Research Paper E-ISSN NO : 2455-295X | VOLUME : 7 | ISSUE : 10 | OCTOBER 2021

MICROALGAE CULTIVATION IN PHOTOBIOREACTOR FOR BIOMASS EXTRACTION

DRISYA D S [1] | SUCHITH CHELLAPPAN [1*] | CHINGAKHAM.C [2]

[1] ENVIRONMENT ENGINEERING & MANAGEMENT DIVISION, DEPARTMENT OF CIVIL ENGINEERING, UKF COLLEGE OF ENGINEERING & TECHNOLOGY, KOLLAM, KERALA-691302

[2] SCHOOL OF MATERIALS SCIENCE AND ENGINEERING, NATIONAL INSTITUTE OF TECHNOLOGY, CALICUT, KERALA-673601

***Corresponding Author:**

SUCHITH CHELLAPPAN: ENVIRONMENT ENGINEERING & MANAGEMENT DIVISION, DEPARTMENT OF CIVIL ENGINEERING, UKF COLLEGE OF ENGINEERING & TECHNOLOGY, KOLLAM, KERALA-691302

ABSTRACT:

Microalgae are small photosynthetic micro-organisms adapted to almost all possible environments. Microalgae are currently cultivated commercially around the world in several small- to medium-scale systems for the production of human nutritional products. Microalgal biotechnology has wide commercial applications. Microalgae are also used as nutrient supplements for human consumption due to their high proteins, vitamins, and polysaccharides content. Some microalgae species contain high levels of lipids which can be extracted and converted into biofuels. They also find use in pharmaceutical industries, as some species of microalgae produce bioactive compounds such as antioxidants, antibiotics, and toxins. Whereas, India and Australia, are smaller producers of microalgal products. Microalgae are the rich source of proteins, carbohydrates, fatty acids, and other nutrients. Microalgae play an important role as primary producers to various consumers such as Rotifer, Copepods, Shrimps, etc. In the present study, an attempt was made to cultivate microalgae, at a pilot scale using a photo bioreactor with varied parameters such as pH, light intensity, cell count, nutrient content, etc. The results were compared with the analysis of dried biomass. The harvesting methods used here are gravity sedimentation, dewatering, filtration, and drying. However, more works need to be done to fully utilize the potential of microalgae biomass for the application in the large-scale production of biofuels, food additives, and nutritive supplements.

KEYWORDS:

MICROALGAE, BIOMASS, PHOTOBIOREACTOR, BUBBLE COLUMN.

INTRODUCTION

Microalgae are microscopic eukaryotic organisms, usually found in freshwater and marine systems that use solar energy to produce ATP, which is converted to lipid, carbohydrate, and proteins by its metabolic function. Microalgae offer great promise in contributing to renewable bioenergy, as they have high lipid content, rapid growth rate, and aquatic growth environment using solar energy and also avoid the food versus fuel debate. In heterotrophic growth, algae cannot synthesize their food and depend on glucose or other utilizable carbon sources for carbon metabolism and energy. Heterotrophic production is not as efficient as using photosynthetic microalgae. This is because the renewable organic carbon sources required for heterotrophic microorganisms are produced by photosynthesis, usually in crop plants.[1]

Microalgae technology has been recognized commonly as an efficient alternative to solve potentially the urgent global challenges that nowadays the human being facing, including energy crisis, climate change, environmental deterioration, and food shortage. However, microalgal biofuels have not been commercially produced yet due to the uncertainties of economic feasibilities. It was reported that microalgal biomass productivity and cost was the most significant determinant of algal biofuel prices.[3]

Many microalgae can produce large amounts of triacylglycerol (TAG) as a storage lipid under certain stress conditions. Microalgae have long been considered as an alternative and renewable feedstock source for biofuels.[4] Many microalgal species can be induced to accumulate substantial quantities of lipids. Humans have always tried to take advantage of these properties through algal mass culture.[5] They reproduce quickly and can be harvested day after day. However, the lipid content in microalgae is required to be high, otherwise, the economic performance would be hard to achieve.[6]

The advantages of microalgae over high plants as a source of biodiesel are numerous;

- Oil yield per area of microalgae culture can exceed the yield of the best oilseed crops.
- Microalgae can be cultivated easily in freshwater, seawater, brackish water, and non-arable land.

- They do not compete for resources with conventional agriculture.
- Microalgae cultivation does not need herbicides or pesticides.
- Microalgae reproduce themselves using photosynthesis to convert sun energy into chemical energy, completing an entire growth cycle every few days.
- Microalgae can be grown in suitable Photo bioreactors with higher annual biomass productivity on an area basis.
- Producing biodiesel from microalgae provides the highest net energy.
- Microalgae can be obtained from wastewater treatment plants growing by using these contaminated water as nutrients.
- Residual algae biomass after oil extraction may be used as feed or fertilizer or can be fermented to produce ethanol and methane.
- Harvesting of algae biomass is a significant and operating cost in the algal process.
- In large-scale microalgae culture systems, productivity can be affected by contamination with unwanted algae and other microorganisms.
- The difficulty of maintaining selected species in outdoor culture systems.[7]

