Production of Biodiesel from Microalgae Collected from Ponds

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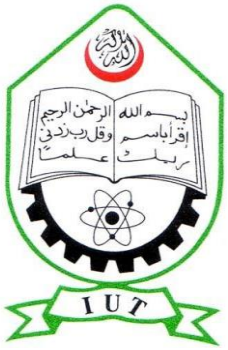

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Abstract

In this paper, authentic empirical informations on the production of biodiesel from different species of microalgae have been demonstrated after a series of extensive experimentations and research. Two different species of microalgae have been collected, isolated, cultured measuring their growth, harvested and finally biodiesel have been extracted from their biomass. Two process have been used in extraction of biodiesel from their biomass: wet extraction process and dewatering method. The result of these experiments have been depicted in lucid and cognitive interpretations. Also the contemporary context of biodiesel and the possibilities of microalgae in mitigating the forecoming global energy crisis have been composed.

Introduction

At present, climate change is a buzz word denoting the most importunate global environmental crisis.[2] World primary energy consumption has raised by 1% in the year 2016, following growth of 0.9% in 2015 and 1% in 2014. This compares with the 10-year average of 1.8% a year [3-1 accessed on 25/07/2017]. With the increase of environmental protection concerns, both clean fuel technologies and new sources of energy are being intensely pursued and explored. [3] In order to meet the upcoming global energy crisis, (as shown below in the graph)

we need to focus more on these new sources of energy which are nearly infinite in amount & that have less effects on the environment.

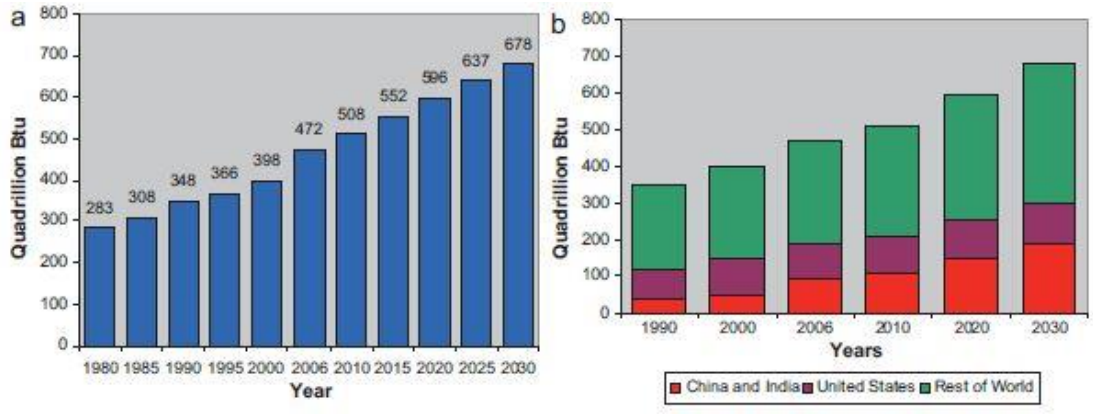

Figure 1: (a) World marketed energy consumption. (b) Marketed energy use by region. Sources: Energy Information Administration (EIA), International Energy Annual 2006 and World Energy Projection Plus (2009).

Renewable energy resources capture their energy from existing flows of energy, from on-going natural processes, such as sunshine, wind, flowing water, biological processes, and geothermal heat flows. [www.sciencedaily.com/terms/renewable_energy.htm on 26/07/2017] Any energy resource that is naturally regenerated over a short time scale and derived directly from the sun (such as thermal, photochemical, and photoelectric), indirectly from the sun (such as wind, hydropower, and photosynthetic energy stored in biomass), or from other natural movements and mechanisms of the environment (such as geothermal and tidal energy). Renewable energy does not include energy resources derived from fossil fuels, waste products from fossil sources, or waste products from inorganic sources.[3-2 http://www.treia.org/renewable-energy-defined/]

The above figure depicts the global energy consumption and the energy use by region from 1980 to the present and projected through 2030. [2-5]. It is clear from the graph that the world will need 60% more energy in 2030 than today. If this ravenous consumption of energy continues, the whole world will be outfacing an energy crisis because the global fossil fuel reserves will be depleted in less than 45 years. Therefore, in order to resolve these important issues, technologies that can act as alternatives of fossil fuel with renewable energy should be harnessed. [2]

Biodiesel:

Biodiesel is a lucrative energy resource for abundance of reasons. Biodiesel is a renewable energy resource that has sustainability in its provision. Biodiesel appears to have many auspicious environmental properties resulting in no net increase in the release of carbon dioxide

and very low sulfur content [5-6,7]. Recent researches have revealed that the use of biodiesel can decrease 90% of air toxicity and 95% of cancers compared to common diesel source [5-64]. Again, biodiesel seemingly has rich economic potential because as a non-renewable energy source that fossil diesel prices will increase incompetence in the near future [5-8]. Also biodiesel is better than diesel fuel in terms of flash point and biodegradability [5-9].

