For the calculation of PCE the energy content of the biomass has to be measured. It can range from 20MJ/kg to 30MJ/kg for oil rich algae. According to thermodynamics oil rich algae could show lower areal biomass productivities than other algae, cited from, but this can nevertheless mean a high PCE, which is at the end the decisive value. PE is used for the photosynthetic efficiency.

**Lighting**

An optimal reactor enhances light intensity/ penetration, as well as the wavelength of light and the frequency of cellular exposure to light. The level of light intensity is critical because at a certain level algae experience light saturation and dissipate the excess energy as heat.

1. Light saturation can be mitigated by the spatial dilution of light, which is the distribution of solar radiation on a greater photosynthetic surface area. Spatial dilution of light also reduces mutual shading of cells in the culture, which results in higher growth rates and lower content of accessory pigments. Thus, a design principle for photobioreactor design is to maximize the surface area to volume ratio.
2. Beyond the surface area and volume, the unique geometry of a reactor influences the light distribution. In a tubular reactor, for example, the light gradient is primarily determined by the diameter of the tube and the biomass density in the medium. The biomass density affects both the light intensity and the light penetration. Optimal cell density is specific to each strain and needs to be maintained in order for light intensity and light penetration to remain at optimal levels. There is an important operating parameter known as the critical cell density, which is the maximum cell concentration without mutual shading in algal cultures. During designing this has to be taken under strong consideration.
3. With respect to light the more the better is not true for microalgae. Most of them are adapted to low light intensities representing only a fraction of the full day light even in mid- latitude regions. After a linear increase of growth rate with increasing light intensity (Blackman kinetics) saturation is approached in the example given here at about 100mE/(m2 s). This is only 10% of the midday sunlight intensity in a European summer. Light in excess is wasted as fluorescence and finally heat.
4. The answer of process engineering is to design vertically mounted photo-bioreactors with a large surface area. These could be flat panels or alveolar panels. The sunlight, falling on a given ground area, is spread over a larger reactor surface area. As a result, the microalgae are irradiated with only a small fraction of the whole intensity of the incident radiation and grow in the non-limited region of the light saturation curve. That means that the surface to ground area ratio AR/AG should be in the range of 10 or higher. The optimum value depends on the strain and the region, where the reactor is in operation. Nevertheless, this is on the cost of requiring more reactor construction material and more volume. To allow the light to reach the transparent surfaces and its dilution in a horizontal direction especially at day times with high radiation, the fences are usually mounted in north/south direction.

   Light and dark cycles strongly influence the growth of algae. In both open ponds and outdoor closed reactors, natural light is subject to changes in time of day, weather, season, and geography. Unfortunately, all reactors using natural light are subject to the absence of light during nighttime. According to Chisti, biomass losses might reach as high as 25% during the night, depending on the light intensity during the day, the temperature during the day, and the temperature at night.
5. Light attenuation and light path length reduction The spatial distribution of the light intensity inside the reactor is, apart from the geometry, mainly influenced by light attenuation caused by mutual shading of the cells via adsorption by the pigments or via scattering by the cells. Some mechanistic formulae for calculating light gradients in liquid particle systems have been published, however, for a small and flat volume element an exponential development depending on biomass concentration can be assumed. As long as the light path length exceeds the plate thickness, more or less

exponential growth can be observed. After the biomass concentration reaches higher values, there will be only linear growth. That does not mean that the process is automatically less efficient. As long as proper mixing and light distribution is achieved, the linear increase in biomass is proportional to incident light.

6. However, a fraction of the total volume is dark. It therefore does not contribute to productivity but to energy cost. In addition, high concentrations can be reached faster with a lower dark volume fraction. Assuming that a given fraction of the incident light is converted to cell mass then the produced cell mass is diluted in a smaller volume in case of thin film reactor leading faster to high cell densities. Of course, medium composition has to be adjusted to these high concentrations. High cell densities have big advantages in saving energy for mixing and during down- stream processing. To achieve high cell densities the reactor thickness should be as small as possible. Short dark/light cycles are a crucial point to obtain such high cell densities.
7. The aspects given above can be summarized by the requirements for a high surface/volume ratio (SVR). Most current reactor designs follow this principle. Installing many plates quite close together increases both SVR and the areal water coverage, defined here as the total fluid volume per ground area. To bring more surface area to a given ground area, it should be carefully counterbalanced with reducing volume per ground area on the basis of the kinetics to provide not more light distribution than necessary and to save volume for high biomass concentration and reduced energy.
8. Light regime strongly influences photoacclimation, which describes the physiological responses of cells to rapid changes in light intensity. An example of a common response to light intensity alteration is a change in pigment content. Also a sudden surge of light can be fatal for many species of algae. Thus, it is important to consider light regime and photoacclimation when designing a reactor, particularly in order to maximize the photosynthetic efficiency.

**Mixing**

The level of mixing in a reactor strongly contributes to the growth of algae. In fact when environmental conditions do not limit growth rates', mixing is the most influential factor contributing to algae growth rates.

1. Local turbulences carry the cells more or less randomly through well-illuminated volume elements near the glass wall and poorly illuminated reactor zones remote from light incidence, so each individual cell is exposed to statistical dark/light cycles. These cycles have a strong effect on algae growth. Several authors observed dependency of growth from mixing time constants in lab scale experiments. So mixing affects growth in two primary ways. Mixing improves productivity by increasing the frequency of cell exposure to light and dark volumes of the reactor and by increasing mass transfer between the nutrients and cells. Mixing attempts to distribute radiation evenly to all cells in the culture and reduce diffusion barriers around the cells. Mixing and lighting are closely related, as mixing is often

responsible for inducing the light and dark cycles beneficial to algae growth. But mixing offers little benefit if lighting is poor.

2. Mixing coefficient depends on the agitation rate, type of sparger, antifoam agents and temperature. The use of fine spargers could result in the formation of large bubbles, which leads to poor mass transfer because of the reduced contact area between liquid and gas. Also the large air bubbles generate turbulence that reduce wall growth. The size of the bubbles and the gas bubble velocity are dependent on the liquid flow rate and has to be controlled properly. Here, mechanical energy has to be supplied in as directed a manner as possible. For proper PBR design computational fluid dynamics should be employed. To achieve mixing with a minimum of auxiliary energy it would be favourable to limit turbulences to one specific frequency may be of several Hz. Several means have been proposed to achieve such highly defined flow patterns.
3. The level of mixing has to be optimized carefully because high levels of mixing will result in cell death from shear. It is found that bubble formation is the main cause for cell death in gas-sparged reactors, and gas entrance velocity could be used as a measure for estimating cell damage in these reactors.
4. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand, aerating (bags, tanks), or using paddle wheels and jet pumps (ponds).

**$CO_2$ Consumption**

In addition to light and water, carbon dioxide is necessary for photosynthesis to occur. Beside light transfer the most important task of photo- bioreactors is to feed the algal cells with carbon dioxide for photosynthesis. The CO2-demand of the culture can be calculated on a stoichiometric basis by the carbon content of the biomass. The carbon fraction varies from 0.45 for algae with high carbohydrate content up to 0.8 for oil rich cells. Accordingly, the stoichiometric CO2 requirement of the algae lies at 1.85g CO2/g biomass or higher. However, an excess of CO2 can also be detrimental to photosynthesis and cell growth.

1. CO2 can be supplied via diffusion through a gas permeable membrane in order to provide sufficient CO2 to the entire culture. CO2 concentrations from 1% to 5% (by volume) often lead to maximum growth. Despite this, laboratories routinely aerate algal cultures with 5-15% CO2, or even pure CO2.
2. The cost of CO2 has to be considered when evaluating the economics of biofuel production from microalgae. Reviews suggest that because supplying CO2 continuously is expensive, it may be necessary to supply it discontinuously.

