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A Comparative Study Using Two Types of Photobioreactor for Cultivation of *Chlorella vulgaris* Microalgae †

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Abstract: Effective vessel design is crucial for the viability and practicality of microalgae cultivation, aiming for high biomass production. This study tested two different 5-litre vessel designs for cultivating *Chlorella vulgaris*, aiming to produce a high biomass and evaluate lipid production. The study varied pH medium (6.5–10.5), light intensity (200–1000 lux), and CO_2 concentrations (5–15%) to assess each reactor's performance. The aerated vessel (tubular shape) produced 21.05% lipid concentration, while the fabricated vessel (oval shape) produced 20.14% at optimum conditions. The aerated vessel performed best at pH 10.5, 5% CO_2, and 1000 lux light intensity, whereas the fabricated vessel's optimum conditions were pH 10.5, 15% CO_2, and a white LED system. The highest biomass was 0.432 g/L in aerated tubular vessels and 0.281 g/L in fabricated oval-shaped vessels. Both systems performed well and are suitable for further study with other microalgae types.

Keywords: *Chlorella vulgaris* cultivation; biomass lipid; photobioreactor

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1. Introduction

The overconsumption of fossil fuels has caused an energy crisis worldwide due to the escalating numbers of vehicle usage. The major problem of fossil fuel is that it is a non-renewable type of energy, and can contribute to global climate change by releasing carbon dioxide (CO_2) as the main product in vehicle combustion. Thus, biofuel can be a solution to replace fossil fuels because it is renewable and safer for the environment. Biofuel produce from microalgae is expected to have a 50% lower lifecycle of greenhouse gas emission compared to petroleum-based fuels. Before the discovery of microalgae as a potential source, food crops were the first generation of biofuels which was not profitable, also causing food competition due to the limited availability of the food crop itself [1].

Photosynthetic microalgae require carbon dioxide and light sources to grow. Excessive light intensity produces a photosystem overload, pigments bleach and finally a break in the photosystem. Furthermore, insufficient light forces microalgae to consume carbohydrates during photorespiration. On the other hand, unstable conditions may not be suitable for conducting experiments while the light intensities vary during the experiment run. Therefore, to promote microalgae growth, the selection of the photobioreactor is crucial to have an overview of the growth profile and simultaneously controlling the selected parameter such as pH medium and CO_2 percentage will enhance the growth to maximum biomass production.

Lipids obtained from microalgae are usually found in both polar and non-polar forms. The non-polar lipids consist of hydrocarbon, waxes, eicosanoids, fatty acids and acylglycerol while polar lipids consist of phospholipid and glycolipid [2]. Reported by Aguoru and Okibe (2015), lipid composition of *Chlorella vulgaris (C. vulgaris)* obtained seven main fatty acids in the cell which are nonanoic acid, decanoic acid, tridecanoic acid, hexadecenoic acid, two profiles of octadecanoic acid and octadecadienoic acid [3]. Lipid composition of microalgae can be affected by the cultivation conditions [4].

Therefore, in the present study, a centred-light photobioreactor (CLPBR) and an aerated bottle (conventional method) were tested. The main objective was to validate the reactor design by comparing their efficiency to cultivate *C. vulgaris*. Biomass lipid was extracted to determine the maximum potential of the cell growing in each reactor. The cell concentration was also determined considering three crucial factors (pH, light intensity and CO_2 concentration) to evaluate their feasibility.

2. Materials and Method

2.1. Materials

C. vulgaris obtained from the CSIRO Microalgae Research Centre (Hobart Australia) was used in this experiment. All chemicals used to prepare the stock solution were analytical grade.

2.2. Methods

2.2.1. Cultivation

The cultivation was performed in a 5-litre aerated bottle (tubular shape) and fabricated vessel (oval shape) [5] using MLA medium (modified algae) growth medium [6]. The details of the dimension and design of the fabricated vessel were reported by Mustapa et al. (2020). The seed inoculum, which is 10% (v/v) with an optical density (OD) 680 nm of 1.00, was used for the cultivation. A total volume of 0.4 litre microalgae and 3.6 litre media were used while other conditions were fixed at 25 $\pm$ 3 °C, pH 10.5 (otherwise specified). The cultivation period was fixed at 12 days with a photoperiod 12:12 h (light:dark) cycle.

2.2.2. Harvesting Biomass

Triplicate samples were collected at 24 h interval for 12 days. The sample was then centrifuged (5000 rpm) for 5 min. The pellet was rinsed with distilled water and crushed in a mortar and then left in the oven to dry (70 °C, 24 h). The biomass concentration is calculated based on the dry weight produced per litre (g/L).