The principal substitution to fossil fuel is biodiesel. The principal substitution to diesel fuel in EU is biodiesel, representing 82% of total biofuels production [6-9] and is still growing in Europe, Brazil, and United States, Malaysia based on political and economic objectives. **Biodiesel** is a renewable, biodegradable fuel manufactured domestically from vegetable oils, animal fats, or recycled restaurant grease. (https://www.afdc.energy.gov/fuels/biodiesel_basics.html published on May 18,2107) Conventional biodiesel mainly comes from soybean and vegetable oils [5-10], palm oil [5-11], sunflower oil [5-6], rapeseed oil [5-12] as well as restaurant waste oil [5-13]. Algae have also been investigated as a source of fatty acid for a biodiesel feedstock. [7] Since biodiesel is mostly produced from vegetable oils and vegetable oils are also used for culinary purposes, it can promote an increase in price of food-grade oils that may result in the cost of biodiesel to increase. [6] In order to meet the economic as well as energy demand, biodiesel should be produced from low-cost feedstock such as non-edible oils, animal fats, used frying oil etc. But, the available quantity of waste oils and animal fats are not enough to match the contemporary demand of biodiesel. Thus, production from second generation biofuels, such as microalgae, can also contribute to a reduction in land requirements as well reduces cost. In addition to that, bio diesel thus produced, has lower impact on the environment. [6-13]

Potential of microalgae as source of biodiesel

The potential sources of biodiesel such as corn, jatropha, canola, soy bean, coconut, oil palm etc. require unsustainably large cultivation areas for their cropping. These sources need almost 50% of the total land area available. [15] So, in this way the mitigation of world energy crisis with the help of biodiesel would become really challenging. On the other hand, if microalgae are used for the same purpose, the whole scenario changes dramatically. Only between 1 and 3% of the total land area would be enough for manufacturing algal biomass that satisfies 50% of the transport fuel needs in the United States [15-Table1]. The following table depicts a relative comparison of oil content (in percentage of dry weight) between different species of microalgae that can be harvested to produce biodiesel.

Microalgae	Oil content (% dry wt.)
Botryococcus braunii	25-28
Chlorella sp.	28-32
Crypthecodinium 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

Unlike other oil crops as previously mentioned, microalgae multiply very fast and many are exaggeratedly rich in oil. It has been found that microalgal oil content can exceed 80% by weight of dry biomass (Metting, 1996; Spolaore et al., 2006). Practically, instead of microalgae, oil producing heterotrophic microorganisms (Ratledge, 1993; Ratledge and Wynn, 2002) grown on a natural organic carbon source such as sugar, can also be used to make biodiesel [15]. But, we cannot get the same efficiency from heterotrophic production as found by the use of photosynthetic microalgae. Naturally, for the mass production of biodiesel we have to choose the microalgae with higher oil content. From the above table it is understood that on average 20 percent dry weight of oil content is available from all the species of microalgae that provide biodiesel.

Selection of Strain

The first and most critical step in developing a reliable and commercially viable process for production of microalgae to obtain biofuel is selection of algal species and strain. From commercial perspective the species must have to be highly productive, contamination resistive in proposed cuture system. Though our goal is to augment the productivity of lipid, it itself is not sufficient for potential commercial production.

About 35000 species of microalgae have been discovered although the available amount is assumed to be significantly higher. In Bangladesh freshwater species are available, among them the most viable strains are : [19]

Oedogonium: It is widely available in Bangladesh and can be collected from nearby ponds or other still water sources. Having a good growth rate it posseses good lipid content which is 29-49% of its dry weight from which the biodiesel can be extracted.

Spirogyra: It is considered as the most common and widely available algae available in Bangladesh. Its growth rate is very good in Bangladeshi atmosphere. Its lipid content is also good but lower than the Oedogonium. It contains around 18-22% lipids of its dry weight.

Navicola: It is also a uni-cellular alga. It is not available promptly but found at some areas. Its Lipid content is around 25-40% of its dry weight.

