



## MIXED MICROALGAE CULTIVATION IN OPEN POND REACTOR FOR BIOMASS EXTRACTION

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### ABSTRACT:

The need for clean, biodegradable, and carbon-neutral alternative energy has led the focus on microalgae as a biofuel source. Microalgae are considered a future source of biofuel, because it does not compete with agricultural land and freshwater resources, and has the potential to be cultivated in quantities required for substituting mineral oil. Microalgae have been recommended as a superior candidate for fuel production because of their advantages of higher photosynthetic efficiency, biomass & lipid productivity, faster growth rate as compared to other energy crops. To meet up all these criteria, we have developed a continuous outdoor microalgal open pond reactor. Open pond reactors are shallow artificial ponds used in the cultivation of microalgae which is the most economical method for algal culture. An attempt to utilize indigenous sources of nutrients to improve the economics also revealed that microalgal culturing can also be used as a mode of nutrient removal and water treatment. The photosynthetic rate and lipid production was enhanced by arresting daytime cell division and promoting night-time cell division. Large-scale microalgal biomass production for application in biofuel production can be promoted through the use of open ponds, combined with economically viable strategies, such as microalgae-based biorefineries. The advantages of open ponds, such as the relatively low investment and lower energy costs, combined with biorefinery concepts can be considered one of the best options for microalgal biofuel production.

### KEYWORDS:

**MICROALGAE, BIOFUEL, BIOMASS, LIPID PRODUCTIVITY, OPEN POND REACTOR, ALGAL CULTURE.**

### INTRODUCTION

Microalgae or microphytes 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 (Jones & Mayfield, 2012)<sup>1</sup>. 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 (Pate et al., 2011)<sup>2</sup>. Although microalgae have a wide range of applications such as food supplements, lipids, enzymes, biomass, polymers, toxins, pigments, tertiary wastewater treatment, and "green energy," in the past few decades, it has been considered a highly potential source of biofuels. Algae are having both plant-like and animal-like characteristics.<sup>3</sup> Plant-like algae are typically found in aquatic environments that contain chloroplasts and are capable of photosynthesis.<sup>4</sup> Unlike higher plants, algae lack vascular tissue and do not possess roots, stems, leaves, or flowers. Animal-like algae are having flagella and centrioles and are capable of feeding on organic material in their metabolism.<sup>5</sup>

The size of algae varies from a single cell to giant multicellular species, and they can survive or grow in multivariate environments such as in freshwater, saltwater, or wet soil, or on the moist rock.<sup>6</sup> They can be reproduced sexually or asexually or by a combination of both processes through an alternation of generations.<sup>7</sup> Algae have been used in the past as a way to recycle some of the nutrients from wastewater sources and also as a step in industrial wastewater treatment. As long as the world's population continues to grow, the source of wastewater only gets greater and greater.<sup>8</sup> Utilizing this harmful product as a nutrient source, microalgae are capable of producing lipid that is considered a potential source of biodiesel.<sup>9</sup>

However, three aspects of microalgae production that will strongly influence the future sustainability of algal biofuel production are the energy and carbon balance, environmental impacts, and production cost.<sup>10</sup> To consider microalgae as a viable feedstock, the overall energy and carbon balance must be favorable. Recovery and preparation of microalgal biomass for trans esterification reaction requires more than 20%-30% of the total cost of

biofuel preparation.<sup>11</sup> Floatation, centrifugation, coagulation-flocculation, and filtration are some of the most used ways for separation, however, no single best method for harvesting is not yet involved.<sup>12</sup> Most methods used to extract the oil from algal biomass rely on a dry biomass product. 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.<sup>13</sup> However, microalgae are given priority over other energy crops because of the following: They can be cultivated in non-arable land.<sup>14</sup> They can be grown on ponds and photo bioreactors. They can tolerate a wide range of pH, salinity, and temperature.<sup>15</sup> They are continually cultivable, not seasonally harvested. They can mitigate CO<sub>2</sub> from industrial and atmospheric sources.<sup>16</sup> Complete usage of biomass (proteins, lipids, and carbohydrates). They are not a finite resource, i.e., renewable and sustainable.<sup>17</sup>

