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Research Article

The effect of photobioreactor height/diameter ratio on *Chlorella variabilis* microalgae growth and oil production efficiency

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ABSTRACT

This study aims to reveal how height/diameter ratio of column photobioreactors affect the growth and lipid content of microalgae. For this purpose, *Chlorella variabilis* cells were grown in aerated column photobioreactors with height/diameter ratio of 1, 2, and 3 in defined (BG11) culture medium. Results obtained showed that maximum microalgae biomass concentration, cell productivity, cell doubling time, and lipid productivity were found to increase as the height/diameter of photobioreactor increased. After 15 days of cultivation, the highest cell productivity (0.139 gdw/L.day), cell lipid content (21.1%) and lipid productivity (29.33 mg/L.day) were obtained in the photobioreactor with the highest height/diameter ratio (3), whereas the highest specific growth rate (0.045 h^{-1}) was obtained in the photobioreactor with the smallest height/diameter ratio (1). These findings contribute to the knowledge on photobioreactor design and pave way for more efficient use of column type photobioreactors in producing microalgae.

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INTRODUCTION

In today's world, where oil prices have increased in recent years and fossil resources are decreasing, the fact that these resources will not be able to meet the needs in the near future has led people to turn to renewable energy sources. In addition, due to the negative effects of these resources on the environment, the search for alternative resources has become inevitable. The basis of the damage caused by fossil fuels to the environment is the increase in the amount of greenhouse gases released when they burn in the atmosphere. Greenhouse gases mainly consist of carbon dioxide (CO_2), methane (CH_4), nitrogen oxide (N_2O), water vapour (H_2O), ozone (O_3) and fluorinated compounds (CFC, HFC, PFC, SF_6). These gases come from the use of fossil fuels, industry, transportation, energy production, various wastes and agricultural activities. With the effects of global climate change showing more and more each passing day, studies to reduce the emission of greenhouse gases, which play the most important role in this change, have also gained importance.

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In this period of increasing environmental awareness such as global climate change, reduction of CO_2 emission and efficient use of water resources, interest in microalgae has increased considerably in recent years and it is seen as one of the promising microorganisms in biofuel production.

The concept of biological fixation of carbon dioxide is a long-lasting technology to manage the sustainability of the environment. The substantial need to reduce carbon dioxide emissions, microalgae have proven to be the best fit for carbon fixation and production of diverse biofuels by converting atmospheric carbon dioxide into biomass via autotrophs. [1].

Microalgae are primary producers of synthesizing organic matter in the aquatic environment. Microalgae take in nutritional salts and combine them into complex molecules necessary for their vital activities with the help of a light source [2,3].

Microalgae are very important living things due to the protein, fatty acid, pigment substances, vitamins and valuable metabolites they have accumulated in their content [3–5].

For this reason, microalgae are used in many areas such as nutritional support for humans and animals, fertilizer in agriculture, wastewater treatment, cosmetics, and biofuel production. [6–9].

There are many reasons why microalgae are preferred as an energy source. These can be listed as being able to reproduce quickly, having a high oil content, being easily adaptable to various environmental conditions, etc. [10]. In addition, microalgae use sunlight 10 times more efficiently than terrestrial plants [11].

Biofuels derived from diverse microalgal species have grabbed a lot of attention from all over the globe due to rapid growth rate, high efficacy, no restriction of feedstock supply, high photosynthetic efficiency, less cost of production, high content of lipids, carbohydrates, etc. [12]. Significantly, microalgal strains are transforming nutrients into appropriate biomass and different cellular components [13,14].

Microalgae are photosynthetic creatures rich in protein, fat and carbohydrate derivatives. In general, they have oil content between 20-50% of their dry weight [6]. The main inorganic form of carbon required by microalgae is CO_2. This situation ensures that the storage materials of microalgae are mainly oil and starch [15]. Although it varies according to the species, microalgae can be produced quickly in open ponds or indoor bioreactors. They are usually autotrophic and perform photosynthesis.

In recent years, there have been significant developments in studies on closed photobioreactors. Therefore, the use of closed systems in the production of microalgae is becoming increasingly important. These studies aim to reduce the light path length and increase the light intensity reaching each cell. In addition, these systems should have features that allow a good mix of cultures [16].