FUNDAMENTAL CHARACTERISTICS OF ALGAE

Algae are broadly classified into two categories: macroalgae and microalgae.[8] Microalgae are a general term for the algae that form multicellular thallium at least in one stage of the life cycle. It can also show differentiation between tissues and reproductive systems than unicellular microalgae. The largest multicellular algae are called seaweed, which can be well over 25 m in length. The oil content of macroalgae usually ranges between 10% and 30%.[9]

Microalgae are simple microscopic heterotrophic or autotrophic photosynthetic organisms, also called phytoplankton. In a batch culture system, algal growth experiences five different phases. The phases are as follows. (1) Lag phase: Initial period of slow growth where microalgae take time for adaptation into the new environment. The length of the lag phase depends on the size of the inoculum as well as the shock of the environment. (2) Exponential: Rapid growth and often cell division occurs when there is an appreciable amount of cells and microalgae grow very rapidly. (3) Declining relative growth: This phase occurs when a growth requirement for cell division is limiting. (4) Stationary: Cell division slows due to the lack of resources necessary for growth. (5) Death/lysis: Cells begin to die due to a lack of nutrients (Fig. 1).[1]

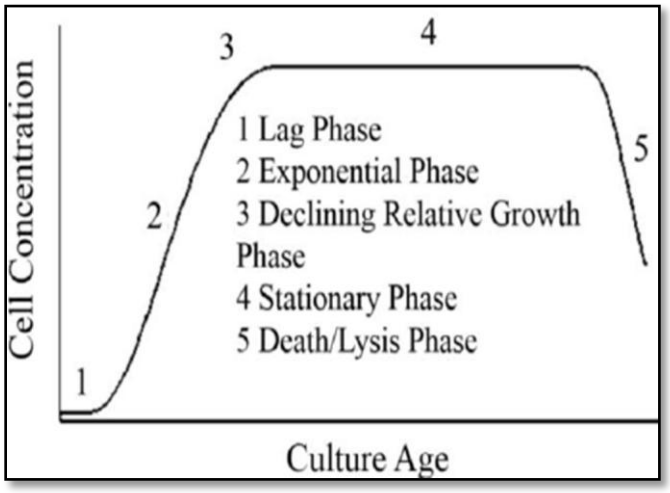

FIG.1 SCHEMATIC GROWTH CURVE OF A MICROALGAL BATCH CULTURE SYSTEM.

Microalgae are easy to cultivate and can tolerate a broad range of pH, salinity, and temperature. They commonly double every 24 hours. During the peak growth phase, some microalgae can double every 3.5 hours. Oil contents of microalgae are usually between 20% and 50% (dry weight), while some strains can reach as high as 80%. It can survive much more extreme conditions, which triggers this to generate higher lipid content.[1] Due to the very high diversity of microalgae, microalgal biomass is a rich source of many biochemical products that can be used in several industries including food, fuel, nutritional, cosmetic, and pharmaceutical industries. The production of the products by microalgae depends on species and culture conditions. Therefore, there are opportunities to recover microalgal biomass and their biochemical products with a certain amount by selecting the suitable species and manipulating the medium components and culture conditions.[10]

The main factors could be carbon dioxide availability, light intensity and photoperiod, culture temperature and pH, salts, and other nutrients. Protein is one of the main components in microalgal biomass. [11]

To consider microalgae as a viable feedstock, the overall energy and carbon balance must be favorable. Comparing biomass production systems in terms of net energy ratio (NER), a positive energy balance should be required. When NER is defined as the sum of the energy used for cultivation, harvesting, and drying, divided by the energy content of the dry biomass, then, if it is less than unity, the process produces more energy than it consumes.[12] Floatation, centrifugation, coagulation-flocculation, and filtration are some of the most used ways for separation.[13] Lyophilization, oven drying, or forced air drying also causes an additional cost. For these reasons, commercial-grade algal oil production is still not cost-effective.[14]

CULTURE SYSTEMS

At present, approximately 5000 tons of dry algae are produced per year worldwide. It is considered green gold for the future. Several cultivation techniques are currently

employed for the large-scale production of microalgae biomass.[15] Microalgae can be cultivated in systems mainly classified into open and closed. An open system exploits sunlight, and it is widely exposed to the environment, resulting in an important advantage due to the usage of free natural resources. The closed systems or photo bioreactors (PBRs) can be classified into tubular, column, membrane, and flat plate. PBR is used due to the higher yield of biomass and better control opportunity. Recently, many researchers try to combine these two to obtain the best case There are advantages and drawbacks for each culture system.[16]

a) CLOSED BIOREACTORS

A PBR is a closed or mostly closed vessel for phototrophic production where energy is supplied via electric lights. PBR can be located indoors or outdoors depending upon the light collection and distribution systems and their commercial feasibility. In PBR, the culture medium is enclosed in a transparent array of tubes or plates, and the microalgal broth is circulated from a central reservoir. PBR can be a different kind like polyethylene bags, glass fibers, cylinder, flat modular photo bioreactor, tubular inclined, segmented glass plate, and annular photobioreactor.[17]