Our goal is to increase the growth of microalgae as well as lipid content and from there, to extract biofuel commercially. But the production of microalgae is function of some factors, these factors are discussed below :

1. Optimum Temperature and Temperature tolerance: Geographical location and its climate is one of the most important factors for production of microalgae. The average temperature of Bangladesh varies from 23.9°C to 31.1°C during summer and from 7.2°C to 12.8°C in winter [20]. Other important factor needs to be considered that microalgae are very sensitive to the temparature and the lethal temperature for microalgae is generally slightly higher than optimum temperature. Strains with broad optimum range are considerabley sustainable and shows better growth [21-22]. As respiration during night time causes loss of biomass significantly [23]. So, length of day is also needed to be considered.

2. CO2 supply, pH and O2 tolerance: High rates of photosynthesis is dependent on the uptake of inorganic Carbon by cell. In the aquatic environment, plants are exposed to four forms of dissolved inorganic carbon: dissolved CO(CO_{32-}), - CO2 , bicarbonate (HCO3-)and carbonate ions. All microalgae can take up CO2 and some can take up carbon in the form of HCO3- [23-24]. Microalgae are pH sensitive, so uncontrolled pH can be lethal. As CO_2 converts into HCO3-, it can change the pH of the medium. It should be noted that, CO2 diffusion from air to the medium is greater at more alkaline pH [26-27].

3. Salinity: To increase the lipid content in microalgae, salinity of water in an important factor. Use of saline water reduces the pressure on fresh water requirement for cultivation [28]. Reduction of contamination can be possible by the use of saline water in fresh water strains in the medium.

4. Respiration rate: Respiration rate is usually dependent on conditions like temperature, nitrogen content, light etc.

5. Competitive strain: For successful cultivation the selected strain must outlive other weaker strains. Some selected species can outcompete contaminating organisms by surviving in high pH and producing DMSO (dimethylsulphoxide).This characteristic can be used as the antibiotic and also leads to the successful long term outdoor production.

Culture medium preparation

Culture medium is required for isolation of dominant strain and initial batch production of microalgae which will be undergone mass production later on. Different researchers have used different types of culture media for isolation & cultivation purpose. In our research we have used Tris-acetate–phosphorus [29] medium as culture medium for isolation and batch production.

Tris buffer solution contains following chemicals as listed in the table:

Tris buffer solution		Trace metal solution	
Chemicals	Amount (mg/L)	Chemicals	Amount (g/L)
1. Tris (hydroxymethyl) aminomethane	2,420	1. Na_2EDTA	50
2. NH_4Cl	400	2. $ZnSO_4 \cdot 7H_2O$	22
3. K_2HPO_4	108	3. H_3BO_3	11.4
4. $MgSO_4 \cdot 7H_2O$	100	4. KOH	16
5. KH_2PO_4	56	5. $MnCl_2 \cdot 4H_2O$	5.06
6. $CaCl_2 \cdot 2H_2O$	50	6. $FeSO_4 \cdot 7H_2O$	4.99
7. Glacial Acetic acid	1mL	7. $CoCl_2 \cdot 6H_2O$	1.61
		8. $CuSO_4 \cdot 5H_2O$	1.57
		9. $(NH_4)6Mo_7O_{24} \cdot 4H_2O$	1.10
		10. $CaCl_2 \cdot 2H_2O$	0.05

It is to be noted that, Erlenmeyer flasks of 100mL volume were used for preparing the stock solutions from these aforementioned chemical reagents. After preparing the separate stock solutions for each reagent, they were autoclaved at a pressure of 2.5 bar & temperature. This was done for sterilizing the stock solutions so that there is no chance of contamination. After autoclaving for 25 minutes, the solutions were taken out & then 1mL Glacial Acetic Acid was added to the final solution, which is 1 liter in volume. The solution thus prepared, makes the culture medium that we have used in our research.[30]

Isolation

In order to explore any algal species, it is necessary to grow algae as a pure culture. That is, a culture of only a single species (called a unialgal culture). To obtain a unialgal culture, isolation of one species from all the rest needs to be done.

Successive dilution showed better growth of uniculture and considerably easy. Firstly, using aseptic technique, 9 mL of culture media was dispensed into each of the 10 test tubes with sterile 10 ml pipette. The tubes were numbered from 1 to 10, indicating dilution factor of 10^{-1} to 10^{-10} .1 mL of enrichment sample was added to the first tube. The sample was allowed to mix with the medium thoroughly. From the first tube, 1 ml diluted media was taken and was added to the second tube. This process was followed until the last tube was filled. The test tubes were nurtured under controlled temperature and light condition inside an incubator. Temperature was kept fixed at 36.45^0C. A photo-period of 10 hours was maintained and steady luminous flux of approximately 320 luxes was maintained.