**$O_2$ Removal**

A high presence of oxygen around algae cells is undesirable. The combination of intense sunlight and high oxygen concentration results in photooxidative damage to algal cells.

1. As a general guideline, oxygen concentrations should be maintained below 400% of air saturation value. Because oxygen does not build up substantially in open ponds, this is one aspect in which open ponds performs better than closed reactors.
2. The time taken by the fluid to travel the length of the degasser must at least equal the time required by the oxygen bubbles to rise out. If practical, the capture and sale of

this oxygen stripped from the reactors may be an opportunity to reduce the cost of biofuel production.

**Nutrient Supply**

In order to grow, algae require more than the reactants in the photosynthesis reaction. Two major nutrients are nitrogen and phosphorus, which both play a role in controlling growth rates and lipid production. Other essential nutrients are carbon, hydrogen, oxygen, sulfur, calcium, mag- nesium, sodium, potassium, and chlorine. Nutrients needed in minute quantities include iron, boron, manganese, copper, mo- lybdenum, vanadium, cobalt, nickel, silicon, and selenium.

1. The experimenters therefore asserted that balancing the nutrients based on the elemental composition of the biomass should be the basis for effective medium design. However, Chisti noted that some nutrients need to be present in excess. For example, phosphorus must be supplied in excess because the phosphates react with metal ions.
2. Applying stress in the form of limited nutrients (especially N or P) can increase lipid percentages within the biomass. However, this stress application also curtails the growth rate and thus may lower overall lipid production.

**Temperature**

Temperatures experienced by algae grown outdoors can vary as much as the extreme outdoor temperatures characteristic to the geographic region of cultivation. Although algae may be able to grow at a variety of temperatures, optimal growth is limited to a narrow range specific to each strain.

1. Research has found that the optimum temperature range is around 20-26 °C. Seasonal and even daily fluctuations in temperature can interfere with algae production. Temperatures can reach as high as 30°C higher than ambient temperature in a closed photo- bioreactor without temperature control equipment. In addition, a lower temperature appears to reduce the loss of biomass due to respiration during the night
2. In Bangladesh temperature control is the most serious issue as in summer the temperature within the photobioreactor reaches around 50°C which is not sustainable for growth. So evaporate cooling or shading techniques need to be employed frequently to inhibit temperatures of that magnitude.

**pH**

Each strain of algae also has a narrow optimal range of pH. The pH of the medium is linked to the concentration of CO2. Experts have mentioned that pH increases steadily in the medium as CO2 is consumed during flow downstream in a reactor. The pH affects the liquid chemistry of polar compounds and the availability of nutrients such as iron, organic acids, and even CO2. Because pH is so influential, commercial pH controllers should be used in reactors to optimize growth.

**Water Consumption**

A noteworthy benefit of producing fuel from many strains of algae, as opposed to conventional crops, is that the cultivation does not have to require freshwater.

1. Algae can grow in a much wider range of water sources than other terrestrial crops. Studies have shown that algae can grow in fresh drinking water, saline or brackish water, and even wastewater effluent.
2. A major disadvantage of open ponds is the loss of water to the atmosphere by evaporation. When water evaporates from the reactor, the concentrations of all species present increases, and this can be a particular problem.

**3.3 Commonly Employed PBR Designs**

**A. Flat plate reactor:** The flat plate reactors are surely the most robust design. Roughly speaking, two sheets have to be glued together to make a flat plate reactor with any desired light path length d in the range from a few mm up to 70mm. Mixing and CO2-supply is accomplished by sparging with CO2-enriched air. For the pilot scale example reactor (0.07m wide, 1.5m height, 2.5m length) the authors report air flow rates of 0.25v/v/min leading to a mixing time of the medium of 150s. Others reported even much higher aeration rates up to 2.0 v/v/min with positive effects. Power supply for bubbling was in the range of 50W/m3. Even in quite compact arrangements of several plates close to each other this value is not too high for an economically feasible production of chemical energy by microalgae. Agitation only by bubbles seems to be the gentlest way with respect to shear stress for the algae.

**B. Annular reactor:** Bubble columns are frequently used especially in larger lab scale for indoor experiments. To work with sufficient volume, the diameters of 20 cm and more are higher comparing to tubular reactors. This leads to considerable high dark fraction in the middle of the cylinder. This part does not contribute to productivity nor has any detrimental effects on growth. To leave this part out of the internal reactor space the so- called annular column has been developed. It consists of two 2m-high acrylic cylinders of 40 and 50 cm in diameter placed one inside the other so as to form an annular chamber. The other way round, this can be seen as a wrapped flat plate reactor. It may be that the inner surface does not contribute too much to overall radiation, but for indoor applications or dark periods additional lamps could be fitted. Consequently, typical aeration rates are with 0.25v/v/min in a similar range than those for flat plates.

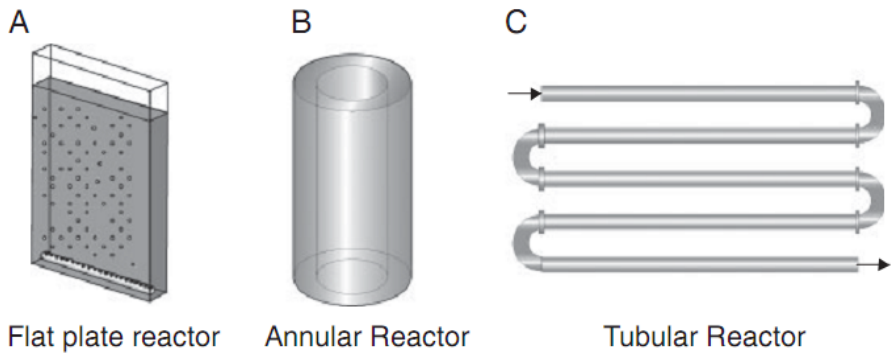


Fig 3.4: Different reactor types

C. **Tubular reactor:** Tubular reactors consist of transparent tubing arranged in parallel lines coupled by manifolds, the so-called solar collector. The single tubes can be straight, they can follow a course either flat on the ground or ordered in panels or coils (so called helical reactor). The tubes have diameters of 10 to maximum 60 mm, and lengths of up to several hundred meters. The employment of tubes leads to a quite high surface to volume ratio SVR over 100 m-1, which is one of the main advantages of this design. Furthermore, the so called ‘‘lens’’ or ‘‘focussing effect’’ has the added advantage of homogenous light distribution. Incident light is diluted along the circumference and is in radial direction focussed onto the axis of the tube. In this way, exponential decrease of light by mutual shading of the cells is to some extent compensated by a geometrically enforced hyperbolic increase of radiation intensity. Of course the arrangement of the tubes has to be calculated to achieve the most homogeneous incident light conditions. Pumping of the medium with linear liquid velocities of 20cm/s to 50cm/s is either done by airlift circulators or by centrifugal pumps. Aeration and degassing is also achieved in the airlift part, while a separate gas exchange station has to be supplied along with the pumps. A velocity of more than 1m/s will cause micro eddies of less than 50mm diameters which potentially can damage the cells. The relatively high velocity is necessary to achieve turbulent conditions leading to acceptable light/dark cycles for helical reactors for example high values for volumetric productivities and efficiencies have been reported. However, the high energy consumption of more than 2000W/m3 required is surely one of the major drawbacks. For scale-up of a single tube only the diameter and the length can be considered. According to aspects increase of the diameter is possible only in narrow limits, usually not more than 40mm. Tubes with much bigger diameter (440cm) have been occasionally tried out but showed only low biomass concentrations and low areal and volumetric productivities, probably because the diameter is much larger than the light path length.

