Effects of geometrical configurations of photobioreactors on the growth of marine benthic diatom *Cylindrotheca closterium*

**Abstract:** Objective: For algal biotechnology, the primary barrier is the developing of cost effective photobioreactor operating in a high-efficiency. In this study, the effects of three different types of photobioreactors (bag, flat plate and bubble column) on the growth and sugar production of the diatom *Cylindrotheca closterium* were simultaneously investigated for 7 days of batch cultivation period.

Methods: The photobioreactors were incubated at 21±2°C under the light intensity of 50 µmol photons m⁻²s⁻¹ with a 12:12 h light:dark cycle photoperiod at the air flow rate of 2 L min⁻¹ for 7 days of batch productions. The turbidity (optical density), chlorophyll-a, exopolysaccharide (EPS) content and reducing sugar concentrations were measured.

Results: The maximum specific growth rate of 0.29 day⁻¹, which corresponded to the doubling time of 2.40 day, was obtained in the bag photobioreactor cultivation of *C. closterium*. It was found that the polyethylene bag photobioreactors are suitable for the production of diatoms as a closed photobioreactor.

Conclusion: The key is not only the selection of geometrical configuration of photobioreactor, but also the determination of the light penetration path for cost effective photobioreactor design operating in high efficiency related to the algal productions.

**Keywords:** Bag, bubble column, *Cylindrotheca closterium*, flat plate, photobioreactor

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Introduction

Knowledge and experience in bioreactor design with efficient control systems have been established for many industrial processes employing microorganisms, mammalian cells and plant cell culture [1]. Although both photobioreactors and bioreactors can be used for the production of algae, they are different systems, and the economics of each system are different. The significant differences between a bioreactor and a photobioreactor are energy source, circulation, O₂ supply and sterility. Furthermore, vessel geometry depends on the light penetration for photobioreactors [2]. The main challenge in photobioreactor design is to create a simple, inexpensive, with high volumetric productivity energy efficient photobioreactor, which is scalable to industrial capabilities [3].

*Cylindrotheca*, an epipelic benthic pennate diatom, holds promise as a nutraceutical source. *Cylindrotheca* species have many good characteristics, such as rapid growth and multiplication rate; they are easy to culture and harvest, and can endure contamination [4]. *Cylindrotheca* species can be used in aquaculture both as a nutritious feed [5] and also as an antibacterial agent [6]. Exopolysaccharide (EPS) secretion for diatoms may also be the result of overflow metabolism, which is defined as an excess amount of carbon dioxide fixed relative to growth requirements. This is a well-known phenomenon in diatoms [7,8]. Also in *C. closterium*, exopolysaccharides accumulated as a result of overflow metabolism [9].

In this study, bag, flat plate and bubble column photobioreactors (PBRs) were investigated for the effect of geometrical designs on the cultivation of the diatom *Cylindrotheca closterium*. This work is a first attempt to evaluate different kinds of PBRs for the growth and sugar production of the diatom *Cylindrotheca closterium*. One of the scientific phenomena remaining in the lack of industrial know-how was tried to be illuminated related to the diatom cultivation for the commercial interest with the rising awareness of a feasible production strategy at low cost.

Materials and Methods

Strain maintenance and inoculum preparation

The diatom *Cylindrotheca closterium* EgeMacc-044 was obtained from Ege University Microalgae Culture Collection, Izmir, Turkey. Stock culture was monoalgal and cultivated in F/2 medium [10] (Table 1) at 22±2°C under continuous illumination (100 µmol photons m⁻²s⁻¹) in 2-L sterile bottle for 15 days. For the preparation of the inoculum, the cells from the stock culture were collected and concentrated by centrifugation (1160 g, 3 min) and the supernatant was removed. The collected cells were transferred, incubated aseptically in 250 mL flasks containing 100 mL of F/2 medium under the light intensity of 40 µmol photons m⁻²s⁻¹ with the agitation rate of 120 rpm at 22±2°C for four days. A 4-day-old culture (100 ml, approximately 2×10⁶ cells mL⁻¹) cells were used as inoculum for all experiments.