## ALGAE MASS CULTIVATION SYSTEM

### a. PHOTOBIOREACTOR

A photo bioreactor (PBR) is a closed or mostly closed vessel for phototrophic production where energy is supplied via electric lights. PBRs can be located indoors or outdoors depending upon the light collection and distribution systems and their commercial feasibility.<sup>18</sup> 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.<sup>19</sup> PBR can be a different kind like polyethylene bags, glass fibers cylinder, flat modular photo bioreactor, tubular inclined, segmented glass plate, and annular photobioreactor.<sup>20</sup> 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.<sup>21</sup>

Closed bioreactors support up to fivefold higher productivity concerning reactor volume and consequently have a smaller 'footprint' on a yield basis.<sup>22</sup> 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. Most closed bioreactors are designed as tubular reactors, plate reactors, or bubble column reactors. Other less common designs like semi-hollow-spheres have been reported to run successfully.<sup>23</sup> The most common type of closed bioreactor is the tubular photo bioreactor. Tubular photo bioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass.<sup>24</sup> The solar collector tubes are generally 0.1 m diameter or less in diameter because light does not penetrate too deeply in the dense culture broth that is

necessary for ensuring high biomass productivity of the photo bioreactor. Microalgae broth is circulated from a reservoir to the solar collector and back to the reservoir.<sup>25</sup>

### b. OPEN POND

Open pond cultivation system typically consists of a simple water tank or bigger earthen-bank ponds where nutrients are added from outsourcing. Natural light plays a role in photosynthesis, and CO<sub>2</sub> comes from the atmosphere.<sup>26</sup>

The pond is usually designed in a raceway or track configuration, in which a paddlewheel or physical mixing provides circulation and mixing of 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.<sup>27</sup> Baffles in the channel guide the flow around bends that also minimize space and loss. Medium is added in front of the paddlewheel, and algal broth is harvested behind the paddlewheel after it has circulated through the loop. They have lower energy consumption and probably lower energy consumption and lower construction and operating costs.<sup>28</sup> Open cultivation system generally consists of a simple water tank or bigger earthen ponds in which nutrients are supplied from outsource.<sup>29</sup>

Natural light plays an important role in photosynthesis and carbon dioxide comes from photosynthesis. The ponds are kept shallow because of the need to keep the algae exposed to sunlight and the limited depth to which sunlight can penetrate the pond water.<sup>30</sup> The ponds are operated continuously; that is, water and nutrients are constantly fed to the pond while algae-containing water is removed at the other end. Open culture is used commercially in the USA, Japan, Australia, India, Thailand, China, Israel, and elsewhere to produce algae for food, feed, and extraction of metabolites.<sup>33</sup> Open-culture systems allow relatively inexpensive production but are subject to contamination. Consequently, only a few algal species can be cultured in open outdoor systems. Species that grow successfully in the open include rapid growers such as *Chlorella* and species that require a highly selective extremophilic environment that does not favor the growth of most potential contaminants. For example, species such as *Spirulina* and *Dunaliella* thrive in highly alkaline and saline selective environments, respectively. Algae produced in quantities in open systems include *Spirulina*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Anabaena*, and *Nostoc*.<sup>32</sup>

### ALGAE GROWTH FACTOR

Major Key components for algal growth are a growth medium with proper nutrients, a light source for photosynthesis, and CO<sub>2</sub> 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. These factors can be divided into three categories (Table 1).<sup>34</sup>

**TABLE.1 GROWTH FACTORS OF MICROALGAE.<sup>34</sup>**

| Categories            | Factors  |
|-----------------------|--|
| Environmental factors | <ul style="list-style-type: none"> <li>• pH</li> <li>• Nutrients</li> <li>• Temperature</li> </ul> |
| Processing parameters | <ul style="list-style-type: none"> <li>• Mixing</li> <li>• Light intensity</li> </ul>              |
| Biotic factors        | <ul style="list-style-type: none"> <li>• Invasive species and predators</li> </ul>                 |

## 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 6.0 to 8.0 and photosynthesis ability in the neutral to alkaline pH range. Variation of pH affects growth in several ways. pH increases during daytime caused by photosynthetic CO<sub>2</sub> assimilation by the algae followed by a decrease in pH at night due to the respiratory process of the community. There is a relationship between CO<sub>2</sub> concentration and pH of microalgal culture medium.<sup>35</sup>