### 3.4 Current Photobioreactor Designs

In present design of PBR, the primary focus was to design and fabricate a working design that would be cost effective. Commercial photobioreactors are very costly and unaffordable for our country perspective.. So, avoiding fully closed system, a semi controlled system was

designed. Present designs are mostly flat plate and annulur type. Tubular system can be considered moderate to large system and was not within the scope of this project. The lower part of PBR is closed but upper cover is open for breathing. Open upper part is used for supply of carbon dioxide, air, and other nutrients.

In our study photobioreactor [Fig 3.5] is made which is controlled but semi closed. Because with the time some algae will grow attached to the internal wall of the photobioreactor tube, thus will prevent light penetration into the tube and reduce bioreactor productivity. So we will need to clean the internal wall of the cylinder. Also it will provide the escaping path for water and aeration air. It consists of (i) transparent bioreactor and (ii) steel structure

Fig 3.5: Photobioreactor

**Bioreactors**

Bioreactors are the main medium where the algae will be cultivated. They should be transparent and light in weight. Transparent acrylic or glass material is used to make the bioreactors. Parameters to considered while selecting the material are:

*Transparency*: In colorless form acrylic plastic is as transparent as the fine glass. Its total white light transmittance is 92%, the highest transmittance physically possible of any material.

*Breakage Resistance* - Acrylic sheet has from 6 to 17 times greater impact resistance than ordinary glass in thicknesses of 0 .125" to 0.250". When subjected to blows beyond its resistance, acrylic sheet reduces the hazard of injury because it breaks into large relatively dull edged pieces which disperse at low velocity, due to the light weight of the material.

*Weather Resistance* - The many years of actual outdoor exposure of acrylics in a wide variety of applications, proving its weather resistance, cannot be matched by any other transparent plastic material. However, over time the translucency decreases for acrylic material while comparing with that of glass material. Glass surface can be polished to get its transparency back.

*Chemical Resistance* - Acrylic plastic or glass has excellent resistance to most chemicals, including solutions of inorganic alkalis and acids such as ammonia and sulfuric acid, and aliphatic hydrocarbons. H

*Light Weight* - Acrylic sheet is less than half as heavy as glass: it is 43% as heavy as aluminum and 70% as heavy as magnesium.

*Ease of Fabrication* – Acrylic can be drilled, and machined like wood or soft metals. When heated to a pliable state, Plexiglas can be formed to almost any shape. . By glass the round shape cannot be made.

*Leakage Proof* – Tubing made of acrylic material cannot be fabricated leakage proof by local fabricator. In this case glass material was superior.

This last criterion determines the selection of material as glass. If we can solve this leakage problem we will surely move on towards acrylic material for other qualities. Our first design with acrylic round cylindrical annular type bioreactors failed only because they cannot store the water for a long period.

**Shape and Size of the Bioreactors**

Initial choice was round shaped tall cylindrical bioreactors for their high surface to volume ratio and low areal cross sectional area. However, this shape can be made locally with acrylic only and the trial failed as mentioned above. So we moved to rectangular shape with glass material. Size used for acrylic glass cylinder was: Length-100cm, Diameter-15.24cm, Capacity of the cylinder=18.2 Liters, Design capacity =10Liters. Size of each cylinder is considered as 10 Liter. Top part of the cylinder is to be kept empty for proper aeration and ventilation.

For the second trial we moved to glass material and rectangular shaped bioreactors. A size of 4’ X 4’ X 3” was selected for large volume. Low depth of the reactor allow light to penetrate inner part of the reactor. It also has high surface to volume ratio. However, stability of the reactor was not so good. It did not have enough strength to hold 100 litre of water. After it was broken down we designed another shape 3 feet tall and 1 feet wide with 3 inch depth. It has good strength and stability.

As said earlier another important operating parameter is known as the critical cell density. For this we had to take optimal cell density under strong consideration while deciding the diameter of the cylinders.

As said earlier another important operating parameter is known as the critical cell density. For this we had to take optimal cell density under strong consideration while deciding the diameter of the cylinders. At the middle lower of the cylinders there is a stopper valve to collect sample from the cylinder to check the pH and nutrient consumption.

**Steel Structure**

Now as discussed earlier lighting is primary factor for the growth of algae. So our goal was to an optimal reactor considering light intensity/ penetration, as well as the wavelength of light and the frequency of cellular exposure to light. For this consideration a lot of study and

calculation was done. At the end, we used a hinge joint at our structure for changing the cylinders into inclined positions. So we are able to keep our cylinder in three different positions and study the effect of sunlight on algae growth at different positions. At the same time we were concerned about the capacity of the system. For this capacity we needed a strong structure which can support around 80Kg weight. So we selected mild steel as our structure material.

Steel structure is the main load barrier of our photobioreactor setup. Also by the setup we can control the cylinder inclination with horizontal plane. By changing the angle of cylinder the sunlight is controlled. At morning the angle will be high like 600 and at noon relatively low like 300. Also the angle can be changed to 450 if necessary. For changing the angle there is a hinge joint between table and the cylinder holder. And a steel support bar for keeping the cylinder at a fixed position. Every cylinder holder is individually supported but hinged with the main structure support.

We preferred the three angles ($30^0$, $45^0$ and $60^0$) because if we use angle lower than 300 the water from the medium cylinder will fall down from cylinder.

Also if we used angle large then $60^0$ the cylinder need to remain almost vertical and the sunlight will be minimum at noon. So for these reasons the cylinder holder's angle is made to be stand at $30^0$, $45^0$ and $60^0$.

Fig 3.6: Cylinders at three different angles

From the change of angle we will be able to study the effect of sunlight at algae growth for fixed carbon dioxide supply and nutrient supply.

**pH Meter**

In the study, control of pH is an important necessary. Most algae strains grow well at light basic condition (pH range 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. So we need to measure the pH for better growth of algae. By measuring the pH we can decide whether pH needs to be increased or decreased by controlling the supply of Carbon Dioxide (CO2). In the case of high-density algal culture, the addition of Carbon Dioxide (CO2) allows to correct for increased pH, which may reach limiting values of up to pH 9 during algal growth. A proper performed pH meter calibration is essential to get an accurate and repeatable measurement.

A calibration procedure will be highly dependent on the manufacturer of the pH meter, so for best results, pH meter should be calibrated. We used a pH meter for our study which was calibrated.

Nitrate Test Kit

Since $KNO_3$ would be used as one of the vital nutrients for culturing algae, a nitrate test kit has been purchased with a view to monitoring the consumption rate of nitrogen in terms of nitrate at different phases of algal growth. It would help to determine the optimum concentrations of $KNO_3$ that need to be maintained at different stages of the mass culture.

**Aeration System**

Aeration 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 and to improve gas exchange between the culture medium and the air. So we can say that the purpose of mixing is served through aeration. Aeration of cultures serves to keep algae in suspension, to supply the $CO_2$ needed for plant growth and pH control, and to strip O2 from the culture media, preventing super saturation.

In our study aeration is done with the help of supplying air and Carbon Dioxide at the bottom of the cylinder. Buoyancy forces cause the bubble to move away from the pipe outlet. Bubble growth continues through a narrowing neck connecting the bubble to the pipe. When this neck closes and the bubble detaches from the pipe outlet, water rushes in to the region of the bubble neck. By this there is mixing of nutrient, control of pH, keeping algae in suspension and a better scope for Carbon Dioxide consumption as the Carbon Dioxide goes through from bottom to top. Still we have to work on our bubble size as it is quite large and causes shear. For us mixing is very beneficial as our lighting is very rich.

Our aeration system consist three components-

1. Aeration compressor

2. Tube

3. T-joints and flow control valves

**Aeration compressor**

A small compressor is used in our study to supply air into the cylinder. Air is taken from the atmosphere and supplied to the medium. By this the medium water consisting algae is always at turbulence.

Supply capacity 2 L/min

Tub**ing and valves**

Tubes were used to carry the air from the compressor to bottom of the medium cylinder. Diameter of the pipe is about 6mm.