Photobioreactors (PBRs) and production conditions

Schematic diagrams of pneumatically agitated photobioreactors are shown in Figure 1. The conical shape of the 5 L polyethylene bag (H: 74 cm, D: 16 cm) with the cell suspension working volume of 1 L was hung from a metal frame. Agitation was provided by bubbling air from the bottom of the perforated tube placed from one side of the bag. The flat plate reactor has cuboidal shape with minimal light path. It is characterized by high surface area to volume ratio and open gas disengagement systems. The vertical flat plate PBR was made of plexiglas (0.5 cm wall thickness). The total volume of the PBR was 1.5 L (25 cm
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Lx2.5 cm Wx25 cm H, working volume: 1 L). Agitation was provided by air sparger from the bottom of the reactor so as to supply enriched air to the culture. The sparger nozzle diameter is 0.4 cm. 1.5 L bubble column was made of glass with a thickness of 0.5 cm. The working volume was 1 L, the height and internal diameter was 75, and 5 cm, respectively. The top and the bottom of the column were closed with O-ring covers made of polycarbonate. Sterile air was supplied through the 0.20 µm (GyroDisc CA) filter. Disk sparger with a diameter of 3 cm, was employed to supply air from the bottom of the column. The PBRs were equipped with the ports for the sample collection and gas outlet. Chemical sterilization was applied by using 1.5% (v/v) hypochlorite for PBRs.

The PBRs were incubated at 21±2°C in the temperature-controlled incubator for 7 days of batch productions. Illumination was provided both by LED downlight lamp (Cata 10 W CT-5254) from the top of the PBRs and by standard cool white fluorescent lamps (Philips TLD/54, 18 W) from one side of the PBRs with a 12:12 h light:dark cycle photoperiod. Measured light intensities by a quantum meter (Lambda L1-185) on the surface of the PBRs were 50 µmol photons m⁻²s⁻¹. Air was supplied to the cultures by air pump continuously and air flow rates were adjusted to 2 L min⁻¹ with flow meters.

**Experimental analysis**

Samples were taken at indicated times, and the following growth parameters were measured immediately; the cell concentration was determined by counting triplicate samples in a Neubauer hemocytometer. The turbidity (optical density) was measured at 556 nm in UV/VIS spectrophotometer (GE Healthcare Ultrospec 1100 pro, UK).

For the chlorophyll-a measurement, cells were harvested at 3500 g for 3 min. Chlorophyll in the cells was extracted with 100% (v/v) methanol until the powder color became gray. The amount of chlorophyll-a was determined spectrophotometrically by measuring the light absorption at different wavelengths (665 and 750 nm) [11]. The chlorophyll content was calculated by using the following equation: Chlorophyll-a (mg/L)= 13.9 (A₆₆₅-A₇₅₀), where A₆₆₅ and A₇₅₀ correspond to the absorbance of methanol extracted supernatant at 665 nm and 750 nm wavelength with 1 cm pathway cuvette, 13.9 is the extraction coefficient.

The exopolysaccharide (EPS) content was determined using the phenol–sulfuric acid method at the absorbance value of 490 nm [12]. Reducing sugar concentration was analyzed using the dinitrosalicylic acid (DNS) method where the absorbance was measured at 540 nm [13] using a UV/Vis spectrophotometer (GE Healthcare Ultrospec 1100 Pro, UK).

The specific growth rate (µ) of the cells was calculated from the initial logarithmic phase of growth for at least 48 h, as µ=(ln C₂ - ln C₁)/dt, where C₁ is the final cell concentration, C₂ is the initial cell concentration and dt is the time required for the increase in concentration from C₁ to C₂. Cell count was used as indicator for the specific growth rate. Doubling time (DT) was also calculated as DT=ln 2/µ.

The data were analyzed using one-way analysis of variance (ANOVA). A probability value of p≤0.05 was considered to denote a statistically significant difference, and p≤0.01 was also used to show the power of the significance. Results were reported as mean values with standard deviations (n=3) unless otherwise indicated.

