Enhancing algal biomass production and nutrients removal from municipal wastewater via a novel mini photocavity bioreactor

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ABSTRACT
This study presents the use of a novel photocavity reactor in order to intensify the growth rate, biomass and lipid productivity in microalgae. The reactor offers an aseptic approach for better control on growth rate in microalgae. The Absorption Factor (AF), Attenuation Factor (AtF), Modified Fluence Rate (MFR) and water factor of the photo reactor were recorded to be 1.581, 0.267, 0.347 mW/cm² and 2.072 respectively. The maximum growth rate recorded was 310 mg L⁻¹ in photocavity reactor. The results clearly indicate that using the stainless steel visible photoreactor can lead to a significant increase in the growth rate (43.3%), productivity of biomass (27-33%) and lipid content (6-8%) in comparison to microalgae cultivated in glass conical flasks (control). COD, total nitrogen, phosphate and bacterial load (colony-forming unit- CFU) were determined in this study. A decrease in COD (180 to 19 mg/l) and CFU (57×10⁸ to 5×10⁸) of wastewater was also recorded in this study.

Keywords: photocavity; reactor; microalgae; wastewater; growth.

1. INTRODUCTION

Microalgae biomass has been increasing attention in recent years as they have high growth rate, high lipid content and CO₂ sequestration ability [1]. However, production of microalgal biomass is still not economically feasible, due to significant amount of energy required during cultivation and harvesting [2]. Microalgae are among the oldest microorganisms on earth that can either be autotrophic or heterotrophic in nature [3]. Photoautotrophic microalgae can survive well under stress conditions due to their unicellular structure. The autotrophic microalgae use CO₂ (carbon source), sunlight, macro and micronutrients for growth, while heterotrophic microalgae require organic compounds as energy source [4]. Photosynthesis is a process by which the chloroplast of autotrophic microorganisms absorbs sunlight as well as CO₂ and converts them into ATP and O₂ [5]. The energy required for photosynthesis is dependent on the sunlight/artificial light travelling through a medium and the number of photons reaching unit algal cell surface area in unit time. Absorbed light energy is then converted into the carbohydrates through photosynthesis used by the algal cells for growth and other metabolic processes [6]. If the intensity of light is too low, algal cell increases the consumption of carbon source which leads to low biomass production. On the other hand, too much high intensity of light leads to inhibition of photosystem-II and production of free radical that decrease the growth rate of microalgae [4]. Light properties such as spectral duration, quality, quantity effect the metabolism and growth of photosynthetic organisms [7]. Blue green light has a shorter wavelength and is able to penetrate the deepest part of water bodies as compared to longer wavelength which is scattered or absorbed by water molecules [8]. Red and blue wavelengths of spectra play important role in photosynthesis and development of photosynthetic organisms [9]. Microalgal cells grow well under blue (λ≈ 420–470 nm) or red light (λ≈ 660 nm) [10]. Now a day’s Light Emitting Diodes (LEDs) lighting has been used in microalgal research due to its desirable characteristics like mercury-free, long-lasting (about 50 000 h) for microalgal growth. Also they can be easily adjusted according to the biochemical composition of the microalgae [10; 11].

Photoreactor is a closed system important for efficient growth and mass cultivation of microalgae for biodiesel and bioactive products production [12]. Photobioreactors have been used since 1950s [11]. Different types of photoreactors are reported in the literature like photobioreactor [13] flat plate [14] bubble column [15] and airlift [16]. Mainly two types of photoautotrophic systems are used commercially for mass cultivation of microalgae which are photobioreactors and open pond, both are dependant as a light source on the sunlight or artificial light [17].

Reactor offers better control overgrowth and contamination than open pond systems. The efficiency of a photoreactor depends on the light intensity and Light distribution [18]. Light reflection, scattering and shading due to microalgae cells are responsible for the non-uniform light intensity distribution [19]. There are three main zones depending on the intensity of light in a photobioreactor viz., strong illumination zone (near to light source), weak illumination zone ( Far away from light source) and dark zone [20]. When density of microalgae increased up to 10g/l the light is unable to reach 5–10 mm [21]. Microalgal growth rate decreases as there is increase in the water depth in open pond or in photoreactor [22].