Tubes were connected with T-joints and flow control valves were used to control the rate of aeration.

**$CO_2$ Supply**

In our study we supplied CO2 from a commercial cylinder through a pipe via diffusion. As it is a very costly process our supply was discontinuous. We designed a relay circuit which automatically starts and cut off supply. This happens every 12 hour. Also to ensure that the supply is at desired level, pH is checked regularly.

**Temperature**

This is the biggest challenge we had to face during our study. For proper lighting we installed our total setup on the roof. Though we had abundant sunlight but the high temperature of the photobioreactor was always a worry during the summer. Our first culture couldn't survive this heat. The strain we culture can sustain at around 35°C and above that growth drastically declines. So for this we are planning to make a shade and install a proper cooling system. At the present we are depending on our aeration system to keep the reactor cool to some extent.

**Nutrients**

Nutrients are major determining factor in how fast algae grows and what composition it develops. As we know algae require dissolved Nutrients, similar to terrestrial flora. Nitrogen and phosphates are two notable nutrients, as well as some sodium and potassium. The Nutrients that would be used are Nitrogen ($KNO_3$), Phosphorous ($K_3PO_4$), Potassium ($KNO_3$).

**Water Consumption**

The algae we cultured are not restricted to any specific type of water. So we used normal tap water for the culture which caused no problems.

**Safety Box**

A wooden safety box was built to keep all the parameter controlling and measuring instruments safe from different environmental aspects such as rain, storms etc.

**3.4 Challenges**

1. **Design constraints:** We studied and calculated the possible designs and options for photo bioreactors. A lot of the study was related to the dimension and flexibility of the reactor. We wanted to build something that can be rotated at different angles for the different incidence of sunlight and which would have tubes with high durability and resistance to factors like weather, brittleness etc. For this reason we chose acrylic as our cylinder material and made a steel structure which could rotate at 3 different angles. The later one was implemented successfully. But the tough part was handling our acrylic cylinders. First of all they were very rare in the local market and costly. We have already discussed our reason for choosing this particular material. The extra added advantage that we wanted something with a tubular or circular shape and that we achieved.

   Our total structure was located on the roof of the EME building and the reason behind this was to utilize the maximum amount of sunlight for algae production. But with the sunlight we got additional heat which caused thermal expansion of our acrylic cylinders. So later on the cylinders got deformed and all our efforts went vain.

   A lot of effort was put in fixing the cylinders as they are like the backbone for our algae production and the total structure circulated around them. But again we had to

face tremendous difficulties due to the limitations of our local market. We tried different methods to seal the bottom part to prevent leakage like use clamps and polybags, special glues but nothing could prevent the large amount of pressure at the bottom.

Fig 3.8: Damaged acrylic cylinders

2. **Temperature Fluctuation of the environment:** This was another big challenge and we discovered this during the production process. After our initial setup was completed we started our first batch of algae production in the photo bioreactor. A selected strain of algae was used for this and with time we were observing its growth. But as discussed earlier different temperatures have a different affect on algae strain. As our reactor was located in roof and had no shades during the summer season it went through higher temperatures than we desired. The medium in which the algae was growing rose to temperatures around 40-45◦C which was above the maximum temperature our algae could withstand. As a result the growth started to deteriorate even there was ample sunlight. This was an unexpected turn for our course of actions and later we had to take different measures for mitigation.

3. **CO2 Supply:** It was a necessary element of the production process but the start-up was quite difficult. The biggest barrier regarding this was its cost, difficulty of purchasing and transportation. In our country CO2 is sold in big cylinders which are quite heavy. Also to complete the purchase we had to go through some serious loops with Linde Bangladesh as our purchase was not commercial and significant enough. Still we did manage but the cost of CO2 was really high and we understood that there is no luxury to waste any of it. Transportation was a big difficulty due to the cylinders size and weight. It took 3 of us to even roll the cylinder to a suitable and safe position.

**Measures taken**

1. We understood that our targeted acrylic cylinders were not the suitable due to its limitations and we moved on to flat bed reactors made of glass. Although glass bioreactors were brittle, they were available in the market and did not have the problems of deforming due to high temperature. Initially a large rectangular reactor of 50L capacity was manufactured that experienced cracks at joints and thus leakage because of high water pressure inside. Finally five smaller flat bed reactors were fabricated - each having a capacity of 10L.

2. As we cannot move our reactor or control the natural environment we had to take serious remedies against the environmental temperature. First of all we placed a shade around the reactor which protected it from direct sunlight which was the primary reason for increasing the temperature to such extents. Though we were blocking the main growth factor of algae, still the amount available was good enough. Also we started making continuous use of our aeration systems which were able to cool the cylinder mediums to some extent.
   But as the process was going on we thought about fabricating another photo reactor and restricting it to the indoor atmosphere. We would use different LED lights and maintain all other parameters as before. But the biggest advantage we will have here is that we would not have to face any difficulty regarding high temperature and would be able to control it time to time.
3. After purchasing CO2 our biggest challenge was to make full use of the resource without wasting any. So to make the supply a controlled one we used a relay circuit through which we could indicate how many hours we want the flow of CO2 to continue. This helped to make best use of the costly and limited resource.

Fig 3.9: Relay circuit and CO2 cylinder

**Microscope and Spectrophotometer**

A biological microscope would be used for identifying algal strains and counting cells during culture and a dual beam UV/Vis spetrophotometer would be used for measuring the optical density of the culture media. Then by relating the cell concentration to the optical density a calibration curve would be generated that would ultimately help determine the growth rate of algae at any phase of culture.

# CHAPTER 4
# HARVESTING AND OIL EXTRACTION

## 4.1 Harvesting

Algae harvesting consists of biomass recovery from the culture medium that may contribute 20-30% of the total production cost. Now harvesting includes the removal of heavy water content and extraction of oil from algal biomass. There is no such single method that can be applied for harvesting and removing their water content. In order to remove large quantities of water and process large scale of algal biomass volumes, a suitable harvesting method may involve one or more steps and be achieved in several physical, chemical or biological ways, in order to perform the desired solid-liquid separation.

In microalgae aquaculture, the conventional processes used to harvest include sedimentation, centrifugation, filtration, ultrasonic separation, ultra-filtration, flocculation, sometimes with a combination of flocculation-floatation. Flocculation is applied to create congregation of microalgae mass so that it can easily be removed from water. The technique may be biological flocculation where, microalgae cells start accumulating themselves, forming flocks due to the presence of non algal microbial entity or it may be chemical flocculation in which certain chemicals such as aluminum sulfate, chatoyant, ferric chloride and ferric sulfate are used to promote the formation of flocks. Both types of flocculation methods are usually followed by sedimentation, filtration or centrifugation. The sedimentation of microalgae biomass though, an effective technique but not suitable in oil production as it is space and time consuming thereby severely affect the overall cost of the procedure. Besides, centrifugation is efficiently used to recover microalgae in large volumes but here also the use of electrically or mechanically driven centrifuge makes this procedure too costly. Filtration is carried out under pressure or vacuum if algae sizes do not approach bacteria sizes. Micro-strainers (typically 25 to 50 m openings) can be used for species like Anabaena or Spirulina, which are filamentous in shape. If flocculation is performed before, higher filtration efficiency will be reached. On the whole, selection of harvesting method depends upon the type of algal species, volume and size of pond.

On the other, filter presses operating under pressure or vacuum can be used to recover large quantities of biomass, but for some applications filtration can be relatively slow which may be quite unsatisfactory. Also this process is better suited for large microalgae but cannot recover smaller dimensions. Alternatively membrane microfiltration and ultra filtration are other possible alternatives for recovering algal biomass, which are more suitable for fragile cells and small scale production processes. But these processes are more expensive for the need of membrane replacement and pumping.