**Results and Discussion**

From an engineering viewpoint, economic analysis is essential for new technology evaluation. For algal biotechnology, the primary barrier is the development of cost effective PBR operating in high-efficiency. In this study, the effects of three different types of PBRs (bag, flat plate and bubble column) on the growth of *C. closterium* were simultaneously investigated for 7 days of batch cultivation period. The polyethylene bags were modified as models for economic photobioreactors for algae cultivation in developing countries [14]. Large scale continuous bag cultures are well-used production systems (www.seacaps.com) in many commercial bivalve hatcheries [15]. Flat-plate photobioreactors have received much attention for cultiva-
tion of photosynthetic microorganisms due to their large illumination surface area [1]. Flat plate reactors were first described in the 1980s [16] and thereafter researched and developed by Tredici et al. [17] and Tredici and Materassi [18]. Also, these reactors are essentially bubble columns, stirred very effectively by streaming of compressed air, the rate of flow of which may be accurately controlled to set the optimal rate of mixing [19]. Bubble columns are one of the most popular types of reactors as they serve simplicity in both construction and operation. This type of reactors is frequently used to carry out photosynthetic processes [20].

The growth profiles of *Cylindrotheca closterium* in different types of PBRs

As shown in Figure 2a, optical density of the culture in bag PBR reached a peak value of 0.72±0.01 on day 7 while the minimum absorbance was obtained in the flat plate PBR only with the value of 0.44±0.02. On the other hand, absorbance values were close to each other in both flat plate and bubble column PBRs at the end of the cultivation. This could be due to the effect of circulatory flow in the bubble column PBR and the mixing characteristics of the PBRs. The main difference between bubble column and flat plate lies in the fluid flow, which depends on the geometrical configurations and the positions of the spargers. One important point to note here is that laboratory-scale reactors exhibit a greater degree of mixing than large-scale reactors.

Liquid circulation is developed in a bubble column because of the introduction of gas and it largely affects the performance of the bubble column. The liquid velocity at the column axis is one of the most important parameters for the design of reactors. Large bubbles rose in zigzag paths rather than in straight paths and trended toward the center of the column due to the wall effect [21]. Furthermore, in the Newtonian fluid systems, bubble coalescence did not control the bubble size distribution. Most bubbles were smaller than 0.01 m and large bubbles could not be observed [22]. In addition to mixing the culture, aeration aids in removing the photosynthetically produced oxygen.

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**Figure 2:** Growth profiles of *C. closterium* in different types of PBRs: (a) optical density (OD), (b) pH, (c) cell count, (d) chlorophyll-a concentration. Bag PBR (◊), Flat Plate PBR (□), Bubble column PBR (∆).
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The initial pH of the cultivation was 8.22 for the growth of *C. closterium*. The pH-oscillations were recorded in the range of 8.2 to 8.5. The pH levels of both bag and bubble column PBRs were similar during the cultivation. pH increase was more distinctive in flat plate PBR reactor than the other PBRs (Figure 2b). On the other hand, Figure 2c shows that the cell concentration was significantly lower in flat plate PBR compared to bag PBR under the light intensity of 50 µmol photons m⁻²s⁻¹. The maximum cell concentration, 10.8±1.1x10⁵ cells/mL, was obtained in bag PBR, which was 31% higher than in bubble column PBR. The maximum specific growth rate of 0.29 day⁻¹, which corresponded to the doubling time of 2.40 day, was obtained in the bag PBR cultivation of *C. closterium* (Table 2). As reported by Imamoglu et al. [24], the maximum specific growth rate of 0.271 day⁻¹ was found in flat plate PBR for the semi-continuous cultivation of Haematococcus pluvialis. The maximum specific growth rate of *C. closterium* was 0.047 h⁻¹ at 20°C under the light intensity of 60 µmol photons m⁻² s⁻¹ with a light:dark cycle of 12:12 h [25].

Smyda [26] reported that a combination of temperature, salinity, and light played an important role in the cell division of diatoms. Moreover, the changes in salinity and temperature appear to influence oceanic phytoplankton abundance [27], and the estimation of the optimal growth rate in different environmental conditions is very important for mass culture of benthic diatoms [28].