A conventional microalgal photoreactor is made up of glass only. Designing reactors is difficult because a number of factors have to be considered like light intensity, lamp type and wall of the reactor [23]. Reactor structure, materials and light distribution
inside the reactor are the important parameters for the design and optimization of algal photoreactors [20].

Earlier, Hong et al., [24] have reported a photo cavity reactor cover with plasmonic nanoparticle layer to prevent the scattering of light. In this study a novel stainless steel photo cavity reactor is designed which prevents the passage and scattering of light.

2. MATERIALS AND METHODS

2.1. Materials.

Chorellaminutissima(MCC-27) was purchased from Centre for the Indian Agricultural Research Institute, New Delhi. Scenedesmusabundans (NCIM 2897) were procured from National Chemical Laboratory, Pune. Bold’s basal solution (PL031-10X) used in this study was acquired from Himedia Chemical Laboratory, Pune. Bold’s basal solution (PL031-10X) used in this study were of HPLC grade. For the preparation of reactor stainless steel S3 was used.

2.2. Estimation of light absorbed by Photocavity reactor.

For the estimation of light absorbed by the photocavity reactor and glass flask WACO 206 Solar power meter was used (Fig.1). Photocavity reactor absorbs whole light, while glass flask absorbs 0.01 wt/cm² of light and emits the remaining. The absorption of light increases in flask with increasing biomass in the flask. On the 14th day, absorption of light was 1.2 wt/cm². The amount of energy reaching the base of photocavity reactor was calculated using exponential set up given in figure 1 and formulas [26]:

Absorption factor (AF) calculated based on LED emission

\[ AF = -\log \left( \frac{\text{Energy}_{out}}{\text{Energy}_{in}} \right) \]

Attenuation factor (AtF)

\[ AtF = \frac{(1 - 10^{-AF})}{AFln(10)} \]

Modified fluence rate

\[ MFR = E'F \times AtF \]

Water factor

\[ WaterFactor = \frac{1 - 10^{-w}}{aLm(10)} \]

2.3. Photocavity bioreactor and microalgae growth.

The stainless steel reactor used in this study measured a working volume of 200 ml (10 cm ID, 7 cm height, 2 mm thickness) (Fig. 1). Chorellaminutissima(MCC-27) and Scenedesmusabundans were maintained in the photocavity reactor with 200 ml of municipal waste water. The reactors were kept at 25 °C, at a photoperiod of 16:8 h (light:dark cycle) with 200 lmol photons m² s⁻¹ LED light. Growth of microalgae in photocavity reactor under sunlight was also determined. Conical flasks under similar conditions were considered as control.

3. RESULTS

3.1. Evaluation of energy reached in photocavity reactor.

In this study a photocavity reactor was designed using glass and stainless steel. The design of this reactor aimed to capture the scattering of light suitable for the photosynthesis. For the control experiment a transparent glass (conical flask) without stainless steel was used. The light intensity was measured inside the photocavity reactor and then conical flask. Hemispherical geometry of photocavity reactor is favorable for microalgal intercellular proximity. Impinging and transmission of direct light by photoreactor walls is an important factor for Wastewater analysis like COD, total nitrogen, phosphate and bacterial load (Colony Forming Unit- CFU) was determined according to the protocol of Jämsä et al., [26].

\[ \text{CFU/ml} = \frac{\text{no. of colonies x dilution factor}}{\text{volume of culture plate}} \]

2.4. Determination of rise in temperature in Photocavity bioreactor.

Change in temperature was determined under sunlight and LED light. 100 ml water was poured in the bioreactor and conical flask. Change in temperature after 1 h under sunlight inside the room, direct sunlight in open field and LED light (200 lmol photons m² s⁻¹) were recorded using a laboratory thermometer.

2.5. Determination of microalgal growth rate and biomass.

The growth rate was monitored every two days by taking the absorbance at 750 nm using a spectrophotometer (Beckman Coulter DU 800 Spectrophotometer). Dry microalgal biomass was determined gravimetrically. Algal biomass samples were collected from the reactor and centrifuged at 5500 rpm for 5 min and further vacuum dried at 100 0C overnight. Biomass productivity (mg/L/d) was then calculated according to the following equation:

Biomass productivity

\[ \frac{\text{Final dry biomass} \text{ (mg) } - \text{ initial dry biomass} \text{ (mg) }}{\text{Cultivation time} \text{ (d)}} \]

2.6. Lipid content and FAMEs Analysis.

Lipids were extracted using dried algal biomass following the Bligh and Dyer [27] protocol.