Cultivation

In the field of algal biotechnology there have been a magnificent advancement. As algae have been proved to emerge opportunities in renewable energy sector, researches have been carried away for the development of its cultivation and production.

Visible growth of microalgae was seen in the isolated test tubes after 24 days. Soon after observing visible growth, microalgae were transferred to the culture medium for their cultivation. This phase in the experiment is the most important one, as the quality and vital strength of the algae at the time of extracting lipid depends solely on their cultivation. Algae were inoculated at 2% ($v_{inoculation}/v_{media}$) in 500 mL conical flasks with 250 mL of liquid medium. Two samples were prepared and preserved in the incubator [**incubator model]** for cultivation in a steady luminous flux of fluorescent light (approximately 2850 lumens) at a constant temperature 27 degree Celsius. This phase was maintained for 2 weeks followed by transferring them to photo-bioreactor for mass production.

Mass Production:

A very important phase of algal cultivation is the mass production. It ensures the healthy growth of algal strains & also multiplication on a large scale. There are several factors affecting mass growth of algae in the culture medium. The most influencing factors are:

- CO_2 concentration **[Chiu et al]**
- pH **[Richmond]**
- Aeration & light intensity **[Kaewpintong]**

Effect of CO_2 concentration:
The more the concentration of CO_2, the more is the growth of the algal colony. This has been proved in most of the experiments carried out in the cultivation of microalgae. Their autotrophic behavior is the main reason here. But, there has been countered a difficulty in supplying CO_2 to the microalgae at the time of mass production.[Richmond] So, we have used the natural way of supplying CO_2 to the growing algae. We have kept the mouth of the conical flask open for 5-10 minutes each at an interval of 24 hours. Thus the contamination has also been prevented along with supply of CO_2 to the algae.

Effect of pH:
Since the culture medium is a buffer itself, so the pH is to be kept neutral. Acidic or basic nature of the medium could hamper the health of microalgae resulting in less growth. So, the pH is to be kept constant throughout the medium throughout the mass production phase. Experimental results show that, it is difficult to control the pH while supplying CO_2 to the algae being grown in shallow culture media. So, we have omitted the simultaneous supply of CO_2 and made them separate so that pH remains constant.

Effect of aeration and light intensity:
Aeration leads to better mixing of microalgal culture preventing the sedimentation of the culture media. It also helps keeping the media homogenous & thus maintaining a better contact between cell and nutrients.[mata] An airlift bioreactor yields better growth than an unaerated column **[Kaewpintong].** In order to provide better aeration, we have used a shaker that was run at 50 rpm for 72 hours. The cell density also increases with an increase in the light intensity up to a certain limit, above which the algal growth is inhibited. So, we have used a constant source of fluorescent light of luminous intensity 5.6 candela. The concept of photo-bioreactor has been used in the installation of the light sources.

Figure 4: Customized Photo-bioreactor for optimum light supply during shaking.

An aggregate of the aforementioned set up has contributed to an outstanding growth of microalgae that have been further filtrated for collecting the biomass as described in the next part of the this book. The use of photo-bioreactor is quiet expansive in the mass production of microalgae. Also, the use of shaker adds extra cost. But, we have managed to achieve both of them in a single installation: our shaker cum photo-bioreactor, which is shown below in the figure.

The shaker has a maximum speed of rpm. And our customized photo-bioreactor has a the intensity of light as required for the higher growth of the microalgae. Photo-bioreactors are used for the mass multiplication of algal cells. And the laboratory shaker was used for ensuring uniform growth of algal colony all over the medium.

Different methods for greater biomass production have been developed including bioreactors and open pond systems.[31]

According to the environmental and commercial issues open raceway ponds might be the most feasible option for commercial cultivation of microalgae. Open raceway Architecture should be selected based upon these points- Cheapest of all current techniques, sustainability to contaminaton challenges, CO_2 utilization, temparature control, evaporation, and maintinability. [32]

In the raceway pond the algae, water, and nutrients circulate around a racetrack. The flow is generated by the paddlewheel and culture will be circulated at about 20-30 cm/s. the algae are thus kept suspended on the water and circulated back up to the surface at a regular frequency which prevents thermal stratification.The algae should be kept exposed to the sunlight and at a limited depth to which the sunlight can penetrate under water, owing to this fact the ponds are kept shallow.[33]

In the raceway pond to ensure adequate exposure to sunlight, the ponds are usually lined with plastic(e.g. HDPE geotextile plastics) or cement, are about 15 to 35 cm deep. The culture media and the algae are circulated continously in the pond lined by the plastic which are between 0.2 to 0.5 ha in size.[32]