It is related to the chemical equilibrium among chemical species such as CO<sub>2</sub>, H<sub>2</sub>CO<sub>3</sub>, HCO<sub>3</sub><sup>-</sup>, and carbonate. The equilibrium of these species is pH- dependent when CO<sub>2</sub> is predominant at pH below 7.0 and carbonate predominant above pH 10.0. Microalgae have been shown to cause a rise in pH to 10.0 - 11.0 because of CO<sub>2</sub> uptake photo synthetically to convert its biomass. Increasing CO<sub>2</sub> can lead to higher biomass accumulation but leads to lower pH value, which hurts microalgal physiology. However, when pH increases too high, photosynthesis can be limited due to the scarcity of CO<sub>2</sub>.<sup>34</sup>

## 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<sub>2</sub> 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. In nature, some of these can be found easily from a mineral source, and some can be found by bacterial metabolism. Nitrogen is abundant in nature because it fixes by bacteria continuously, while phosphorus is limited, which is effectively bound as orthophosphate in sediment.<sup>35</sup>

Nutrient levels, especially nitrogen and phosphorus combined, are very important in the production of lipids. Many articles have shown that a nitrogen-deficient growth

medium triggers the algae to produce higher levels of lipids. With the same species, nitrogen limitation only induced a lipid content of 30%. Nutrient deficiencies and excess nutrients, both, can cause physiological and morphological changes in microalgae since they can inhibit some of the vital metabolic pathways. 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.<sup>34</sup>

### Temperature

Most algae of interest for lipid production have a temperature tolerance between 15 and 40°C. Researchers have shown that many of the oil-producing algae species grow best between 25°C and 30°C. The optimal growth temperature varies by species and the desired algae response. But controlling the temperature in the outdoor condition is tough and expensive. Not only the extreme temperature but also the 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. Contrarily, many researchers found there is no effect of temperature upon lipid accumulation. So, there exist contradictory views about this issue.<sup>34</sup>

### 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. It also helps to maintain a homogeneous cell concentration in the medium. Mixing can be provided in several ways such as open ponds use a mechanical stirrer (a paddlewheel in raceways) and bubbling in gas (air, CO<sub>2</sub>) to provide mixing. PBRs use pumps and bubbling in gas for mixing. It is also noted that many microalgae species cannot tolerate vigorous mixing.<sup>36</sup>

### 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.<sup>32</sup> Overheating caused by both natural and artificial illumination is also unexpected and should be avoided. It is suggested that light intensity of 1000 lux 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.<sup>31</sup>

#### 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. Open ponds are susceptible to invasion by low oil-producing algae strains, while PBRs prevent this by keeping the algae contained from the outside environment.<sup>34</sup>

## 2. MATERIALS AND METHODS:

### COLLECTION OF ALGAL STRAINS

The mixed microalgae culture was collected from the fish pond located at an aquarium shop. The algal blooms are collected in polyethylene bottles and were centrifuged. For preliminary culture, 1 liter of microalgae growing from the fish pond was transferred to the diluted BBM medium. After seven days onwards, the algal colonies started growing in the medium and their growth multiplied. The inoculums of the algal cultures to be used for outdoor mass cultivation have been prepared. The cultures were grown at  $24 \pm 10^\circ\text{C}$  in a thermostatically controlled room illuminated with cool white fluorescence lamps (Philips 9W, Cool daylight 6500K) at an intensity of 900 lumens in a 12 hrs light and dark regime. In an open pond reactor system, the temperature is not typically controlled and is, therefore, dependant on the sunlight regime, evaporation, and ambient air temperature.

#### Open pond construction for Microalgae cultivation

An outdoor algal raceway pond was constructed with a wall thickness of 10 cm. The inner dimensions of the pond were such that the length was 40cm and the width was 20 cm. The depth of the pond was kept at 15 cm keeping in mind the penetration of sufficient light for the growth of algae. The floor was constructed with a slight slope on either side of the partition in the opposite direction to enable proper mixing of the culture. The base floor was constructed using earthen bricks as due to its cheap availability and its ability to act as a strong base as shown in Fig.1. The bricklayer is completely covered using a tarpaulin sheet which is a strong, flexible water-resistant material so that water may not seep into the ground as shown in Fig.2.

The main advantages of this open pond reactor are lower energy consumption, lower construction and operation costs as the materials are easily available and cost-effective. Most widely used for commercial cultivation since they are cheap and less maintenance, easy to clean. The algal inoculums were added into the medium and grown with

daily stirring and harvesting of samples at every 5-day intervals. The growth properties of algae can be determined by measuring the cell number per unit volume of cell suspension. The pond requires constant stirring, mixing, and recirculation of the culture. The stirring system provides homogenous light to extreme microalgae.