Now experts have suggested the one main criterion for selecting a proper harvesting procedure is the desired product quality. For low value products one procedure has to be followed and for high value products a completely different procedure has to be followed. Also another basic criterion for selecting the harvesting procedure is its potential to adjust the density or the acceptable level of moisture in the resulting concentrate right to the optimum subsequent process.

After separation from the culture medium algal biomass (5-15% dry weight) must be quickly processed unless it will spoil only in a few hours in a hot climate.

### 4.2 Extraction of Oil

This report describes the three key stages of our project to develop a viable extraction process. First, we needed to study various extraction processes to determine which process held the most promise for future development. Second, once it was discovered that a dry extraction process using hexane as a chemical medium would worth pursuing, we then needed to begin work on a system for drying the algae. Finally, we needed to design an efficient system for processing the algae to release its oils.

Experimentation: The difficulties of microalgae oil extraction

Extensive study and research was conducted to determine the best extraction method over the two methods of extraction. The experiments themselves consisted of two types.

1. Wet extract processes that focus on disrupting the algae cells in solution.
2. Dewatering methods which remove the algae from aqueous water solution and then mechanically or chemically disrupt the cells.

The two Chlorella spp. that were used grows in solution at 99% water by mass, which makes a wet extraction process extremely advantageous; however, achieving results proved difficult.

**Wet Extraction Methods**

***A. Freezing Method***

**Theory**

It is known ice is 8% greater in volume than liquid water. The desired effect is that the expansion of the water inside the cell as well as the water around the cell will cause the cell walls to rupture from the inside out or be disrupted by the compressive forces.

**Procedure**

1. Two algae solutions of high and low concentration are dispensed into two 10 ml beakers and placed within a freezer until the solutions became frozen.
2. The solutions of algae are then thawed by placing the beakers into warm water.
3. A sample of the lower concentration of algae is collected and placed under a microscope for inspection.
4. The remainder of the less concentrated solution as well as the high concentrated solution is placed back into the freezer for a second session.
5. The two solutions are then thawed and samples of each are taken and inspected under a microscope.
6. The remainders of the two solutions are then placed back into the freezer for a third session.
7. Samples of the two solutions are thawed for a third time and were then inspected under a microscope.

**Results**

After inspecting the two samples under the three freeze cycles, it appears as though the algae cells began to cluster together. However, when the samples were compared to the control sample (the algae sample that did not undergo any freezing) it seems evident that the cell walls are still intact without experiencing rupture.

***B. Homogenization Method***

**Theory**

Homogenization is the process of reducing the size of particles in a mixture so that the media is uniform throughout. The process is usually done by expelling mixtures through small valves at high pressure.

**Procedure**

Two tissue grinders are used. The grinders are comprised of glass pestles that fit tightly inside glass cylinders. The spacing between the pestles and cylinders are tightly toleranced. Both grinders are designed to homogenize mammalian cells: one to break up the cells and one to break up nuclei. Because the homogenizers are designed for use with mammalian cells, which, unlike algal cells, do not have cell walls, the effectiveness was uncertain.

**Results**

Using two different concentrations of decanted aqueous solution of algae, referred to as slurry, in the two separate tissue grinders, what appears to be homogenized samples of algae are produced. After examination under microscope, the non- homogenized algae cells look the same as the homogenized algae cells. It is clear that the mammalian cell homogenizers are ineffective in breaking the cell walls, and no oil was released. During the grinding process, it was noticeable that small flakes of algae are escaping past the grinding pestle in the glass stator. The problem could be that the tissue grinders used are not of fine enough tolerancing to destroy the algae cells. Homogenizers having nano-tolerancing are a potentially viable source of extracting oil on a lab scale; however, they are extremely difficult to reproduce on an industrial scale.

***C. Ultrasonic Technique***

**Theory**

Ultrasonic waves could be used to induce cavitation bubbles adjacent to the algae cell wall. The cavitation bubbles should create a pressure gradient great enough that the cell wall will collapse and release its' contained oil.

**Procedure**

Algae are dispensed into six separate beakers, each consisting of 40 ml of algae. Each of the six tests vary within time and output from a Branson Sonifier 450, while keeping the duty cycle at a constant setting.

Test 1: Algae are kept in the sonifier for 1 minute with an output of 1.

Test 2: Algae are kept in the sonifier for 1 minute with an output of 5.

Test 3: Algae are kept in the sonifier for 1 minute with an output of 10.

Test 4: Algae are kept in the sonifier for 5 minutes with an output of 1.

Test 5: Algae are kept in the sonifier for 5 minutes with an output of 5.

Test 6: Algae are kept in the sonifier for 5 minutes with an output of 10.

**Results**

Using a 100X power microscope, the sonified algae specimens are observed in conjunction with a control sample.

**Dry Extraction**

**Hexane Extraction**

The mentioned experiments all required a wet extraction method; however, none of them yield the desired results, release of oil from the algae cells. As a result, one final method was studied, Hexane extraction. This method unlike the other requires dry algae flakes. Therefore, a drying system would have to be created in conjunction with a hexane extraction system.

**Theory**

Oil that is present inside of the single cell algae is trapped by the cell wall and plasma membrane, which inhibits its ability to easily be exported from the cell. When the algae cell is dried, the plasma membrane is degenerated and weakens the cells ability to retain the oil. When the hexane, an organic solvent, is introduced to the dry algae sample, the cell wall is penetrated by the hexane and the oil within the cell is dissolved. When the hexane is removed from the algae sample, the oil dissolved in the hexane is transported through the cell wall and effectively removed from the Algae cell. The collection of the oil is done by evaporating the hexane off, which will leave the algae oil behind.

**Procedure**

The algae used in the experiment needs to be dried under a heat lamp and crushed into a fine powder. The algae powder has been dispensed into a paper container and enclosed to withstand any solid algae discharge. The container has to be arranged within an extraction chamber and successfully prepared for hexane extraction.

The extraction process follows these systematic steps.

1. Hexane is heated within the miscilla tank, creating vapor rising to the condenser.
2. The hexane then condenses and is released into the extraction chamber with the algae.
3. The hexane begins to break down the cellular wall, releasing lipids into the extraction chamber
4. The hexane/ lipid mixture then reaches a critical height level within the extraction chamber. This initializes the siphoning process.
5. Once siphoned back into the miscilla tank, the process starts over, turning the hexane into vapor under specified temperature and pressure while retaining the algae oil within the miscilla tank.
6. Steps 1-5 run for roughly 2.5 hrs, until the cellular wall has been completely broken down.
7. The hexane/lipid mixture is then heated once more, converting the liquid hexane into vapor.

8. The hexane vapor is run through a condenser and released into the hexane chamber.
9. Steps 7 and 8 run for a subsequent amount of time until all hexane is released from the chamber leaving only algae oil

**Results**

The experiment above has been run at labs using 10g of oven dried algae, which produced 1.4g of oil, a yielding of 14% of the overall algal mass.

### 4.3 Present Method of Extraction

The extraction process was planned by a combination of two methods

i. Mechanical Method- Drying and disruption of algae

ii. Chemical Method

In our present study algal sample would be dried so that it retains its oil content. The algal powder would be pressed in manual Hydraulic Press and Ball Mill. These would help in cell disruption by applying pressure on the wall of algae. It has been observed that dried matter does not show any significant results in mechanical extraction. Therefore, we would have to apply moist sample to the hydraulic press.

After the cell disruption using hydraulic press and ball mill, oil extraction with different solvents like n- hexane, benzene, and petroleum ether would be attempted. The algal sample would be dissolved in respective solvents and allowed to stir for 5- 6 hours on magnetic stirrer. The residual pulp would be removed from the solvent through filtration. Then it would be allowed to separate in a separating funnel. Continuous shaking of sample would result in the separation of two layers on the top of separating funnel. After that the solvent would be allowed to evaporate.