Effects of height to diameter ratio of bioreactors are multifold: As the H/D increases, air residence time thus the mass transfer coefficient of oxygen and carbon dioxide also increases, mixing time which indicates homogenization efficiency of culture decreases, surface area per volume increases which increases manufacturing cost, lateral heat transfer rate and external illumination efficiency. On the other hand, as the photobioreactor height increases, liquid pressure at the bottom also increases which increases air solubility, air pump cost and power usage. All these factors create a complex problem which is a hurdle in design of photobioreactors. Moreover, their combined effect on microalgae growth needs to be shown identified for an efficient bioprocess operation. Therefore, studies that investigate H/D ratio on culture performance are important to ensure an efficient bioprocess and to provide basis for scale up studies.

Studies have been carried out to increase the oil accumulation of microalgae produced in photobioreactor systems with high production efficiency, and it has been concluded that the oil rate of the species produced in the environment where the nutrient is limited, and the production cost is lower [17].

In this study, the effect of photobioreactor height/diameter ratio on growth and oil content of *Chlorella variabilis* microalgae was investigated under laboratory conditions. In this scope, column photobioreactors with a diameter of 9 cm were operated at liquid levels of 9, 18, 27 cm corresponding to height/diameter ratios of 1, 2, 3, and *Chlorella variabilis* was cultured in BG11 defined medium under continuous illumination. Growth and lipid data were collected; specific growth rates, cell productivities and doubling times, lipid contents and productivities were calculated and reported. To the best of our knowledge, there is no similar research published on the literature. Thus, the findings of this study help fill the knowledge gap in a neglected aspect of photobioreactor design (height/diameter ratio), and pave way for more efficient use of column type photobioreactors in producing microalgae.

MATERIALS AND METHODS

Microalgae Strain and Nutrient Medium

Chlorella variabilis, belonging to the green algae group used in the research, was obtained from the Molecular Biology and Genetics department of Istanbul Medeniyet University. Blue-Green (BG-11) medium was used as the nutrient medium. Chemical composition of this medium was (g/L): $NaNO_3$ 1.5000, KH_2PO_4 0.0400, $MgSO_4.7H_2O$ 0.0750, $CaCl_2.2H_2O$ 0.0360, H_3BO_3 0.0029, Na_2CO_3 0.0200, Citric acid 0.0060, Iron citrate 0.0060, Vitamin 0.0200. Chemicals were analytical grade and were purchased from Merck.

EXPERIMENTAL SETUP AND OPERATING CONDITIONS

Microalgae were cultured in cylindrical bioreactors with different height/diameter (H/D) ratios. The bioreactors used are 9 cm in diameter and 9, 18 and 27 cm in

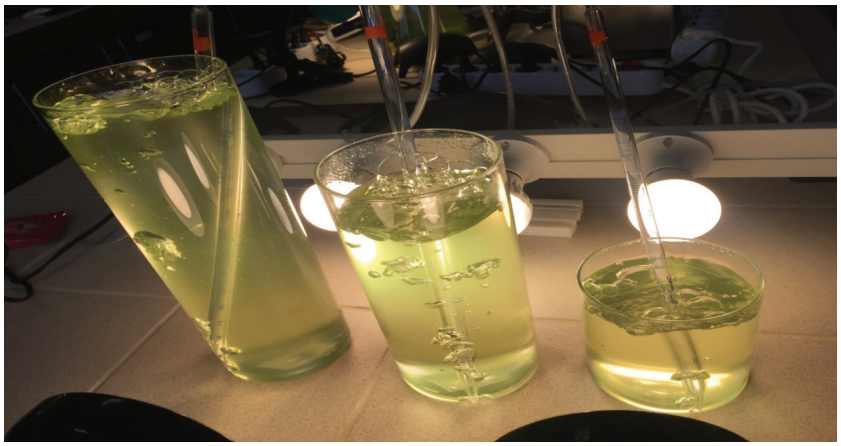

Figure 1. The photobioreactors with different height/diameter ratio.

height. Accordingly, the photobioreactor height/diameter ratios are 1, 2, and 3, respectively (Figure 1). The volumes of the bioreactors are 573, 1145 and 1717 mL, respectively. An average of 6% of the stock culture was inoculated. The illumination of the cultures was provided continuously (24 hours) by OSRAM brand-led bulbs with a 7W 2400K value, which would provide a surface light intensity of 3.80 Klux. The temperature was kept constant at 25 ± 2° C. The aeration of the samples was provided by an air pump at a rate of 300 L/h. The air from the air pump was sterilized by filtration (0.45μm) before being fed into the photobioreactors.