The main objective of any PBR is the reduction of biomass production costs. To achieve the goal, numerous studies have been done on catalysts improvement, shaping of the PBR, controlling environmental parameters during cultivation, and aseptic designs. The controlling of operational parameters such as pH, temperature, and gas diffusion is also a vital issue in PBR.[4]

Closed bioreactors support up to fivefold higher productivity concerning reactor volume and consequently have a smaller 'footprint' on a yield basis. Besides saving water, energy, and chemicals, closed bioreactors have many other advantages which are increasingly making them the reactor of choice for biofuel production as their costs are reduced. Closed bioreactors permit essentially a single-species culture of microalgae for prolonged durations.[18] The most common type of closed bioreactor is the tubular photobioreactor.[14] Tubular photo bioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass. The microalgal broth is circulated from a reservoir to the solar collector and back to the reservoir.[19]

- Column photo bioreactors (bubble and airlift) Airlift and bubble column PBRs are simple cylinder devices with a radius, which should not exceed 0.2 m to avoid problems related to the light availability in the center of PBR and a height limitation of about 4 m for structural reasons, due to the strength of the transparent materials employed and to avoid shading effects. They are commonly used in bioprocessing and wastewater treatment because of their low cost. They have low shear forces, absence of wall growth, high mass transfer, consequently high efficiency of CO_2 use. The operating efficiency and the maximum biomass productivity are strictly related to the column dimensions, to the specific growth rate or algae strains doubling time, the intensity of light, and surface area.[20] Based on aeration mode, column PBRs can be divided into bubble column and airlift reactor (Fig.2). However, the bubble size is a function of several factors such as the properties of sparger, liquid, and gas phase physical properties and the ratio H/D of the column. It has to take into account also phenomena of bubble coalescence or breakage and the possibility of clogging effects due to the presence of micron size algae, more common at high biomass concentration and high-pressure drop.[21] The bubble size distribution in the column is very important because the size of gas bubbles at the top decides the down comer gas holdup, which then results in specific liquid circulation velocity and the light/dark cycles.[22]

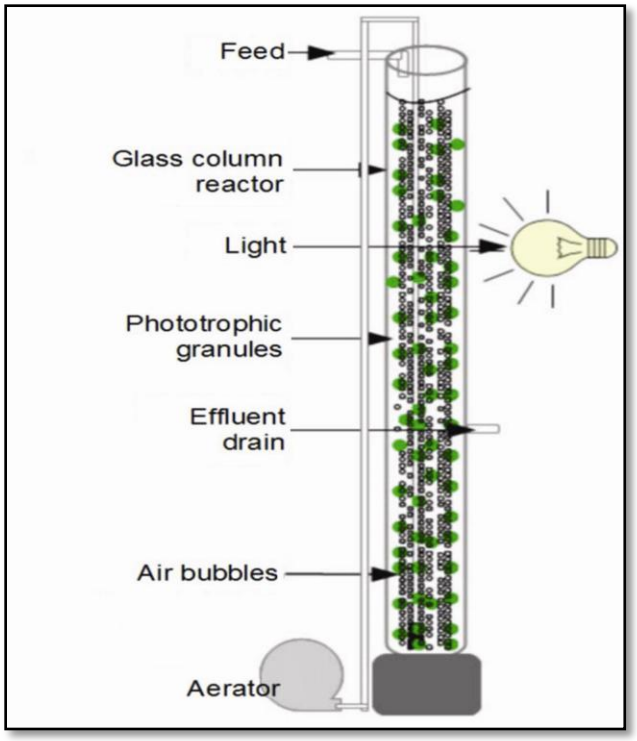

Fig.2 Bubble column photobioreactor

Airlift PBRs have the substantial advantages of low-operational power consumption and less shear stress exerted on the cells. Furthermore, the airlift reactor has a defined circular fluid flow and relatively better gas-liquid mass transfer performance. Column airlift PBR is one of the most promising configurations for the industrial production of microalgae. However, their high capital cost and cleaning problems are the main obstacles to their large-scale implementation.[23]

b) OPEN POND SYSTEM

Open ponds are the oldest and simplest systems for the mass cultivation of microalgae. 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.[24] The system is often operated in a continuous mode.[11] Open-pond systems are shallow ponds in which algae are cultivated. Nutrients can be provided through runoff water from

nearby land areas or by channeling the water from sewage/water treatment plants. The water is typically kept in motion by paddlewheels or rotating structures, and some mixing can be accomplished by appropriately designed guides.[25] Open architecture approaches while possibly the cheapest of all current techniques, and suffer challenges with contamination, evaporation, temperature control, CO_2 utilization, and maintenance ability open-pond "algae farm" systems.[26]

Photosynthesis is the most important biochemical process in which plants, algae, and some bacteria harness the energy of sunlight to produce food.[27] The photobioreactor system of biomass culture must be approved to achieve high and sustained growth rates and oil yields that are essential to developing the algal-based biofuel industry.[28]

c) Mixed Microalgae Culture System

Heterotrophic and mixotrophic algae are very interesting to some researchers, as they grow 24 hours a day compared with the autotrophic algae that grow 12 hours a day.[29] Heterotrophic algae are independent of the light source, which makes their growth condition energy-intensive and their surface-to- volume ratio lower than PBRs and open ponds. Some researchers suggest that heterotrophic growth systems may be better for producing high biomass and high lipid contents.[30,31]