Besides the use of solvents a known amount of dried algal consortium powder would be kept for steam distillation. The temperature would be maintained at 500 C and the whole system would run for 8-10 hours with continuous running water. The ring of oil gathered on the top of condensed material in the apparatus would be eluted out. The algal cake left after extraction would be kept for further use as a bio fertilizer for soil reclamation of barren land.

The extracted oil after the evaporation of respective solvents would be dissolved in DMSO (Di Methyl Sulphoxide) and kept for storage for further studies.

**Mechanical Method- Using Ball Mill**

The Ball Mill concept was established to bring algae from aqueous solution form to dry flakes in preparation for the extraction process. One of the underlying objectives for the dryer, besides dewatering the algae, was to have the process that could be easily modified in the future. The project is in the early stages of development and changes and/or optimization of the ball mill in the future is necessary.

**Design Constrictions**

The final design of a small, prototype ball mill was one of the tasks we had to handle. Due to funding constraints, the ball mill was scaled down dramatically in effort to conserve funds and changed from a full-scale dryer to a tabletop proof of concept. The same general principles govern the ball mill on both the large-scale and the small-scale design. The

difference, however, lies in the ability to fully dry algae. The dimensions of the full scale ball mill allows for more grinding to enable more water removal from the algae before final air drying A complete process re-design may have enabled the algae to exit the dryer in dry flake form, but time constraints forced a scale down of the original design.

**Performance Targets**

Early on in the design process, goals were set in order to evaluate concepts generated by the drying group. The five main goals considered during design development were as follows:

- Dewatering of algae sufficient for extraction process
- Low energy requirements
- Affordability
- Ability to process large batches of algae solution

**Alternative Designs**

The three main alternatives for drying processes were centrifuge, evaporative dryers, and ball mills. Each of these methods has multiple variations in their designs. Continuous centrifuges can be efficient at dewatering but are very complicated, expensive, and energy intensive. More simple centrifuges are very inefficient due to the large acceleration of the massive liquid. Due to these factors the centrifuge drying process was not perused. Evaporative driers are energy intensive due to the increase in water temperature and the phase shift required to drive off water. Low- pressure systems can be made, but those too require significant energy input. Ball mills use grinding actions to draw water out which is an attractive option due to their low energy input.

**Final Ball Mill Description**

Fig 4.1: Ball Mill setup for drying and disruption of algae

The small-scale ball mill described below is shown in Figure. The small-scale ball mill skeleton is made from steel framing. The rollers are made from 2” steel rollers and end caps, joined together by friction. We used a motor of 1 horsepower which could allow a number of

speed ranges. An automatic speed control device was attached to control the speed. To give the structure rigidity and prevent frictions bearing houses are used.

A belt attaches the one of the rollers with the motor. The motor makes the roller to rotate when initiated. A strong glass jar is filled with algae and put in between the roller. Besides algae the glass jar is filled with marbles. As the roller rotates so does the glass jar and the marbles start to grind the algae solution. The structure is very compact and can be easily handled.

**Chemical Method- Using Soxhlet Extractor**

The basis of the extraction system design is an existing laboratory extraction device, called a Soxhlet extractor, which will be used for chemical extraction to remove the algal oil. The system, shown in Figure, recirculates hexane by constantly boiling and condensing it.

Fig 4.2: Soxhlet extractor used to extract oil

Dried algae are placed in the Algae Reservoir and liquid hexane is then added. The hexane fully immerses the algae and dissolves a small amount of it. When the hexane fills the reservoir to a certain level, a siphon is created, and the hexane, along with whatever oils it has dissolved, drains into the bulb, labeled Hexane/Oil Reservoir. Here, a hot plate heats the hexane and oil mixture. The hexane is boiled to vapor and rises through the tubes indicated by the dashed path in the diagram; because oil has a higher boiling point, it does not vaporize. When the vapor hexane reaches the Condenser Tube, cooling water that encases the tube removes heat from the hexane, causing it to condense. The condensed hexane drains to the Algae Reservoir, where it immerses the algae and dissolves more oil. The recirculation continues in this way until sufficient oil extraction has occurred. At the end of this extraction process, a mixture of hexane and oil is left in the Hexane/Oil Reservoir and the leftover algae, called mill, is left in the Algae Reservoir, soaked with residual hexane. This leaves two

problems: separating the hexane from the oil, and recovering the hexane from the dried algae. In the laboratory setup, the hexane and oil mixture is exposed to a vacuum and then heated in order to remove the hexane. The vaporized hexane passes through a condenser so that it can be recovered. However, there are high hexane losses in this process, and there is no laboratory process to recover the hexane contained in the mill.

### 4.4 Tests for the Presence of Lipids

There are some qualitative tests for the presence of lipids in a sample. Some of these tests will be performed for the confirmation of lipids in the extracted sample.

#### Translucency test

This test involves a piece of filter paper and a hot plate and ether. Here, we have to take the piece of filter paper and place a drop of the test solution on it. Then place the filter paper on the hot plate and heat to 60 degrees Celsius or 140 degrees Fahrenheit for 5 minutes. Remove the filter paper and immerse in ether. After the paper is air dried, look at the spot. If the spot is translucent, there are lipids in the solution.

#### Sudan Red Test

Sudan red is a lipid soluble dye. When Sudan red is added to a mixture of lipids and water, the dye will move into the lipid layer coloring it red. Add 2 ml of water in 2 ml of the test sample. Then add 5-6 drops of Sudan reagent. Red color of the sample confirms the presence of lipid in the sample.

# CHAPTER 5
# STUDY OF ALGAL GROWTH

## 5.1 Algal Density Assessment: Calibration Curve for Unicellular Algae

Standard routines to estimate algal concentration include direct cell counts, chlorophyll content measurement, and absorbance or turbidity numerical correlations. New method includes use of a calibrated spectrophotometer to estimate the density.

In the project, the microscope is used to directly count the number of algae cells in a given volume as a means to determine algal density. It is considered to be an easy and quite accurate a method. This however is a tedious and time consuming method.

The spectrophotometer is used as an indirect method that correlates algal density to light absorbance at specific wavelengths. This is not only reliable but also easy to setup for automatic monitoring systems. So, the main usage of the spectrophotometer is to generate a regression model to estimate density of algae in water samples by using the absorbance values of the spectrophotometer.

## 5.2 Cell Count using Microscope

1. Initially, the algal concentration is estimated using the mean number of cells that are obtained from direct cell count of the samplefrom the microscope. This is done by first measuring the volume of a drop of the algal solution by measuring the number of drops of the sample in a 1 ml solution.
2. Next, a drop is taken on a clean glass slide and another glass slide is used to press and spread the drop into a flat plane. This ensures that all the algae cells are in a single plain and minimizes any error that may occur from the overlapping of cells.
3. The pressed glass slides are next placed under the microscope and the number of algae cells is counted.
4. From the above, the density of algae cells, as numbers of cells per ml are determined.
5. The process is repeated thrice for a particular sample to minimize errors.

Fig 5.1Biological Inverted Microscope

**5.3 Spectrophotometric Analysis**

When spectrophotometrical absorbance is the chosen method, the spectrophotometer is calibrated initially for a particular cell species.

1. First, the maximum absorbance for a particular algal species is inspected by scanning the culture sample between 600 and 800 nm wavelength of light in the spectrophotometer. These values are correlated to the light absorbance of chlorophyll, which could be best determined at a wavelength around 664 nm. Hence, for a particular algae sample, the peak with the highest absorbance is obtained. This represents the wavelength of maximum sensitivity to quantify that species in the algal samples. Once this is determined all further analyzed samples for that particular species are read in this wavelength.