The Geometrical Interpretations of the Effect of Photobioreactor Height

For a typical cylindrical (column) photobioreactor, surface area shows the material amount needed to construct the bioreactor. Here, the shape that has the smallest surface area and therefore requires the least material to construct for a given volume has a height to diameter ratio of 1, and the material cost increases as this ratio increases. Moreover, for externally illuminated photobioreactors (almost all of the indoor photobioreactors, since internal illumination has its own challenges and not usually preferred), surface area per volume of the photobioreactor needs to be high to improve illumination efficiency which means height to diameter ratio should be high. Similarly, for jacketed vessels, heat transfer occurs through lateral surface of the cylinder, therefore high surface area (high aspect ratio) increase heat transfer rate and should be high. On the other hand, as the photobioreactor height increases, liquid pressure at the bottom also increases which increases air solubility, air pump cost and power usage. All these factors create a complex problem and combined effect on microalgae growth should be shown, which the objective of this study is.

Analyses

The growth of microalgae was monitored spectrophotometrically throughout the studies. A 3mL sample was taken from the photobioreactors to determine the optical density. Jenway 6800 UV-VIS spectrophotometer was used for analysis, samples were placed in quartz cuvettes and measurements were made at 600 nm wavelength. The pH (Mettler Toledo Seven Easy model) values of the culture medium were measured. After the studies were completed, the photobioreactor output was centrifuged at 4000 rpm for 30 minutes to determine the dry weight of the microalgae biomass. It was then dried at 60 °C until constant weight.

Lipid analysis was performed according to the method applied by Bligh and Dyer (1959) [18]. 120 mL of methanol-chloroform (1/2 ratio) mixture was added to 0.2 grams of homogenized algae samples. Then, 0.4% $CaCl_2$ solution was added to these samples. After these samples were mixed in a mechanical mixer at 200 rpm for 3 hours, they were filtered into tared flasks with the help of filter paper Figure 2 and Figure 3.

These flasks were kept for one day by closing their mouths in such a way that their mouths did not get airtight, and the top layer consisting of the methanol-water mixture was separated the next day. The chloroform was evaporated from the chloroform-lipid part remaining in the flasks and the remaining lipid was cooled to room temperature and the lipid ratio was calculated.

Calculations

The specific growth rates (μ) of the grown microalgae were calculated according to equation (1)

$$\mu = \frac{\ln X_{2-} \ln X_1}{t_{2-}t_1} \quad (1)$$

In the formula, X_2 and X_1 indicate the biomass concentrations at t_2 and t_1 times during the exponential (logarithmic) growth phase.

The doubling time (τ D) of the cells was found with the help of equation (2)

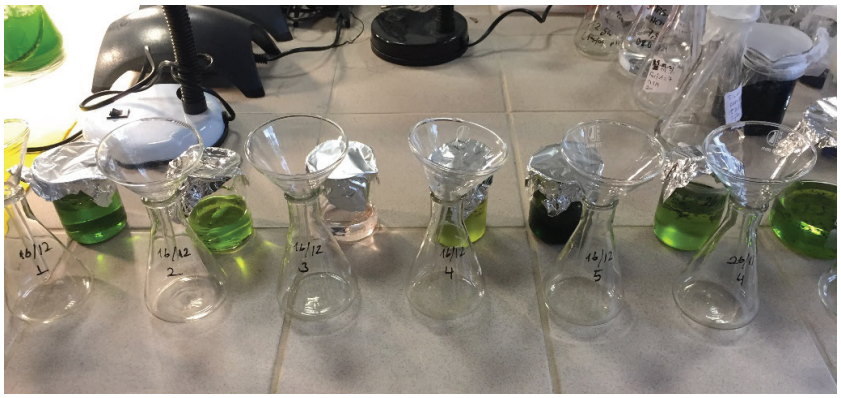

Figure 2. Oil analysis.