Contamination and management of contamination:

There are a lot of challenges which have to be overcome for the commercial production of biofuel from algae. One of the major challenges is the management of contmination in open ponds.
Open ponds are excellent habitat for the growth for variety of algae contaminants. Several common biological contaminents are obseved during culture of algae in raceway ponds which eventually resulted in failure. These contaminents include unwanted compeititive algae, mould, fungi, bacteria, yeast, protozoans. [06]

In order to overcome this contamination problem several techiques have been developed some of them are described below :

1. Maintaining extreme culture environment : One of the most effective techniques to mitigate contamination have been introduced to produce microalgae in open pond system is to maintaining extreme culture environment. In this technique, the extreme environment can be achieved by maintianing high salinity, high alkalinity or high nutrition status. [34] But the main drawback of this technique is cost ineffectiveness. Due to high maintanence of this technique it is not that much suitable for producing biofuel commercially[35]

2. Deploying chemicals in pond : Several chemical techniques were introduced to abort the contamination such as use of hyperchlorite, Ammonia, deploying Glyphosate and ozone, use of pesticides like Dipterex, Parathion, and dichlorodiphenyltrichloroethane (DDT). Use of these chemicals alter the pond environment to gain a differential advantage on the target algae strain, or to disadvantage the undesirable contaminants. But chemical techniques aren't successfull in commercial field.[36]

3. Sapphire Energy Inc. technique : As the other techniques have some issues regarding commercial application, Sapphire Energy Inc. optimised those and derived a technique which has been more successfull and viable for commercial production of algae. Sapphire Energy Inc. technique involves some stages – Isolating pest, identifying the mechanism responsible for reducing the production, pest tracking, designing chemical strategies, application of the process in the pond and this technique can be applied for long term. [36]

Growth rate measurement

The growth control of microalgae is very important for commercial production. The growth of microalgae is dependent on some key factors such as light intensity, optimum temparature, nutrient concentration, so this factors must be optimised for preventing economic losses[37]. Owing to these fact, simple and efficient method for meauring the growth rate is required to ensure economic viability. During selection of the method some characteristics should have to be observed carefully such as stability, speed, easiness, sporadic calibrations [38].

There are numerous methods used to determine progressive growth in microalgae cultivation which can be classified into two types : Direct methods and Indirect methods. Direct methods are algal biomass, packed cell volume, settled cell volume, cell counts, fresh cell weight, dry cell weight, cell density and detecting pigment contents[39]. Indirect methods are primary

productivity and changes in chemistry of the aqueous environment used to express algal growth quantitatively.[40]

The most common direct methods for measuring growth rate are performed by direct cell counting by microscope or by dry weight determination, besides these methods there are other methods which are effective, efficient and economically more viable.[41]

Cell counting method:

After sedimentation of algae in the culture medium, the algae cells are counted directly by the Utermöhl inverted microscope techique.[A comparision of eight methods for estimating the biomass and growth of planktonic algae]. In this Utermöhl chamber the sample of mesured volume is added and settled for 1 hour to 24 hours for accuracy if there is a little amount of algae, it will take longer time to sediment. Then under microscope about 20 Whipple fields have to count and the area of Whipple field is measured accurately. A magnification of X400 is used during counting. [43]

An electronic particle counter is required to measure the growth rate having large counts with high accuracy. The principle of this method is that,the algae particles are suspended in an electrolyte solution then they are sized and counted by passing them though an orifice having a particular path of current flow for a given length of time. As the algal particles are passing through the orifice they displace the volume of electrolyte equally and create a resistance to the path of the current thus creating a current and voltage difference. The magnatude of change in the current and voltage is directly proportional to the number of particles in the sample.

In the beginnning the stopcock creates a vaccume and draws the particles through the orifice. The deflection in manometer due to the entrance of particles resets the counter to zero. when the column of mercury flows past the “stop” position the eletronic counter starts counting and ceases when it passes the ‘stop’ position. The distance traveled by the mercury column can be calibrated to provide the sample volume. The electrolyte used is 1% NaCl which has been filtered to remove particulates. The algae samples are dispersed into electrolyte in special sample beakers. The dilution factor is determined experimentally, and is designed to yield the best distribution. Variously-sized aperture tubes are available for use in counting variously-sized particles; the aperture size is chosen to match that of particles.