2. The highest absorbance value is then used to generate and calibrate the curve of algal density.

   The absorbance values for various cell densities are measured at the determined wavelength. To do this, first the direct cell count method with a microscope is used to determine the algal density in a particular sample. Next, the absorbance for that particular wavelength for that sample is found using the spectrophotometer. In this way, for the same species, numerous cell density and their corresponding absorbance is found for the determine wavelength.

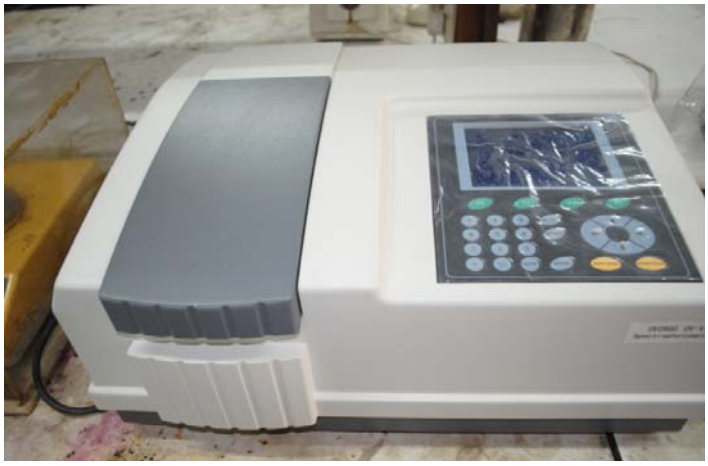

Fig 5.2 Spectrophotometer

The relationship between spectrophotometer absorbance and the counted number of cells is found to follows a general power equation:

$$\text{Absorbance} = a.(\text{Cells/mL})^b$$

where a and b are calibration coefficients, estimated using standardleast squares procedures for linear regression after log transformation of absorbance and density data.

# CHAPTER 6
# ECONOMIC ANALYSIS OF BIODIESEL PRODUCTION

## 6.1 Cost Analysis of Biodiesel Production

Algae are the solution to the problem of depletion of natural resources, and preservation of environmental and ecological balance. So now we stand with the challenge of using the measuring tools of environmental and social accounting, to measure the change in cost of production for using new methods for the production of biofuel from algae. In this report a cost structure and aggregate cost elements are summarized by using accounting terminology to evaluate the production cost, and the potential economic viability of algae in producing fuel instead of producing it from conventional sources.

**Cost elements**

1. Total capital cost
    a. Cost of base (steel structure)
    b. Cost of bioreactors
    c. Cost of aeration system
    d. Cost for Ball Mill setup
    e. Cost for Soxhlet Extractor
2. Total maintenance cost
    a. Labor cost
    b. Cost of nutrients
    c. Cost of fresh water supply
    d. Cost of electricity
3. Cost of CO2 supply
4. Cost of Harvesting
5. Cost for lipid testing and oil extraction
6. Indirect Costs
    a. Depreciation
    b. Others

**Cost equations**

Total capital cost= Total bioreactor number*(Cost of bioreactor preparation+ Contingency) + Cost of Ball Mill setup+ Cost of Soxhlet Extractor

Maintenance cost= Total growth days*(Total labor cost+ Total cost of nutrients+ Total cost of fresh water supply + Cost of power)

- Total labor cost= Labor cost of technical hands
- Total cost of nutrients= Cost of nitride+ Cost of Phosphorus
- Total cost of fresh water supply= Total bioreactor number*Average rate of evaporation*Average Rain
- Total cost of electricity= Total power usage*Unit cost*Total growth days*(1-Down Time)

Total cost of CO2 supply= CO2 cost*CO2 needed for different pH*Total number of bioreactors*(1-Down Time)
Cost of Harvesting= Productivity*Unit harvesting cost
Cost for lipid testing and oil extraction= Productivity*Chemicals for lipid testing + Productivity* Amount of Hexane needed for processing + Power cost
Total operational cost= Total capital cost + Maintenance cost + Total cost of CO2 supply + Cost of Harvesting + Cost for lipid testing and oil extraction
Indirect Cost= Depreciation (Total capital cost*10%) + others (Total operational cost*1%)
Revenue= (Oil yield*Price)
Benefit= Revenue- Total operational cost

**Important factors**

It has to be noted that any factor can increase the net productivity of the plant on annual biases. Some discussion regarding cost factors are given below-

- Photobioreactor selection is the most important part. A microalgae plant should be designed and built for optimal growth conditions for the longest possible period.
- Selection for faster growing, more productive strains optimized for the prevailing conditions is also important. To do this it is essential to have a good understanding of those factors which limit growth and productivity.
- Productivity can also be improved through better culture systems; however the incremental cost of these culture systems in relation to the improvement in productivity must be evaluated carefully.
- The cell concentration of the products is also very important. Increased product concentration not only decreases the effective unit cost of the raw biomass, but it also generally reduces the cost of extraction and purification.
- Harvesting system represents a significant capital and operation cost component. So the choice of harvesting system also depends on final product desired. It is therefore desirable to select algae with properties which simplify harvesting i.e. large cell size, high specific gravity in comparison to the medium.
- Labor is required for pond and equipment maintenance, monitoring of the cultures, harvesting, extraction and further processing. So any improvements in the design of the process and automating the operations of the plant which decrease the labor requirement, without unreasonably increasing the capital costs must be considered carefully as a possible means of reducing production costs.

### 6.2 Feasibility of Low Cost Agricultural Biodiesel Production Unit

The aim is of project is to provide an affordable low cost and low maintenance photobioreactor system for biodiesel production from microalgae that can be used in rural and sub-urban areas of Bangladesh. Algae oil can be used as a replacement for the diesel oil needed for irrigation. The culture broth would be of 10L and the oil extracted from the biomass would be converted into biodiesel. This would be a backyard project shared among several numbers in a community. Photobioreactor system for production of algae would be of

an individual's effort and the ball mill set up for oil extraction should be shared by a group of individuals. Hence, cost of production of biodiesel can be minimized. The possible costs that should be taken into consideration:

Table 6.1: Cost analysis for algal biodiesel production

| Name of items | Cost (BDT) |
|---|---|
| 10 L Glass cylinders (5 cylinders, BDT 800 each) | 4,000 |
| Frame (Wooden or plastics) | 1,500 |
| Ball Mill (2% of BDT 25,000, cost being distributed over a community of producers) | 500 |
| Other costs including cost of pH meter, thermometer, nutrient, electricity supply etc. (10% of BDT 10,000, cost being distributed over a community of producers) | 1,000 |
| TOTAL | 7,000 |

Cost of land, labor, water supply, $CO_2$ supply has been neglected in the above analysis. Each culture will take approximately 20 days to complete growth if proper maintenance is ensured. Around one or two litres of the algae solution will be left in the cylinder to be used again as a culture for continued algae production. Hence, each cycle of 20 days will give a minimum of 40 litres of algae solution. A total of 15-18 cycles per year would be possible. For this project *Cholorococcum* an available strain in Bangladesh has been chosen. This algae strain is known to have lipid content of 19.3% and the lipid productivity is 53.7 mg/L/day (Mata et al, 2010). So for every cycle the approximate amount of collected algae will be 43 gm. The amount of algal oil extracted per annum will be 0.9 kg and 0.7 L of biodiesel can be produced per year. For assuming 10 years service life, cost of per litre biodiesel will be around 1000 BDT. Although it is ten times expensive than current diesel price, the cost of production can be significantly reduced with a bigger size photobioreactor.

# CHAPTER 7
# CONCLUSION

## 7.1 Summary

The investment in algae research has been increasing significantly over the few years and this is not only for the production of biodiesel but also for the reduction of CO2 from the atmosphere. At present production of biodiesel from algae is costly but by doing this a large amount of CO2 can be reduced which will help to prevent climate change and global warming. But commercial production in Bangladesh has a huge potentiality to produce microalgae as labor is cheap and the climate is favorable. Both open pond and photobioreactor is possible in Bangladesh, but raceway pond can be introduced by mass level people in villages as the cost is lower and a huge supply of microalgae can be made available from this source and thus a revolution in energy sector can be ignited. As our study was a lab scale one, we preferred photobioreactor as our culture medium. Here we were able to observe different parameters and their influence during the culture period.