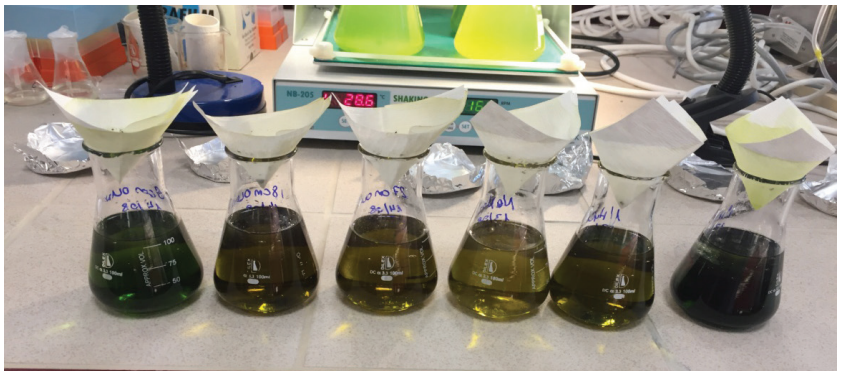

Figure 3. Filtration of solvent-added algae for oil analysis.

$$\text{Doubling time} = \frac{\ln 2}{\mu_{max}} \quad (2)$$

Cell productivity (P_x) is given as the amount of dry cells produced per liter per day (g_{dw}/L.day).

Oil productivity (P_{lipid}) was calculated after 15 days of incubation. Using Equation 3, the amount of lipid recovered (% of lipid) was calculated gravimetrically. Microalgae lipid productivity (mg/l/day) is the product of the lipid content value and the biomass productivity value, as shown in Equation 4.

$$\text{Lipid Amount (\%)} = [\text{Beaker Dare (g)} + \text{Lipid (g)}] - [\text{Beaker Dare (g)}] \times 100 \quad (3)$$

$$\text{Lipid Productivity (mg/l/day)} = \text{Lipid content (\%)} \times \text{Biomass Productivity} \quad (4)$$

Where P_x is cell productivity (gdw/L.day); P_{lipid}, lipid efficiency g/L.day); and % lipid indicates the lipid content (%) by mass of the cell.

RESULTS AND DISCUSSION

The effects of different height/diameter ratios on the growth of microalgae in the bioreactor were studied in detail. The design of a reactor for microalgae cultivation is a very important issue. Because mass transfer, light-dark region areas, and mixing factor affecting oxygen concentration are closely related. The height/diameter ratio (H/D) is closely related to the working efficiency of the reactor due to mixing in the photobioreactor. In this study, the reactor diameter was set to 9 cm, and the reactor heights were set to 9, 18 and 27 cm. The relationship between the maximum growth rate and height/diameter ratio of *Chlorella variabilis* cultured in bioreactors with different heights was investigated. The studies were carried out in 2 sets. The effects of photobioreactor height/diameter ratio (H/D) on the growth of *Chlorella variabilis* microalgae are shown in Figure 4 and pH changes are shown in Figure 5. The graph is shown as the average of two sets.

As can be seen from the graph, the specimens in all height/diameter ratios grew until the 69th hour and continued to grow at a steady rate after this hour. After the 284th hour in all samples, it was determined that the microbial activities of the microorganisms began to decrease and the death phase began.

The pH levels of microalgae are a critical parameter of growth conditions in bioreactors. The pH values, which were initially measured around 8.50, showed a tendency to increase for a certain period of time. Then, they varied between 10-10.5 during the study period. pH changes can

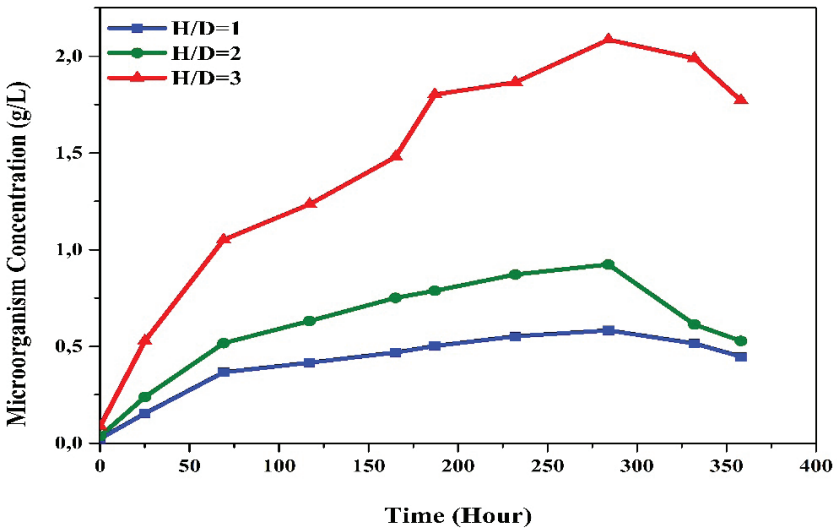

Figure 4. Effect of photobioreactor H/D ratio on the growth of *Chlorella variabilis*.