**FIG.1 BASE CONSTRUCTION OF OPEN POND USING EARTHEN BRICKS**



**FIG.2 LAYER OF BRICKS COVERED USING TARPAULIN SHEET**

Reactor configuration has an important effect on microalgal biomass and by product yield. The depth of the pond is 15 cm deep enough to allow the sunlight to penetrate. Mixing, especially vertical mixing is considered one of the primary factors affecting the performance of raceway reactors since mixing is related to light-use efficiency here the mixing is provided by physical means that is by the use of a stirrer. Atmospheric  $\text{CO}_2$  uptake is not sufficient to meet the carbon demands of microalgal cultivation in open ponds, especially during periods of higher irradiation. To avoid carbon limitation, a carbon source in the form of glucose is added regularly. The open pond reactor is exposed to sunlight, the position of the reactor relative to the sun is also important so that algae receive the necessary amount of light throughout the day. Temperature is maintained between  $25^\circ\text{C}$  and  $35^\circ\text{C}$ . In open ponds, the temperature is not typically controlled and is therefore dependent on the sunlight regime, evaporation, and ambient air temperature.

#### **BOLD'S BASAL MEDIUM (BBM MEDIUM)**

The medium is highly enriched and is used for many of the green algae. For preliminary culture, 1 liter of microalgae growing water from the fish pond was transferred to the diluted BBM medium (10 liter tap water and 1 liter 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 sieved with plankton net and was centrifuged for the collection of microalgal species. The ingredients of the medium is shown in Table 2.

**TABLE 2 INGREDIENTS OF BBM MEDIUM USED IN THE MICRO ALGAE CULTIVATION**

| Stock solution reagent          | Stock solution concentration (per 100ml) | ml   |
|---------------------------------|--|------|
| KH <sub>2</sub> PO <sub>4</sub> | 1.75g/100ml                              | 10ml |
| CaCl <sub>2</sub>               | 0.25g/100ml                              | 10ml |
| MgSO <sub>4</sub>               | 0.75g/100ml                              | 10ml |
| NaNO <sub>3</sub>               | 2.5g/100ml                               | 10ml |
| K <sub>2</sub> HPO <sub>4</sub> | 0.75g/100ml                              | 10ml |
| NaCl                            | 0.25g/100ml                              | 10ml |
| Trace metal solution            | 0.25g/100ml                              | 1ml  |

**HARVESTING AND PROCESSING OF ALGAL BIOMASS**

Algae after cultivation in open raceway ponds or closed PBRs exist as a dilute solution of algae (0.1- 10 g/L). Recovering algae biomass from such dilute solutions poses many challenges, especially for open pond cultivation systems. Here the processing of algal biomass includes the following stages:

**GRAVITY SEDIMENTATION**

The algal medium is transferred to 33 bottles each of 1 liter, where the algae sink to the bottom while the supernatant is scooped off and can be reused as a growth medium. The collected algal water is kept for settling for about 48 hrs, which is nearly 2 days. The tank capacity was about 40 liter and after harvesting about 7 liter of the solution has been lost due to the evaporation taking place. Later the biomass is filtered out and oven-dried. Fig.3 shows the collection of algal water kept for gravity settling.



**FIG.3 COLLECTED ALGAL SOLUTION KEPT FOR**

**GRAVITY SEDIMENTATION.**

**DEWATERING AND FILTRATION**

After gravity sedimentation, the algal solution is taken and the extracellular water is drained off as shown in Fig.4. Draining of water is done by using a physical strainer of a certain nano meter. The biomass obtained in the strainer and at the bottom of the bottles are collected in a petridish. The weight of the biomass along with the petridish is measured and noted. The empty weight of the petridish is also measured and noted. Then the biomass estimation is calculated by gravimetric weight.



**FIG.4 THE FILTERED BIOMASS OBTAINED AFTER SETTLING FOLLOWED BY DEWATERING.**

**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 80°C for about 4 to 5 hrs. After drying, the algal- biomass was finely powdered using a blender, weighed, and then stored for further analysis as shown in Fig.5.