As a student of Mechanical Engineering background, I had no previous knowledge regarding the biological features and compositions of algae. So to design a proper low cost photobioreactor and be able to select and collect proper algae strains for my operation was a big challenge. But I have been able to achieve a certain number of my objectives as followed-

1. A total photobioreactor setup has been completed and is already running. All the necessary parameters which affect algae growth have been measured and their maintenance for a proper growth has been ensured.
2. A successful collaboration has been established with the Botany department of Rajshahi University for assistance regarding the selection and supply of algae strains.
3. For the extraction of algae a Ball Mill setup has been constructed and it is also successfully running. Also a Soxhlet Extractor has been ordered for the complete extraction.
4. Cost equations have been created for the economic analysis and comparison of algae biodiesel with conventional fuels.

## 7.2 Scopes and Challenges

### 1. Emissions

First of let us consider if Algae fuel is good for combating global warming. The first answer will be surely and here lies the reasons-

- **Algae biofuel is carbon neutral; only emits C02 that it absorbs.** Growing algae absorbs C02 in the process of photosynthesis. It is a carbon sink. This is why, when algae biofuels are burned and emit some C02, the emission balance is C02 neutral; it emits only C02 it previously absorbed, adding no new C02 into the atmosphere. Because it is carbon neutral in this way, it is a renewable energy source that can be produced and burned for energy sustainably.

- **Algae reproduces quickly, maximizing biofuel yields** Another good thing about algae is they multiply very fast. They can double their weight many times in a single day.

- **Algae biofuel can scale to replace oil**. Different research suggests that algae could supply enough fuel to meet all of America's transportation needs in the form of biodiesel.

But then we have some concrete points which suggest otherwise. They are given below-

- **Producing algae biofuel requires too much energy**. There are very detailed figures on the amount of energy that will come out of the process, yet it is very hard to find any information on the energy and resources needed to make this energy output possible and is considered to be really large.

- **Energy-intensive production of algae biofuel emits greenhouse gases.** Research suggests that almost all biofuels used today cause more greenhouse gas emissions than conventional fuels if the full emissions costs of producing these "green" fuels are taken into account.

- **Industrial algae depend on dense C02 from coal** Algae can obtain carbon from atmospheric carbon dioxide, but the amounts present are insufficient to promote rapid growth. That requires something like smokestack effluents containing more than 10% CO2, and in fact some of the earliest attempts to grow algae as a fuel source were predicated upon the development of pervasive industrial carbon dioxide capture. That's not happening, and unless it does, real mass production of algal biofuel is scarcely possible.

So there are two sides of the story. And it is us who have to face the challenges and utilize the scopes here.

2. **Land –use**

Now let us consider if algae biofuel take up much land which is not convenient for us.

- **Algae yields much more biofuel per acre than other fuels**. Compared with second generation biofuels, algae are high-yield high-cost (30 times more energy per acre than terrestrial crops) feedstocks to produce biofuels. Since the whole organism uses sunlight to produce lipids, or oil, algae can produce more oil in an area the size of a two-car garage than an entire football field of soybeans.

- **Algae photo-bioreactors require very little land**. For the algae-culture projects which use large growing ponds, the potential biodiesel production per acre is 30 to 100 times greater than obtainable with corn, soy and palm oil. However the most

efficient systems, called photo-bioreactors, stack clear tubes of water with algae in the sun, requiring very little acreage for significant production.

The only problem with land is when it is cultivated in ponds. The reason is ponds need a lot of space, because sunlight only penetrates the upper layers of a water body. It's the surface of the pond that counts, not the depth.

### 3. Economics

It is the most important factor to consider and a lot of research and work is going to make algae biofuel economically viable. Let us take a look at the present scenario.

- **Algae biofuel is commercially viable on an industrial scale.** Different top notch research suggests that they have found a way to inexpensively bring third-generation biofuels to industrial scale. A company named Alganol Biofuel believes its seawater-based process can generate up to a billion gallons of algal ethanol per year from a facility in Mexico.

- **Algae biofuel can become price competitive with oil.** Jennifer Holmgren, director of the renewable fuels unit of UOP LLC, an energy subsidiary of Honeywell International Inc stated if it is possible to get algae oils down below $2 a gallon, then it will be where we need to be. And there are a lot of people who think we can achieve that.

- **There are no soil requirements for algae biofuel.** Biofuels that are created from land-plants all have specific soil-quality requirements. If soil in an area does not meet the specific nutrient requirements of a biofuel plant-type, that plant cannot be built and used to produce the biofuel in the land-area. Algae, because it grows in water (of almost any kind and quality), is not limited by soil-quality.

- **Biproducts of algae biofuels are useful fertilizaers**. Different agricultural specialists have state that fertilizer for other food crops can be produced by using the leftover nutrients that aren't used to make the biofuel after the necessary oils have been extracted from the algae, we can use the byproducts (phosphorus and nitrogen) as fertilizer for the food crops that feed the nation--all while extracting C02 from the air.

- **Algae biofuel does not damage food prices**. Research shows, for algae there is no 'food vs. fuel' tradeoff. The process is not dependent on crops or valuable farmland. It is highly water efficient, delivering 10 to 100 times more energy per acre than cropland biofuels.
- **Algae biofuel industry growing quickly with bright future** The momentum behind algae has grown tremendously since 2008. New companies, new methods, and a changing landscape indicate that biofuel from algae is poised to play a larger role.

We talked about the positive end of the story which is really encouraging. But facts shown below should be considered strongly as well.

- **Producing algae biofuel is relatively expensive**. The cost of various algae species is typically between US$5–10 per kg dry weight. This is relatively expensive, and not really commercially viable.

- **Hi-tech algae biofuel plants require too much energy/money.** First you have to build an array of structures, the glass or polycarbonate containers themselves, the metal frames, the greenhouses. The production of all this equipment might consume less energy (and money) per square meter than the production of solar panels, but you need much more of it because algae are less efficient than solar plants. Moreover, in closed bioreactors, CO2 has to be added artificially. This is done by bubbling air through the water by means of gas pumps, a process that needs energy. Furthermore, the containers have to be emptied and cleaned regularly, they have to be sterilized, the water has to be kept at a certain temperature, and minerals have to be added continuously All these processes demand extra energy.

- **Algae fuel ponds must be expensively covered**. Random natural algae tend to start taking over from artificially seeded algae fairly rapidly unless the pond is covered and covering ponds costs money.

- **Algae pools can become contaminated and less efficient**. Low-tech methods are being left behind for more efficient ones, using closed glass or polycarbonate bioreactors and an array of high-tech equipment to keep the algae in optimal conditions. Even though some companies still prefer open ponds but this method has serious drawbacks. The main problem is contamination by other kinds of algae and organisms, which can replace the energy producing algae in no time.

- **Industrial algae biofuel requires too many nutrients**. It should be pointed out that some experts suggest that algae just be introduced into a properly designed, water filled bioreactor and the organisms will multiply until the unit is packed to overflowing with tons upon tons of green biomass, all in the space of days. This is not correct. Algae can grow quickly, but only in the presence of sufficient nutrients. Just like any other organism, algae require carbon, nitrogen, phosphorus, and various other minerals.

### 7.3 Recommendations for Future Work

From my experience of the thesis work I can suggest following continuation of this project-

1. Effect of sunlight on algae growth can be determined.
2. Rate of Consumption of CO2 can be calculated.
3. Algae biodiesel can be made from harvested algae.
4. Properties of the algae biodiesel can be determined.

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