ALGAE GROWTH FACTOR

Major Key components for algal growth are a growth medium with proper nutrients, a light source for photosynthesis, and CO_2 or airflow. All of these growth factors must be specified for successful microalgae cultivation for a specific purpose, which can vary from species to species. The factors can be divided into three categories (Table 1).[32]

TABLE 1 GROWTH FACTORS OF MICROALGAE

Categories of Factors	Factors/parameters
Environmental factors	• pH • Nutrients • Temperature
Processing parameters	• Mixing • Light intensity
Biotic factors	• Invasive species a

a) ENVIRONMENTAL FACTORS

- **pH**

pH measures the level of acidity or alkalinity that a body of water has. Most algae have pH optima for growth generally and photosynthesis ability in neutral to alkaline pH range. Variation of pH affects growth in several ways. pH increases during daytime caused by photosynthetic CO_2 assimilation by the algae followed by a decrease in pH at night due to the respiratory process of the community. Microalgae have been shown to cause a rise in pH to 10-11 because of CO_2 uptake photo synthetically to convert its biomass. When pH increases too high, photosynthesis can be limited due to the scarcity of $CO2$.[33]

- **NUTRIENTS**

To grow algae, macronutrients should contain nitrogen and phosphorus mainly silicon is also required for saltwater algae. In addition, trace metals, such as Fe, Mg, Mn, B, Mo, K, Ca, and Zn, are also needed. CO_2 fixation is also necessary to build up a balanced medium for optimum growth. Microalgae biomass usually consists of around 40%-50% carbon, 4% -8% nitrogen, and 0.1% phosphate by dry weight. Recently, researchers are focused upon a two-phase growth system, where in the first phase, algae are grown in a nutrient-abundant medium and then transferred to a nutrient-deficient medium where lipid accumulation is boosted up [33].

- **TEMPERATURE**

Most algae of interest for lipid production have a temperature tolerance between 15 and 40 degrees Celsius. Researchers have shown that many of the oil-producing algae species grow best between 25 and 30 degrees Celsius. The optimal growth temperature varies by species and desired algae response. But controlling the temperature in outdoor conditions is tough and expensive. Not only extreme temperature but also evaporation of media, overheating and cooling at outdoor conditions, and lipid composition are also major issues for algal growth. Later it was found that higher temperature is responsible for saturated lipid accumulation and lower temperature for unsaturated lipid accumulation.[33]

b) PROCESSING PARAMETERS

- **MIXING**

Mixing is important to prevent sedimentation of algae and to move the algae between the light and dark regions of the pond/reactor. Without any forced mixing, algae at the surface absorb all the available light and can become photo inhibited, while algae deeper in the media are light deprived. Mixing can be provided in several ways such as open ponds using a mechanical stirrer (a paddlewheel in raceways) and bubbling in gas (air, CO_2) to provide mixing. PBRs use pumps and bubbling in gas for mixing. It is also noted that many microalgae species cannot tolerate vigorous mixing.[34]

- **LIGHT INTENSITY**

When algae are cultivated photo synthetically, the efficiency of photosynthesis is a crucial determinant in their productivity since it affects the growth rate, biomass production, and lipid accumulation. The effect of light intensity depends on the depth of the culture medium and the density of the algal biomass. If the depth and cell concentration of the culture is higher, light intensity must be increased to penetrate through the medium. On the other hand, direct sunlight or high-density artificial light may act as a photo inhibitor. Overheating caused is also unexpected and should be

avoided. It is suggested that light intensity of 1000 lumens is suitable for the culture in Erlenmeyer flasks; 5000-10000 is required for larger volumes. A light/dark system is required for the efficient photosynthesis of microalgae because the light is needed for the photochemical phase to produce ATP and NADPH and dark for the biochemical phase to synthesize essential biomolecules for microbial growth.[35]

c) BIOTIC FACTORS

- **PREDATORS**

Invasive species and predators can be any kind of living organism that is unexpected in the microalgae culture area because they inhibit microalgae growth, pollute the culture medium, and deficit the nutrient. Predators may be fungus, bacteria, insects, and even unwanted microalgae species. To avoid any potential issues from invasive and predator species, industrial algae growth is mostly limited to extremophile algae species, which can grow in extreme environments in which competing species are unable to survive. PBRs prevent this by keeping the algae contained from the outside environment.[34]

PIGMENTS IN MICROALGAE

One of the most obvious and arresting characteristics of algae is their color. In general, each phylum or group has a particular combination of pigments and individual colors. Aside from chlorophylls, microalgae also have various accessory or secondary pigments, such as phycobilin proteins and a wide range of carotenoids. These natural pigments function as antioxidants in plants as well as Humans. Therefore, microalgae are recognized as an excellent source of natural colorants and nutraceuticals.[36]