In using an aperture of 50um of less to count very small particles, extreme care must be taken to reduce background counts. Electrolyte should be filtered at least 3 times. When large particles are to be counted, care must be taken to reduce error due to settling. [43]

Dry weight method:

The growth of microalgae are characterised by five stages. These are

1. Lag or induction phase 2. Exponential Phase 3. Phase of declining relative growth. 4. Stationary phase 5. Death phase. [44]. Dry cell weight method is more easier than the cell

counting method. This method requires the manipulation of samples in nonsterile condition.[45]

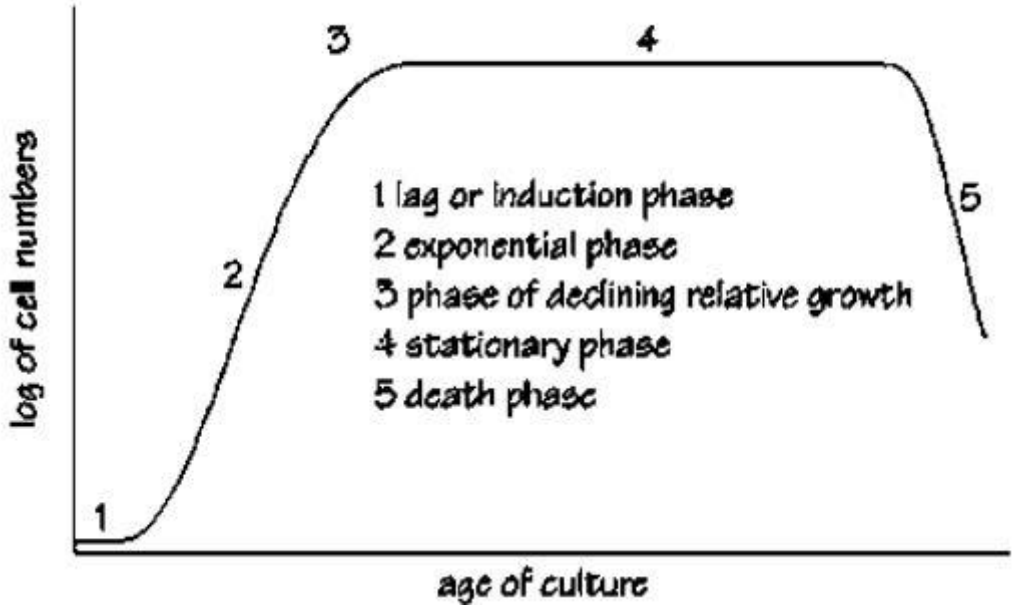

Figure 2: Different algal cycle

Procedure:

1. The algae are harvested on the media surface and weighted on a balance. To avoid contamination the culture jar is opened only during proccessing.

2. Then the cell mass are collected by filteration using a Buchner funnel under vaccum.

3. The cell package is washed by distilled water and retain under vaccum or laminar air flow for a fixed time. Then the package is weighted quickly to reduce the weight variation due to water variation.

4. Then the sample will be kept on the oven at 104 C for 4 hours.

5. After 4 hours the sample are cooled in the desiccators and the weight is measured.

Percentage of dry weight = weight of dry algae/ weight of total algae.

Optical density method :

Optical density method is an indirect method which is being used widely because it can be easily conformed to automated measurement systems. [41].

For each analyzed microalgae species, the maximum absorbance was inspected by scanning sample cultures between 550 and 800 nm. The maximum absorbance value for each microalga was used to perform the growth curve by optical density (OD)[41]

Samples were taken from the culture media every day for measurement of optical density at 680 nm (OD680) using a spectrophotometer (Genesys 5, Spectronic Instruments, UK) as the algal density indicator. The growth rate (GR, per day) was calculated by fitting the OD for the first 3 days of culture to an exponential function:

$GR = (\ln OD_t - OD_0)/t$

where OD0 is the optical density at the initial day, ODt is the optical density measured on day t. Each recorded ODt was corrected by taking away that of the corresponding blank sample. Algae were centrifuged and harvested before the wastewater was discharged.