**FIG.5 BIOMASS OBTAINED AFTER OVEN DRYING FOR ABOUT 4- 5 HRS**

**3. RESULTS AND DISCUSSION:**

The growth of microalgal culture was observed at 7 days intervals using a change in color and nutrient solution (BBM) of 2ml was added at 3 days intervals. From the initial to the 7<sup>th</sup> day, there was no visible growth and the

growth was observed after the 7<sup>th</sup> day. On the 14<sup>th</sup> day, the biomass started to show color changes and thickening. After 56 days, the culture is set for sedimentation for 48 hrs. The culture is settled at the bottom and was the water at the top layer was drained out and the sediment was collected. The collected biomass was dried under sunlight followed by oven-dried at 80°C for 4 to 5 hrs. After drying, the algal-biomass was finely powdered using a blender, weighed, and then stored for further analysis. The dry weight of biomass is estimated as 2.4 g/L.

$$\text{Biomass (dry weight in g)} = (B - A) / 33 \quad (1)$$

$$= (228 - 150) / 33 = 2.4 \text{ g/L}$$

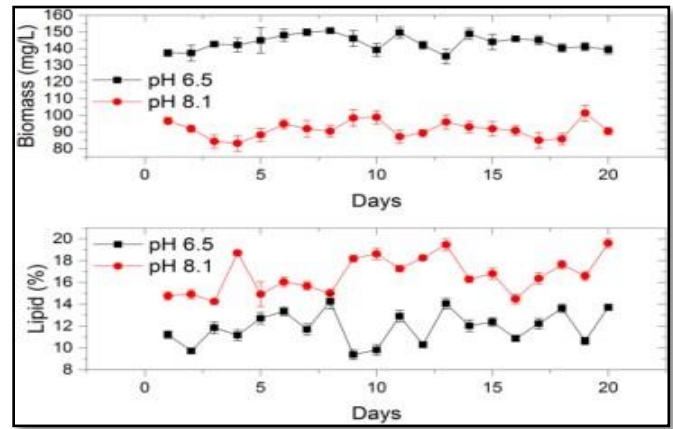
Where B = Total weight of dish with the biomass

A = Empty weight of the dish

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 cells the increase of biomass in the open pond reactor is also affected by the light intensity and temperature the present study aims to screen the potential of certain local freshwater microalgae for biomass production. It has been observed that the pond has a quite good production of microalgae species. An open pond reactor was fabricated with a 40 liter and 10ml of the diluted BBM medium (as a nutrient) poured into it in 3 days. The isolated microalgal culture from the pond was inoculated into the medium, mixing was done regularly. Carbon dioxide is provided externally by adding glucose which acts as a carbon source. Light availability and temperature have been balanced by the atmosphere. The algal biomass production was monitored at an interval of 7 days. The culture medium was collected after 56 days of harvesting and allowed to undergo sedimentation. The sedimented biomass after dewatering followed by filtration is then oven-dried at 80°C for about 4 to 5 hours. The dried biomass is weighed and estimated. It is observed that 2.4 g of biomass can be obtained per liter from the harvesting done in an open pond reactor system.

**pH**

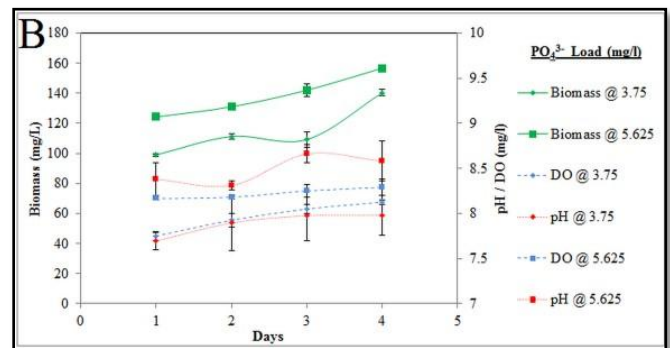
Sreekumar et al. 2018 discussed the effect of pH in lipid Enhancement in Microalgae by Temporal Phase Separation: Use of indigenous sources of nutrients. At pH 6.5 condition of biomass production was 140 - 150 mg L<sup>-1</sup> d<sup>-1</sup>. Whereas at pH 8.1 it was observed that the biomass production is around 90 -100 mg L<sup>-1</sup> d<sup>-1</sup> (Fig.6). At pH 6.5, the total lipid production of biomass was up to 14%. But at pH 8.1, total lipid production of biomass improved up to 20%. Under optimum conditions with pH 6.5 total lipid production of biomass was up to 14% while with pH 8.1 it was 20% (Fig.6). A 30% higher total lipid production was obtained under pH 8.1 and higher biomass was obtained at a pH of 6.5.