MICROALGAE AS AN ALTERNATIVE

The composition of the biomass is useful for characterizing the best use of microalgae species. Algal biomass contains three main components: carbohydrates, proteins, and lipids/natural oil. For example, with the knowledge that biodiesel is made from oils, a microalga with very high protein content and low lipid content would not be useful as a biofuel feedstock.[37] It is found that the bulk of the natural oil made by microalgae is in the form of triacylglycerides (TAGs), which is the right kind of oil for producing biodiesel. The fatty acids attached to the TAG within the algal cells can be both short- and long-chain hydrocarbons. The shorter chain length acids are ideal for the creation of biodiesel, and some of the longer ones can have other beneficial uses.[38]

MATERIALS AND METHODS

COLLECTION OF ALGAL STRAINS

The mixed microalgae culture was collected from the pond which is used for growing Lilly, was used for mass cultivation in a column photo bioreactor. The algal blooms were collected in a polyethylene bottle and were centrifuged. This was then cultivated in Bold basal media (BBM). The inoculum for the photo bioreactors was grown indoors under artificial light in a 2-liter bubble column photo bioreactor.

DESIGN OF COLUMN PHOTOBIOREACTOR FOR MICROALGAE CULTIVATION

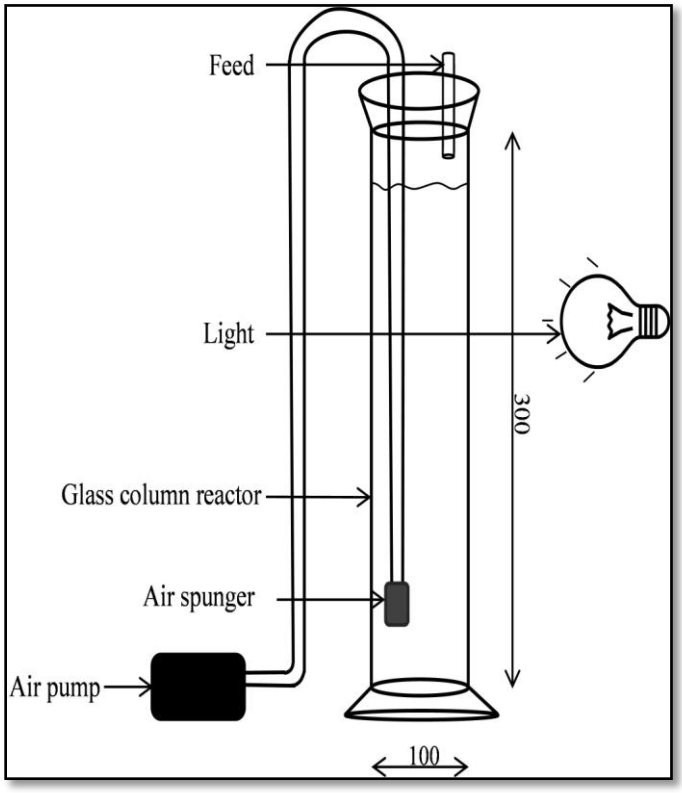

FIG.3 SCHEMATIC REPRESENTATION OF PHOTOBIOREACTOR (ALL DIMENSIONS IN MM)

The schematic diagram of the experimental apparatus used in this study is shown in Fig.3. Measurements were made in a bubble column photo bioreactor. The system was built in 4 mm thick borosilicate glass, an internal diameter of 10 cm, a height of 30 cm, and a nominal working volume of 2 liters. The dispersion system for the reactor consisted of a 0.5 cm diameter air diffuser located in the center of the column. The reactor was continuously illuminated with a 9 W LED fluorescent lamp with 900 lumens light intensity. The light arrangement gives the desired light intensity.

The initial pH is given as 6.5 ± 0.2. The temperature of the system was uncontrolled and closely monitored as 28 ± 3 °C. Airflow into the photo bioreactor was provided via filtered air through an air pump. An arrangement for inlet feed is given on top of the reactor system.

BOLD'S BASAL MEDIUM (BBM)

TABLE 2 INGREDIENTS OF BBM MEDIUM

Reagent	Stock solution concentration (g/100ml)	Amount in culture medium per liter
KH_2PO_4	1.75	10ml
$CaCl_2.2H_2O$	0.25	10ml

$MgSO_4.7H_2O$	0.75	10ml
$NaNO_3$	2.5g	10ml
K_2HPO_4	0.75	10ml
NaCl	0.25	10ml
NA_2EDTA with KOH	1.9	1ml
$FeSO_4.7H_2O$ with H_2SO_4	0.498 + 0.1	1ml
H_3BO_3	0.25	0.7ml
Trace metal solution	-	1ml

The medium is highly enriched and is used for green algae. For preliminary culture, microalgae growing water from the pond was transferred to the diluted BBM medium. It was kept for 10 days for the development of microalgal colonies and the pH was maintained. After seven days onwards, the algal colonies started growing in the medium and were allowed to grow for another 7 days. After 14 days, the fully grown algal culture medium was ready for cultivation. Table 2 shows the ingredients in BBM medium.

MICROALGAL CULTIVATION

For microalgal culture, a photo bioreactor was constructed having a capacity of 2 liters, in which two-liter water along with 20 ml BBM medium was filled as shown in Fig.4.