H: Bioreactor height D: Bioreactor diameter

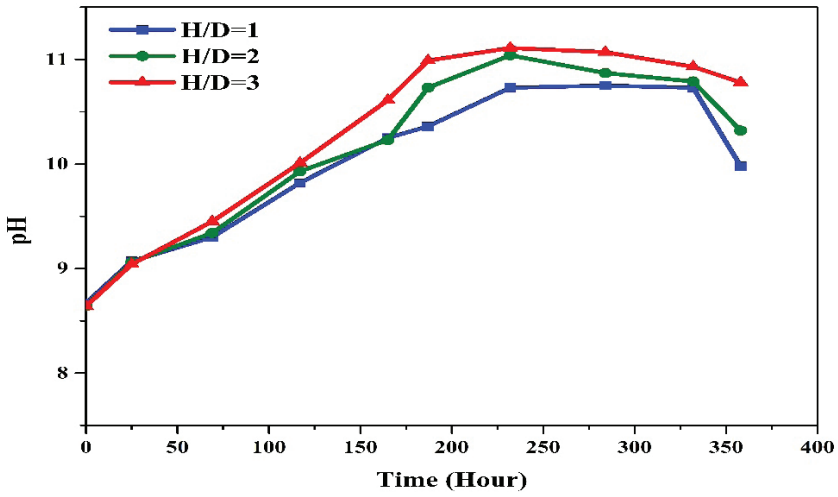

Figure 5. Effect of photobioreactor H/D ratio on pH change of *Chlorella variabilis*.

also affect the biochemical composition and lipid production of microalgae, an important factor in industrial applications such as biofuel production.

The effect of length-diameter ratio on microalgae growth is shown in Table 1. The values in the table were calculated as the average of the two sets.

The highest biomass amount was reached in the bioreactor with H/D=3 with 1.67 g/L. The biomass amounts of microorganisms in other bioreactors were 0.43 and 0.75 g/L, respectively. In this study, it was found that as the height/diameter ratio increased, the amount of biomass increased approximately 2 times. The maximum specific growth rate

Table 1. Effect of photobioreactor height/diameter ratio on the growth of *Chlorella variabilis* microalgae

H/D ratio	Dry weight (g L^{-1})	Px (gdw/L.day)	Growth rate μ ($hour^{-1}$)	Doubling time (hour)
1	0.43	0.039	0.045	15
2	0.75	0.062	0.039	17
3	1.67	0.139	0.037	19

Table 2. Effect of photobioreactor height/diameter ratio on the lipid content of *Chlorella variabilis* microalgae

H/D ratio	Fat Content (g/L)	Fat content (%)	Plipid ($mgL^{-1}day^{1}$)
1	0.046	18	6.98
2	0.087	17	10.6
3	0.22	21	29.33

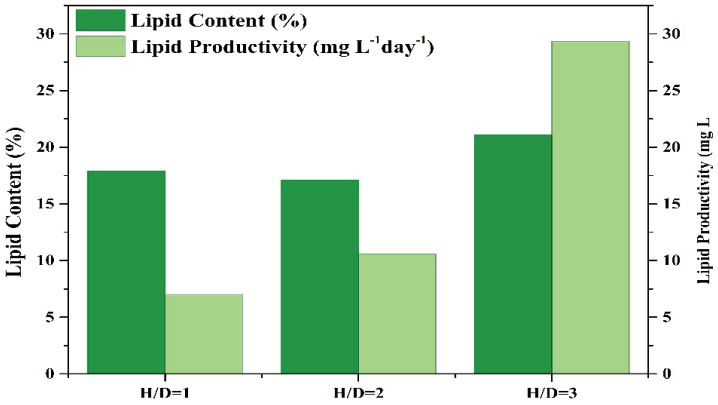

Figure 6. Lipid content and productivity of Chlorella variabilis microalgae grown at different height/diameter ratios.

was determined in the bioreactor with a growth rate of 0.045 h^{-1} and H/D=1. The doubling times were respectively; It is determined as 15, 17 and 19 h. These findings emphasize the importance of bioreactor design. In particular, it shows that the effect of H/D ratio on cell concentration is decisive. Higher H/D ratios generally provide more surface area, which facilitates better diffusion and transfer of nutrients and oxygen needed for cells. At the same time, less mechanical stress and lower shear forces can be applied compared to smaller diameter bioreactors, promoting healthy growth of cells. High H/D ratios also provide a more homogeneous distribution of nutrient substrates within the bioreactor and can contribute to the reduction of the gravity effect, which increases cell productivity and thus oil production efficiency.