Growth Efficiency :

Dry weight, are measurements of tissue's absolute biomass at a given sampling time. No reference to the actual growth capacity is taken in consideration. Growth index is a relative estimation of such capacity as it correlates the biomass data at the sampling time to that of the initial condition. It is calculated as the ratio of the accumulated and the initial biomass. The accumulated biomass corresponds to the difference between the final and the initial masses.

$$GI=(W_f-W_o)/W_o$$

where GI represents growth index, and WF and W0, represent the final and initial masses, respectively.[46]

Specific Growth Rate :

It is generally accepted that growth of a cell culture with respect to time is best described by the sigmoid curve theory. At the beginning, the cell population grows relatively slow (lag phase). As the population size approaches one half of the carrying capacity (defined by the nutrient status of the culture medium), the culture's growth per time unit increases. The rate of growth is measured by the steepness of the curve, and it is the steepest when the population density reaches one-half of the carrying capacity (in the middle of the sigmoid). After this point, the steepness of the curve decreases, keeping this tendency as the population increases, until it reaches the carrying capacity (stationary phase). The specific growth rate (m) refers to the steepness of such a curve, and it is defined as the rate of increase of biomass of a cell population per unit of biomass concentration. It can be calculated in batch cultures, since during a defined period of time, the rate of increase in biomass per unit of biomass concentration is constant and measurable. This period of time occurs between the lag and stationary phases. During this period, the increase in the cell population fits a straight line equation :

Miu = (lnx-lnxo)/t

where *x*0 is the initial biomass (or cell density), *x* is the biomass (or cell density) after time *t*, and *m* is the specific growth rate.[46]

Doubling Time :

Doubling time (dt) is the time required for the concentration of biomass of a population of cells to double. One of the greatest contrasts between the growth of cultured plant cells and microorganisms refers to their respective growth rates. While the pattern of growth may be the same, plant cells have doubling times or division rates measured in days, while this parameter in many microorganisms is in the order of minutes to hours. The doubling time (dt) can be calculated according to the following equation :

$$dt=\ln 2/miu;$$

where miu represents the specific growth rate.[46]

Harvesting

In simple terms, harvesting is separating the algae from its supporting medium and/or concentrating the algal biomass. The harvesting technology that needs to be chosen, depends on the type of algae being cultivated. e.g. characteristics of the microalgae, size, density etc.

[47] The most common harvesting methods are screening, coagulation, flocculation, flotation, sedimentation, filtration and centrifugation [48]. The choice of technology for algae harvesting should be energy efficient and inexpensive for feasible biofuel production.

In various algae-harvesting technologies such as sedimentation, flotation, filtration and centrifugation, coagulation & flocculation of algal cells is carried out as an upstream treatment. [49]

Coagulation and *flocculation* are two distinct processes. Coagulation is used to condition suspended, colloidal and dissolved matter to help in subsequent processing. It involves the addition of a chemical coagulant(s). The aggregation of the destabilised particles and the precipitation products that are formed by one or more coagulants, into larger particles is known as flocculation. Coagulation-flocculation causes algal cells to become aggregated into larger clumps, which are more easily filtered and/or settle more rapidly to facilitate harvesting. Coagulation and flocculation may be initiated through the use of inorganic coagulants, organic coagulants (often polymers) or by using autoflocculation, bioflocculation, ultrasound and electrocoagulation procedures.

Inorganic coagulants can include Alum ($Al_2(SO_4)_3 \cdot 18H_2O$), Ferrous Sulphate ($FeSO_4.7H_2O$) etc. each having a particular optimal pH range for proper functioning. Organic coagulants may include Chitosan, Praestol® etc.

Coagulation and flocculation improves the ease of solid-liquid separation processes involved later. However, all microalgae may not require flocculation to be removed effectively.

Solid-liquid separation techniques constrain either the liquid or the solid.

Liquid constrained systems:

Liquid constrained systems include gravity thickening, centrifugation, sedimentation and flotation [23].

1) **Gravity Thickeners:** Gravity thickeners provide a final solid concentration of generally 2–3%. Most commonly used gravity thickeners are circular in shape. The influent is pumped up through the middle section of the thickener and is released. With time the particles settle, and several zones are created. The top zone consists of relatively clear media and the lower zones consist of particles. Gravity thickeners frequently have a sloped base and scrapers to collect the settled solids for removal [50].
2) **Centrifugation:** Centrifugal processes generate a centrifugal force which acts radially and accelerates the movement and separation of particles based on a density difference between the particle and the surrounding medium. If the particle is denser than the media the particle migrate outwards otherwise it migrates inwards. The maximum discharge concentration typically achieved in continuous centrifugation processes are 10–20% solids. The decanter centrifuge and the disk stacked centrifuge seem to be the most promising centrifugal devices for the recovery of microalgae. In Table I, the two centrifuges are compared [50].