FIG.4 MICROALGAE CULTIVATION IN BUBBLE COLUMN PBR

From the previously collected algal biomass, a total of 2 grams were inoculated into the photo bioreactor. Mixed thoroughly and proper aeration was given. pH and temperature were given constant which varies accordingly and light intensity was given 9W, 900 Lumens until the experiment.

HARVESTING & PROCESSING OF ALGAL BIOMASS

a) GRAVITY SEDIMENTATION

Algae harvesting was done using gravity sedimentation. The algal medium is set to stand in a glass reactor of 2 liters, where the algae sink to the bottom. The collected algal water is kept for settling for about 48 hours, which is nearly 2 days. The bubble column photo bioreactor capacity was about 2 liters. Later the biomass is filtered out and oven-dried. Fig.5 shows the collection of algal water kept for gravity settling.

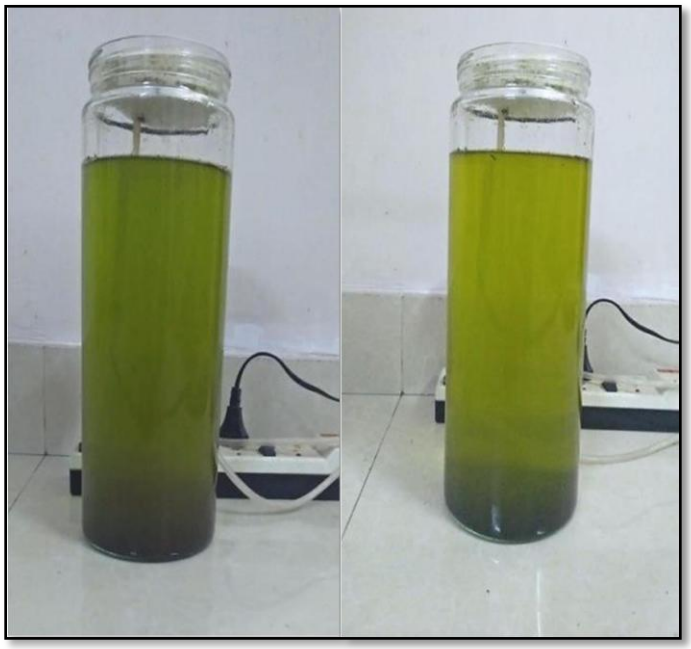

FIG.5 ALGAL WATER KEPT FOR GRAVITY SEDIMENTATION. I) AFTER 24 HOURS, II) AFTER 48 HOURS

b) DEWATERING AND FILTRATION

After gravity sedimentation, the algal solution is taken for the dewatering process. The extracellular water is drained off. Draining of water at the top layer is done by using a physical strainer. The biomasses obtained in the strainer and at the bottom of the bottles are collected in a vessel, shown in Fig.6. The weight of the biomass along with the vessel is measured and noted. The empty weight of the vessel is also measured and noted.

FIG.6 THE FILTERED BIOMASS OBTAINED AFTER SETTLING FOLLOWED BY DEWATERING.

c) DRYING

It may be necessary to dry the biomass almost completely (up to 95% dry matter). The intracellular water remaining in the cells after drainage of the algal suspension must be removed by oven drying. The collected biomass is oven-dried at a temperature of 50ºC for about 1 to 2 hours. After drying, the algal- biomass was finely powdered, weighed, and then stored for further analysis. Fig.7 shows biomass obtained after oven-drying for 1 - 2 hours.

FIG.7 BIOMASS OBTAINED AFTER OVEN DRYING FOR ABOUT 1- 2 HOURS

d) BIOMASS ESTIMATION

The gravimetric method was employed for the quantitative estimation of biomass From the photo bioreactor, nutrient was taken and filtered through a pre-weighed What man No:1 filter paper. It was then dried in an oven at 50^{0}C for 1-2 hours and reweighed. The differences between observations were calculated as per the following equation (1);

$$\text{Biomass (dry weight in Kg)} = \frac{B-Æ}{s} \qquad (1)$$

Where,

B = Weight of vessel with dried biomass

A = Dry weight of the vessel

x = Volume of photo bioreactor

RESULT AND DISCUSSION

BIOMASS ESTIMATION

The growth of microalgal culture was observed at 7 days intervals using a change in color and nutrient solution (BBM) of 2 ml were added at 3 days intervals. From the initial to the seventh day, there was no visible growth and the growth was observed after the seventh day. On the tenth day, the biomass started to show color changes and thickening (Fig.8).