Total lipid productivity was measured using following equations.

Lipid content = (Final lipid extracted - Initial lipid)/Dry algal weight.

Lipid productivity = Lipid content X Biomass productivity/100.

The total lipids were transesterified using methanolic sulphuric acid (6%) for one h into fatty acid methyl esters (FAMEs) [28]. FAMEs were analyzed using GC-MS (GC–MS; Agilent technologies, USA) according to the protocol Kumar et al., [29].

2.7. Statistical analysis.

Data analysis was carried out by repeating experiments three times (n = 3). One way ANOVA (Graphpad Prism software version 7:0) was used for data analysis. Data are presented in mean value ± SD with p < 0.05.
A photocavity reactor was developed with favorable optical characteristics for photosynthesis in microalgae cultures. This reactor was made from stainless steel and provided appropriate intensity of light during the designing of photoreactor. Multiple reflection of the light in photoreactor increases the light distribution in algal cells for growth [32].

No significant change in temperature was recorded inside the bioreactor under sunlight in room and in LED light. A rise in temperature by 2 °C was recorded in photocavity reactor as compared to flask due to stainless steel in direct sunlight.

3.2. Biomass, lipid productivity and clean water.

The present study was aimed to design a photocavity reactor with favorable optical characteristics for photosynthesis in which microalgae were cultivated. For this purpose the photocavity reactor was made from stainless steel.

Biomass productivity of microalgae cultivated in photocavity reactor was higher as compared to control. This is indicated by the increase in growth rate which was found to be 43.3 % higher in photo cavity reactor (Fig 1, 2, S1, Table 1). Also there was an increase in biomass production which was found to be 27-33 % higher in photo cavity reactor. Biomass production depends on the photosynthesis efficiency. Hsieh et al., [33], developed an open photobioreactor tank with rectangular transparent chambers which provide appropriate intensity of light leading to 56% higher biomass productivity. Microalgae fix the CO₂ and produce biomass which can be converted into energy by photosynthesis [34]. Rate of photosynthesis of microalgae is affected by the duration and intensity of light [11]. Carvalho et al., [35] have reported that photobioreactors provide appropriate light wavelength, duration, and intensity for microalgal cells. Chlorella sp. have been reported to achieve maximum growth at high intensity (6000 lx (84 lomol m⁻² s⁻¹) of light and decreased growth rates with decreasing intensity of light [36].

During the study the influence of photocavity reactor on lipid productivity was also examined. Lipid productivity was increased by 6-7% per gram of algal biomass cultivated in photocavity reactor over conical flask. Pancha et al. [37] conducted a similar study in Scenedesmus sp. where they have recorded an increase in biomass productivity with increasing light intensity however, no significance change in lipid content was recorded by them. Photosynthesis, growth and lipid accumulation are the metabolic factors influenced by irradiance [38]. FAME analysis did not show any significant change in the types of lipids obtained (Table 2). The two main fatty acids C16:0 and C18:0 were obtained from both the strains of the microalgae with no significant change in the area of retention. These two fatty acids are very important in terms of biodiesel production [39; 40]. Nutrients removal capacity from waste water in photocavity reactor was high in Scenedesmusabundans. COD and Colony Forming Unit (CFU) were also decreased (180 to 19 mg/l and 57×10⁹ to 5×10⁸) (Table 3).

3.3. Growth rate and Photosynthetic pigments.

In order to demonstrate the advantages of photo cavity reactor, mainly two parameters were considered during the log phase: first was the change in cell number and second the chlorophyll content. Enhanced growth rate as well increased chlorophyll content (125%) was recorded in photocavity reactor microalgae cultures as compared to conical flask cultures (fig.2, Table 4).

Growth rate in photocavity reactor started increasing on 5th day of cultivation. Maximum growth rate 310 mg L⁻¹ was reported in photon cavity reactor. Table 4 displays the chlorophyll content of biomass harvested from photocavity reactor and conical flask. The values of chlorophyll a, b and carotenoids were high in biomass of photocavity reactor as compared to conical flask in both the microalgae strains. High chlorophyll content and growth rate of microalgae in a photobioreactor coated with gold nanoparticles has also been reported by Hong et al. [24].
Figure 2. Biomass productivity in Flask (A) and in photocavity reactor (B) mg/l/d.