Table 1.0

Comparison between two Centrifuges (Adapted from [50])

Device	**Energy Requirements (kWh/m^3)**	**Biomass concentration in solid discharge (%)**	**Reliability**
Disc stacked and nozzle	0.7–1.3	2–15	Very good
Decanter	8	22	Very good

3) **Flotation:** Flotation is a separation process which uses air or gas bubbles. The bubbles adhere to the particles, which are then carried to the liquid surface where they can be separated, usually by skimming. The gas bubbles can be generated within the flotation cell or it can be introduced in to it. The most common gas used for flotation is air. Dispersed air flotation, dissolved air flotation (DAF), bio-flotation and electrolytic flotation are the most popular flotation mechanisms [50].

Particle constrained systems :

Particle constrained systems include cake filtration, screening, attachment etc.

1) **Filtration:** All filtration processes need a filter media. The processes generally operate under gravity, pressure or vacuum [50]. Algal suspension is forced to pass across the filter medium by a suction pump. The medium retains the algae biomass. Filtration process is able to harvest microalgae or algal cells of very low density. But the deposited cells often cause fouling/clogging of the medium [49].

2) **Attachment:** Sometimes the microalgae cells can be removed by physical, chemical or electrical attachment to other materials, which can be directly submerged into the culture medium. Magnetic particles can also be added to separate microalgae by magnetic separation techniques [50].

The harvested biomass slurry consists of 5–15% dry solid content and needs to be processed rapidly after harvest.

Dehydration or drying is commonly carried out after harvesting. Sun drying, low-pressure shelf drying, spray drying, drum drying, fluidized bed drying and freeze drying are some of the methods that have been used.

Sun drying is the cheapest dehydration method but brings certain disadvantages into the scenario, e.g. long drying times, loss of material etc. Spray drying is relatively expensive. Freeze drying is equally expensive but extraction of oils is easier from freeze-dried biomass [47].

In our research, we have used the filtration method for harvesting. The general procedure is quiet similar to conventional filtration. No. #1 filter papers were used in our process. The physical properties of the room are given below:

Physical Properties	**Parameter**
Temperature	20 degree Celsius
Pressure	1 atm.
Air flow	Laminar
Air Speed	0.28 m/s

At first, 500 mL conical flask was taken and glass funnel was introduced into it. Filter paper was then folded into the right proportion and placed onto the funnel. Conical flash from the shaker cum photo-bioreactor set-up was fetched and the medium containing algae was poured onto the filter paper gently. This process was repeated for all 26 filtration set-ups. Algal biomass was collected on the filter papers after 2 hours of filtration. Then the papers were gently taken away from the filtration set-up and fresh weight was taken immediately. BDK Laminar flow system was used for providing laminar flow of air during the filtration process.

Figure 3: Filtration set-up.

After taking fresh weight of all the samples, the filter papers containing biomass were preserved in petri-dish. In order to get the dry weight, the petri-dishes were stored in an oven at constant temperature of 70 degree Celsius for 72 hours. After drying for 72 hours, dry weight of the collected biomass was recorded. The biomass thus harvested, can be stored for a long span of time. This is a major advantage of filtration process.

Although filtration process is economical, there are several drawbacks of the process. 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 unsatisfactory. Also filtration is better suited for large microalgae such as Coelastrum proboscideum and S. platensis but cannot recover organisms with smaller dimensions such Scenedesmus, Dunaliella [06].

Lipid Extraction

The extraction of oil from crops such as soy is relatively easy due to the macroscopic size of the media being used. Simple inexpensive grinding devices with relatively imprecise machining tolerances can be used and will still extract oil. The microscopic nature of chlorella spp. and the tough flexible cellulose cell wall, however, make extraction extremely difficult.

The use of solvents for the extraction of lipids from algal biomass has been a method of choice for many years. The Soxhlet Extraction Method was chosen because of its simplicity in operation, relative safety and potential for upscaling to industrial plant level.

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.

Methodology :

The algae used in the experiment has been 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 been 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.

Upcoming Results and Discussion

1. 14% efficiency (producing14 liter of biodiesel from 100 kg of algae)
2. Possibility of production of jet fuel and petrol.
3. Comparative results from two different species.
4. High volume of production of algal biomass & higher yield of lipid.
5. Fuel demand of Bangladesh can be greatly met, if large-scale culture of microalgae is encouraged.
6. Algae can be cultivated on marginal land, fresh water, or sea water, so it will become a huge prospect in this period of energy crisis.
7. Algal market has limited market competition.

8. Unfortunately, biodiesel from oil crops, waste cooking oil, and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Microalgae appear to be the only source of renewable
biodiesel that is capable of meeting the global demand for transport fuels.
9. Oil supply based on theoretical claims that 47,000 – 308,000 L/ha/yr of oil will be produced form algae.

10. The calculated cost per barrel would be $20, where currently the oil barrel is sold by $100 in US.

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