FIG.8 COLOUR CHANGE OBSERVATION I) INITIAL STAGE II) FINAL STAGE

After 56 days, the culture is set for sedimentation for 48 hours. The culture is settled at the bottom and the water at the top layer was drained out. The culture was dried under sunlight followed by oven-dried at 50^{0}C for 1 to 2 hours. After drying, the algal-biomass was finely powdered, weighed, and then stored for further analysis. The dry weight of biomass is estimated as 8 grams per liter

$$\text{Biomass (dry weight in g)} = \frac{126-110}{2}$$

$$= 8 \text{ gram per liter}$$

Biomass production increased with the availability of carbon dioxide and nutrients from the medium. The

physicochemical parameters like pH and dissolved carbon dioxide have a significant role in the biomass production of Microalgal. The increase of biomass in the photo bioreactor is also affected by the light intensity and temperature. The ions in the BBM medium get disintegration due to the growth and uptake of nutrients from the medium, which is later dissolved into the surrounding water. Higher concentrations of inorganic salts harm the lipid accumulation capacities of algae. The variation in the growth can be observed at regular intervals of time. The harvesting process should be done carefully for a good result.[14]

CONCLUSION

Due to the increased need for fossil fuels, the world looks into alternative sources of energy to meet the increase. Research is going on the developed nations. Developing countries like India have a vast area of water bodies, with more species of microalgae. Limited research work is going on in different parts of the country as per the available literature. The present study aims to screen the potential of certain local freshwater microalgae for biomass production for lipid enhancement. It has been observed that the pond has quite a good production of microalgae species. The observations from the pond and the literature available could understand that the possibility for the culture of microalgae in a photo bioreactor, which can be later used for the production of biofuel. The physicochemical parameters like pH, alkalinity, dissolved carbon dioxide, etc. have a significant role in the biomass production of microalgal cells. The alkalinity has shown an increasing trend corresponding with algal growth. Biomass production increased with the availability of nutrients from the medium. The results of the present study showed that the biomass content is comparatively less than the published reports. It may be due to the lack of proper extraction and the absence of the sonication process. The present study is considered as a preliminary screening test for the production of biofuels by the microalgal species and the analysis part has certain limitations as it is a trial and error study. Hence it can be concluded that the present mixed microalgal culture has the potential for the production of lipid enhancement.

REFERENCES

1. Yousuf A. Fundamentals of microalgae cultivation. In: *Microalgae Cultivation for Biofuels Production*. Elsevier; 2019:1-9.

2. Collotta M, Champagne P, Mabee W, Tomasoni G. Wastewater and waste CO2 for sustainable biofuels from microalgae. *Algal Res*. 2018;29(November 2017):12-21.

3. Huang J, Wan M, Jiang J, Zhang A, Zhang D. Evaluating the effects of geometry and arrangement parameter of flat panel photo bioreactor on microalgae biomass production and economic performance in China. *Algal Res*. 2021;57(October 2020):102343.

4. Plouviez M, Shilton A, Packer MA, Guieysse B. N2O emissions during microalgae outdoor cultivation in 50 L column photo bioreactors. *Algal Res*. 2017;26(June):348-353.

5. Bajpai R, Prokop A, Zappi M. Algal biorefineries: Volume 1: Cultivation of cells and products. *Algal Biorefineries Vol 1 Cultiv Cells Prod*. Published online 2014:1-324.

6. Jacob-Lopes E, Cacia Ferreira Lacerda LM, Franco TT. Biomass production and carbon dioxide fixation by Aphanothece microscopic Nägeli in a bubble column photo bioreactor. *Biochem Eng J*. 2008;40(1):27-34.

7. Chokshi K, Pancha I, Maurya R, et al. Growth medium standardization and thermo tolerance study of the freshwater microalga Acutodesmus dimorphus—a potential strain for biofuel production. *J Appl Phycol*. 2016;28(5):2687- 2696.

8. Pavlik D, Zhong Y, Daiek C, et al. Microalgae cultivation for carbon dioxide sequestration and protein production using a high-efficiency photobioreactor system. *Algal Res*. 2017;25(June):413-420.

9. Moreno-Garcia L, Gariépy Y, Barnabé S, Raghavan GSV. Effect of environmental factors on the biomass and lipid production of microalgae grown in wastewaters. *Algal Res*. 2019;41(April):101521.

10. Borowiak D, Lenartowicz P, Grzebyk M, Wiśniewski M, Lipok J, Kafarski P. Novel, automated, semi-industrialmodular photo bioreactor system for cultivation of demanding microalgae that produce fine chemicals—The next story of H. Pluvialis and astaxanthin. *Algal Res*. 2021;53(June 2020).

11. Sreekumar N, Haridas A, Godwin GS, Selvaraju N. Lipid enhancement in microalgae by temporal phase separation: Use of indigenous sources of nutrients. *Chinese J Chem Eng*. 2018;26(1):175-182.

12. Chu F, Cheng J, Zhang X, Ye Q, Zhou J. Enhancing lipid production in microalgae Chlorella PY-ZU1 with phosphorus excess and nitrogen starvation under 15% CO2 in a continuous two-step cultivation process. *Chem Eng J*. 2019;375(June):121912.

13. Sreekumar N, Giri Nandagopal MS, Vasudevan A, Antony R, Selvaraju N. Marine microalgal culturing in open pond systems for biodiesel production - Critical parameters. *J Renew Sustain Energy*. 2016;8(2).

14. Tan J Sen, Lee SY, Chew KW, et al. A review on

microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids. *Bioengineered*. 2020;11(1):116-129.