**FIG 6 BIOMASS AND LIPID PROFILE AT DIFFERENT pH.<sup>31</sup>**

**NUTRIENT CONCENTRATION**

Sreekumaret al. 2018 discussed the effect of nutrient concentration in Lipid Enhancement in Microalgae by Temporal Phase Separation. Fig.7 depicts the Biomass, Dissolved oxygen, and pH profile of microalgal culture in a PBR at different nutrient loadings. The light exposure was 16 hours of light and 8 hours dark. The nutrient load provided was NH<sub>4</sub>CL -52.22 mg/L; KH<sub>2</sub>PO<sub>4</sub> - 3.75 mg/L; Trace nutrients - 1.2 mg/L and a one-time addition of 1g NaSiO<sub>2</sub> for enhancing the growth. The nutrient levels were set following the Redfield ratio, an atomic ratio of nutrients found in phytoplankton, N: P = 16:1. The continuous analysis of the microalgal sample collected from the raceway pond reactor, under seawater as a nutrient source, twice daily over 10 days was carried out in triplicates.

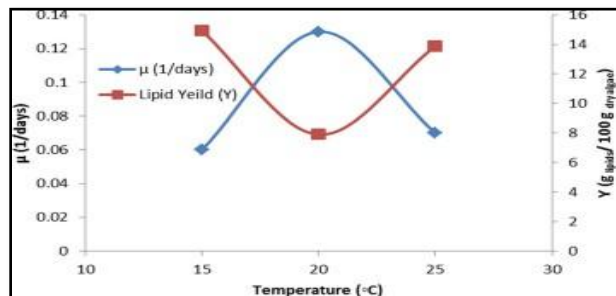


**FIG 7 BIOMASS PROFILE OF SEAWATER CULTURE AT VARIOUS NUTRIENT CONCENTRATIONS.<sup>32</sup>**

**TEMPERATURE**

Sreekumar et al. 2016 discussed the effect of temperature in Marine microalgal culturing in open pond systems for biodiesel production. Ideal growth temperatures allow the cell to undergo photosynthesis without modifying any inherent biochemical or physiological parameters. Microalgae species are capable of carrying out photosynthesis and cellular division over a wide range of temperatures generally stated between 15°C and 30°C but with optimal conditions between 20°C and 25°C.

Temperatures providing maximum growth rates are stated between 20°C and 25°C for mesophilic species but can increase up to 40°C for thermophilic strains (*Chaetoceros*, *Anacystis nidulans*) or decrease down to 17°C for psychrophilic strains (*Asterionella Formosa*) as shown in Fig. 8. A prominent oligogenic marine algae *Nannochloropsis oceanica* is reported to have an optimum temperature of 26.7°C with upper and lower limits as wide as from 0.2°C to 33.3°C. Nevertheless, microalgae are reported to affect lipid content concerning temperature. An increase in temperature from 20°C to 25°C practically doubled the lipid content of *Nannochloropsis oculata* (from 7.90% to 14.92%).



**FIG 8 EFFECT OF TEMPERATURE OVER GROWTH RATES AND LIPID YIELD.<sup>32</sup>**

#### 4. CONCLUSIONS:

In the quest for sustainable alternate energy, microalgal biodiesel is the most promising outcome which could connect the old technologies of internal combustion engines to new age pollution-free technologies. In the outdoor open pond, the yield was around 12-15% lipid and biomass extraction around 2.4 g L<sup>-1</sup>. The total energy absorbed by the microalgal system is divided for biomass production as well as for storage food production. The goal of any production system should be to increase energy intake. In particular, a system for lipid production should invariably be to improve the lipid yield without the loss of biomass. The effective exposed area defines the total energy of the system. To maximize the total yield, the culture needs to be grown in an open pond reactor, which is reported to be the only viable culturing method for industrial scale. The incident solar energy is channeled into chemical energy through the complex photochemical pathway by the microalgae. A proper culturing system is to be designed, which optimizes both the lipid and biomass yield. The critical parameters, which need to be considered, while designing such a system are discussed in detail: such as strain selection, the temperature of the culture system, pH and CO<sub>2</sub> levels, nutrient status and the applied nutrient pressure, incident light, dissolved oxygen, and agitation systems. The most important point to be considered while designing a well-engineered culture system is that all of the above parameters are interdependent so that adjusting any of them will affect one or more of the other parameters.

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