The lipid content of microalgae grown in bioreactors with different H/D ratios is shown in Figure 6. Different H/D ratios favored lipid accumulation by microalgae (Table 2). Lipid contents were H/D=3 >H/D=1>H/D=2, respectively. At the end of the experiment, the highest amount of lipid was obtained at H/D=3 ratio (21%). This is 15% more than the amount of lipid in H/D=1 ratio.

Lipid productivity is as shown in Figure 6. The findings in this study showed that lipid productivity ranged from 6.98 to 29.33 mg L^{-1} day^{-1}. Lipid productivity was found to be higher at H/D=3 ratio. These differences in lipid productivity may be mainly due to differences in biomass as well as different H/D ratios. Because the deviations in lipid content were smaller.

Those findings are generally on a par than the ones reported in the literature previously; Rajapitamahuni et al., (2019) reported highest *C. variabilis* cell concentration as 0.76 g/L with a productivity of 0.022 mg/L/day in a continuous culture [19], Tran et al., (2020) determined maximum biomass concentrations of 1.52 - 1.72 g/L, with specific growth rates of 0.009 - 0.014 h^{-1} when *C. variabilis* was cultivated in domestic wastewater [20], Altın et al., (2018) reported *C. variabilis* maximum biomass concentration and specific growth rate as 1.30 g/L and 0.038 h^{-1}, respectively [21], and Uyar et al., (2016) calculated highest biomass concentration and specific growth rates as 0.30 g/L and 0.037 h^{-1} in a compartmentalized photobioreactor system [22].

Studies that focus on growing microalgae in larger scales are necessary to assess the performance and economy of the process. Notable literature with *C. variabilis* include a pilot scale study that used 35L closed panel photobioreactor in which 11 gdw biomass was obtained after 24 days [23], and industrial scale cultivation of *C. variabilis* in open solar pans that covers a vast area of 772 m^2 with a total cultivation volume of 360 m^3, where an average biomass productivity of 34.6 $g/m^2/d$ was achieved and through the application of solar the authors stated that the entire process was cost effective and energy efficient leading to the sustainable development of microalgae-based biofuel for future commercialization [24]. Other studies that employed different microalgae species in pilot scale photobioreactors, such as *Chlorococcu*m sp in 50 L outdoor photobioreactor [25], and *Tetraselmis* sp. in 80 L indoor photobioreactors [26], were also conducted to define and address issues related to design and technology of photobioreactors. In addition, scientific publications dealt with the analysis of production costs, energy profitability and product life cycle, which are especially important for the production of biofuels, and research results suggest that the economic

viability of producing microalgae solely for biofuel purposes is not cost-effective, highly uncertain and risky, whereas a financially viable process is possible if energy products are generated as by-products in a multifunctional biorefinery system, combined with, for example, carbon dioxide capture and wastewater treatment [27].

Stirred tank bioreactors usually have a height to diameter ratio in the range of 1 to 4, whereas airlift and bubble column bioreactors have higher ratio (4 to 8) to accommodate lower mass transfer of O_2/CO_2 due to lack of mechanical stirrer. This study was limited to H/D ratios of 1 to 3, thus higher ratios should be tested for a more comprehensive understanding of the effects. Studies with other microalgae strains are also required to complement these results.

Another problem that challenges large scale and low cost production of microalgae is the low production efficiency; microalgae require specific conditions for growth, including appropriate light, nutrients, and temperature. Scaling up the production of microalgae to an industrial level while maintaining optimum conditions can also be challenging. However, ongoing research and development is continually improving our understanding of microalgae's potential and as technology advances and new applications are discovered, algae may become a more viable option for technological applications in the future [6,13,14,28,29]

CONCLUSION

In this study, the relationship between the maximum growth rate and height/diameter ratio of *Chlorella variabilis* microalgae cultured in bioreactors with different heights was investigated. No such study has been found in the literature for this species. It was found that as the height/diameter ratio increased, the amount of biomass (and productivity) increased approximately four times, and the oil yield increased approximately five times. This methodology, described in short-term experiments, can be easily tested for other microalgae species. Because there is variation between species. It helps to optimize reactor designs to increase the performance and economic efficiency of microalgae-based industries.

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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