15. Singh AK, Sharma N, Farooqi H, Abdin MZ, Mock T, Kumar S. *ACCEPTED MANUSCRIPT Phytoremediation of Wastewater by Alga Phytoremediation of Municipal Wastewater by Microalgae to Produce Biofuel*.

16. Cui X, Yang J, Cui M, Zhang W, Zhao J. Comparative experiments of two novel tubular photo bioreactors with an inner aerated tube for microalgal cultivation: Enhanced mass transfer and improved biomass yield. *Algal Res*. 2021;58(January):102364.

17. Liang Y, Kashdan T, Sterner C, et al. *Algal Biorefineries*. Elsevier B.V.; 2015.

18. Yen HW, Hu IC, Chen CY, Chang JS. *Design of Photo bioreactors for Algal Cultivation*. Elsevier B.V.; 2013.

19. Abbott MSR, Brain CM, Harvey AP, Morrison MI, Valente Perez G. Liquid culture of microalgae in a photo bioreactor (PBR) based on the oscillatory baffled reactor (OBR) technology - A feasibility study. *Chem Eng Sci*. 2015;138:315-323.

20. Fu J, Huang Y, Liao Q, Xia A, Fu Q, Zhu X. Photo-bioreactor design for microalgae: A review from the aspect of CO2 transfer and conversion. *Bioresour Technol*. 2019;292.

21. Musa M, Ayoko GA, Ward A, Rösch C, Brown RJ, Rainey TJ. Factors Affecting Microalgae Production for Biofuels and the Potentials of Chemometric Methods in Assessing and Optimizing Productivity. *Cells*. 2019;8(8).

22. Acién FG, Molina E, Reis A, et al. Photo bioreactors for the production of microalgae. In: *Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-Products*. Elsevier Inc.; 2017:1-44.

23. Huang Q, Jiang F, Wang L, Yang C. Design of Photobioreactors for Mass Cultivation of Photosynthetic Organisms. *Engineering*. 2017;3(3):318-329.

24. Banerjee S, Ramaswamy S. Comparison of productivity and economic analysis of microalgae cultivation in open raceways and flat-panel photo bioreactor. *Bioresour Technol Reports*. 2019;8:100328.

25. Karthikeyan D, Muthukumaran M, Balakumar BS. *Mass Cultivation of Microalgae in Open Raceway Pond for Biomass and Biochemicals Production*. Vol 3.; 2016.

26. Pankratz S, Kumar M, Oyedun AO, Gemechu E, Kumar A. Environmental performances of diluents and hydrogen production pathways from microalgae in cold climates: Open raceway ponds and photo bioreactors coupled with thermochemical conversion. *Algal Res*. 2020;47(May 2019):101815.

27. Handler RM, Canter CE, Kalnes TN, et al. Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: Analysis of the prior literature and investigation of the wide variance in predicted impacts. *Algal Res*. 2012;1(1):83-92.

28. Slegers PM, Lösing MB, Wijffels RH, van Straten G, van Boxtel AJB. Scenario evaluation of open pond microalgae production. *Algal Res*. 2013;2(4):358-368.

29. Bartley ML, Boeing WJ, Dungan BN, Holguin FO, Schaub T. pH effects on growth and lipid accumulation of the biofuel microalgae Nannochloropsis salina and invading organisms. *J Appl Phycol*. 2014;26(3):1431- 1437.

30. Zuccaro G, Yousuf A, Pollio A, Steyer JP. Microalgae cultivation systems. In: *Microalgae Cultivation for Biofuels Production*. Elsevier; 2019:11-29.

31. Holdt SL, Christensen L, Iversen JJL. A novel closed system bubble column photo bioreactor for detailed characterization of micro-and macroalgal growth. *J Appl Phycol*. 2014;26(2):825-835.

32. Assunção J, Malcata FX. Enclosed are "non-conventional" photo bioreactors for microalga production: A review. *Algal Res*. 2020;52.

33. Venkata Mohan S. *Reorienting Waste Remediation Towards Harnessing Bioenergy: A Paradigm Shift*. Elsevier Ltd.; 2014.

34. Costa JAV, Freitas BCB, Santos TD, Mitchell BG, Morais MG. *Open Pond Systems for Microalgal Culture*. Second Edi. Elsevier B.V.; 2019.

35. Zhou W, Lu Q, Han P, Li J. Microalgae cultivation and photo bioreactor design. In: *Microalgae Cultivation for Biofuels Production*. Elsevier; 2019:31-50.

36. Mubarak M, Shaija A, Prashanth P. Bubble column photo bioreactor for Chlorella pyrenoidosa cultivation and validating gas hold up and volumetric mass transfer coefficient. *Energy Sources, Part A Recover Util Environ Eff*. Published online 2019.

37. Amaral MS, Loures CCA, Naves FL, Baeta BEL, Silva MB, Prata AMR. Evaluation of cell growth

performance of microalgae Chlorella minutissima using an internal light integrated photo bioreactor. *J Environ Chem Eng*. 2020;8(5).

38. Fal S, Benhima R, El Mernissi N, Kasmi Y, Smouni A, El Arroussi H. Microalgae as a promising source for integrated wastewater treatment and biodiesel production. *Int J Phytoremediation*. Published online 2021.