As shown in Figure 2d, there were significant differences on the chlorophyll-a concentration of cells beginning of the day 2 for the cultivation period in different PBRs. The maximum chlorophyll-a concentration of 1.88±0.05 mg/L was found in bag PBR under the light intensity of 50 µmol photons m⁻²s⁻¹ for *C. closterium*, which indicated that cells could adjust well to the growth conditions. The low chlorophyll-a content was found in the flat plate PBR. This might be due to the over-saturation point of light. Flat plate PBR had minimum light path and maximum illuminated surface area depending on the geometrical configuration (Table 2). Additionally, the decrease in chlorophyll-a content leads to lower photosynthesis efficiency or *vice versa*, and thereby the inhibition of algal growth occurred.
The main parameter that affects reactor design is provision for light penetration, which implies a high surface-to-volume ratio. Bubble column reactors are sufficiently transparent to allow good light penetration [29]. When bubble column is used for cultivation of photosynthetic cells, liquid circulation in the bubble column, upward in a central region and downward in a region close to the wall, supports flotation of cells and gives light and dark illumination cycles with a high light intensity close to the wall [30]. Flat plate reactors are conceptually designed to make efficient use of sunlight; hence, narrow panels are usually built so as to attain high area-to-volume ratios [29]. The bag reactors are generally thin to allow deep light penetration [3]. Literature suggests [31], the light availability in the reactors depends on its diameter; the larger the diameter, the lower the light availability at the reactor center [32]. It is also important to underline that the design of cost effective PBR has complicated harmony between the effect of the reactor wall thickness, and the design and the position of the sparger affected the shear rate.

Sugar Production of Cylindrotheca closterium in different types of PBRs

Figure 3 illustrates the effects of different types of PBRs on the sugar production of C. closterium. As shown in Figure 3a, exopolysaccharide (EPS) contents significantly increased after 4 days of cultivation period in different PBRs. Efficient EPS secretion was recorded with the value of 53.84±2.4 mg/mL in bag PBR, whereas the lowest value (31.32±2.2 mg/mL) was obtained in flat plate PBR. Stationary trends were monitored for the reducing sugar concentrations of C. closterium in different types of PBRs, especially between the cultivation days of 1 and 6 (Figure 3b).

As reported by Affan et al. [28], the highest cell density (7.20x10^6 cells/mL) was found on day 10 at 20°C for C. closterium with the polysaccharide content of 7.11 mg/100 g in the viscozyme extract. In another study, C. closterium was cultivated in Kester medium at 20°C under the light intensity of 60 µmol photons m⁻²s⁻¹ with a light–dark cycle of 12:12 h. After 16 days of cultivation period, the maximum cell density of 2.25x10^6 cells/mL was obtained with the EPS concentration of approximately 3 µg EPS x10⁶ cells⁻¹. Furthermore, accumulation of both exopolysaccharides and intracellular carbohydrates were subject to overflow metabolism [9].

Polyethylene bags have frequently been used, with advantage taken from their particularly low cost, high transparency and good sterility [29]. Bag cultures have a relatively short life because the internal surface attracts culture debris and bacteria, which collectively reduce light penetration and are a source of contamination. At the end of a culture run, it is necessary to renew the bag. Large diameter bags are inefficient but those less than 30 cm diameter can be effective because the surface area to volume relationship for light penetration is improved [33]. A major advantage of this system is the possibility to grow several species in separate bags coupled together and its relatively easy maintenance [15]. Flat plate photobioreactors have important advantages for mass production of photoautotrophic microorganisms and may become a standard reactor type best suitable for mass production of several algal species [34]. Accumulation of dissolved oxygen concentrations in flat-plate photobioreactors is relatively low compared to horizontal tubular photobioreactors [1]. Flat panel reactors may be easily cleaned, both from outside and inside, all panels being readily accessible. Wall growth inside the reactor and salt deposition outside are thus easily handled [19]. Bubble columns are widely used in industry as reactors, fermenters, absorbers and strippers because of their relatively low cost and simple construction. However, their design and scale-up are very difficult due to the complexity of their hydrodynamics [22]. The distribution of shear rate is rather uniform throughout the column [21].

Conclusion

Bag, flat plate and bubble column PBRs were evaluated in this study. These are a special class of pneumatic PBRs which are currently receiving considerable attention for their potential application to various bioprocesses, including the more specialist areas of algal cultures, where susceptibility to mechanical damage is high. The key is not only the selection of geometrical configuration of PBR, but also the determination of the light penetration path for cost effective PBR design operating in high efficiency related to the algal productions. For more accurate design purposes, it may be necessary to take other factors, such as low energy consumption and improved mass, momentum and heat transfer characteristics, into account. It was found that the polyethylene bag PBRs are suitable for the production of diatoms as a closed PBR. The geometrical design of the bag PBR has a strong influence on the growth of diatoms.

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