Table 1. Production of algal biomass and algal lipid in conical flask and photocavity reactor.

| S.No | Microalgae Strain     | Conical Flask | Photocavity Reactor |
|------|-----------------------|---------------|---------------------|
| 1    | *Chlorella minutissima* | Suspended microalgae biomass (mg/l) | 785±0.25 | 870±0.5 |
|      |                       | Attached microalgae | 0 | 125±0.4 |
|      |                       | Total Biomass | 785±0.12 | 995±0.1 |
|      |                       | Algal lipid content(%, dry biomass) | 13.06±0.3 | 14.0±0.4 |
| 2    | *Scenedesmus abundans* | Suspended microalgae biomass (mg/l) | 905±0.25 | 1061±0.5 |
|      |                       | Attached microalgae | 0 | 143.5±0.2 |
|      |                       | Total Biomass | 905±0.12 | 1203.4±0.1 |
Table 2. FAME profile of microalgae by GC-MS.

| FAME          | Chlorella minutissima (Area %) | R- Chlorella minutissima (Area %) | Scenedesmus abundans (Area %) | R- Scenedesmus abundans (Area %) |
|---------------|--------------------------------|----------------------------------|--------------------------------|----------------------------------|
| C16:0         | 19                             | 20                               | 28.3                           | 25                               |
| C18:0         | 1.3                            | 3                                | 4                              | 3                                |
| C20:0         | -                              | -                                | 2                              | -                                |
| C16:1         | 4                              | 2                                | -                              | -                                |
| C16:2         | 4.05                           | 5                                | 1                              | 2.4                              |
| C16:3         | 6.50                           | 3                                | 1.5                            | -                                |
| C18:1         | 18                             | 23                               | 1.2                            | 3                                |
| C18:2         | 9                              | 7                                | 17                             | 21                               |
| C18:3         | 2                              | 10                               | 8                              | 9                                |
| C20:1         | -                              | -                                | 4                              | 3.1                              |

Table 3. Nutrients removal efficiency Chlorella minutissima and Scenedesmus abundans in photocavity reactor.

| Characteristics of waste water | Initial | After treatment with Chlorella minutissima (14 Days) | After treatment with Scenedesmus abundans (14 Days) |
|--------------------------------|---------|-----------------------------------------------------|---------------------------------------------------|
| COD (mg/l)                     | 180±01  | 19±0.2                                              | 17±0.2                                            |
| Total Nitrogen (mg/l)          | 7±0.21  | 0.55±0.1                                            | 0.23±0.02                                         |
| Total Phosphorus (mg/l)        | 5±0.1   | 0.49±0.15                                           | 0.82±0.13                                         |
| CFU Unit                       | 57×10⁴  | 17×10⁴                                              | 5×10⁴                                             |

Table 4. Chlorophyll content of microalgae strains cultivated conical flask and photocavity reactor.

| Strain            | Chlorophyll a | Chlorophyll b | Total carotenoids | Chlorophyll a+b |
|-------------------|---------------|---------------|-------------------|------------------|
| F-Chlorella minutissima | 2.41±01       | 0.83±03       | 0.87±01           | 3.24±02          |
| R-Chlorella minutissima | 5.3±02       | 1.91±02       | 1.44±04           | 7.21±02          |
| F-Scenedesmus abundans | 2.92±02       | 1.08±01       | 0.90±01           | 4.0±03           |
| R-Scenedesmus abundans | 6.29±01       | 2.72±02       | 1.64±01           | 9.01±02          |

4. CONCLUSIONS

The present study recommends that photocavity reactor can increase the growth rate, biomass productivity and chlorophyll content in microalgae. Photocavity reactor provides the optimal environments for the photosynthesis of microalgae. The demonstration here using two microalgae strains clearly indicates that photocavity reactor can enhance metabolic activities of microalgal cells and thus increase the growth and biomass productivity. However, this reactor is suitable only under LED light, in the presence sunlight it reduces microalgal growth due to a rapid increase in temperature of the reactor.

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