2.2.3. Lipid Extraction

The dried biomass is transferred inside a cellulose thimble in the Soxhlet extraction apparatus unit. A mixture of solvent with the ratio 2:3 (v/v) of ethyl acetate and hexane was placed in the boiling flask and heated. The lipid extraction is considered complete when the solvent in the thimble compartment becomes colourless. Then, the solution containing the lipid was transferred into a vacuum evaporator to separate the solvent from the lipid. The lipid solution was then transferred into an empty beaker and dried in an oven (70 °C) overnight. The lipid content was determined using Equation (1) [7];

$$\text{Lipid content } (\%) = \frac{(W_2 - W_1)}{m} \times 100\% \quad (1)$$

where W_2 is the weight of the empty beaker and lipid content (g), W_1 is the weight of the empty beaker (g) and m is the biomass weight (g) of microalgae used for lipid determination.

3. Results and Discussions

Figure 1 shows *C. vulgaris* growth that was cultivated using MLA medium prepared into three different pHs (6.5, 8.5 and 10.5). Initially, the pH for MLA medium measured was 7.8 [8]. The highest cell concentration in Figure 1a was 0.432 g/L in 12 days for an aerated bottle, while Figure 1b shows a lower cell concentration of 0.281 g/L in the fabricated vessel at pH 10.5. In general, the trend line shows for both types of photobioreactor, an increasing line being observed with the increasing pH medium. The same trend was reported by Anbalagan et al. (2023) that the biomass density increases for the cultivation of *Hallochlorella rubescens* (*H. rubescens*) in a tubular reactor as the pH varies from pH 6 to pH 9 [7]. From this observation, *C. vulgaris* works well in an alkaline medium with the highest cell concentration produced in an aerated bottle.

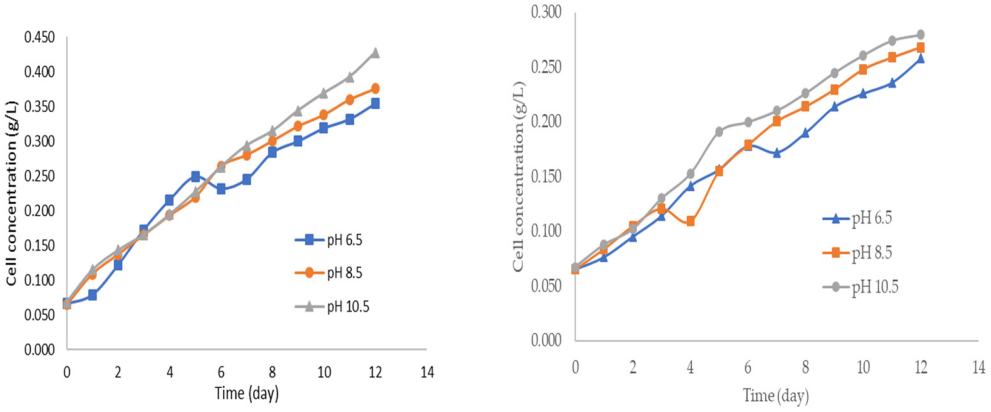
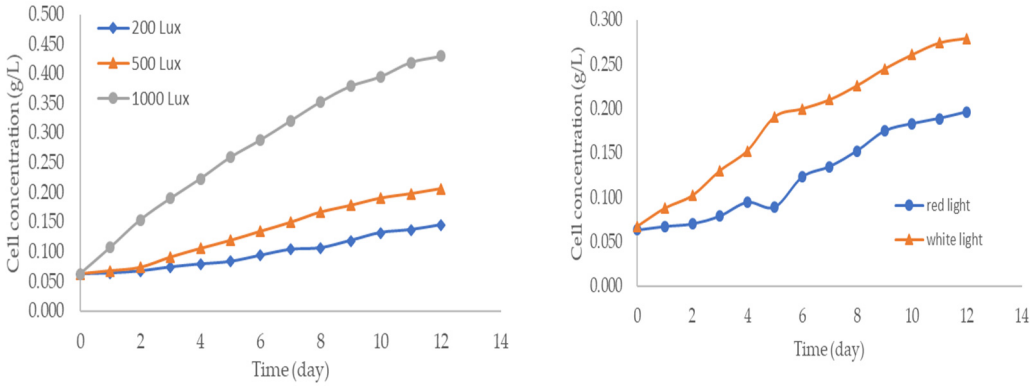


Figure 1. Effect of different pH medium for cultivation in (**a**) aerated bottle (**b**) CLPBR.

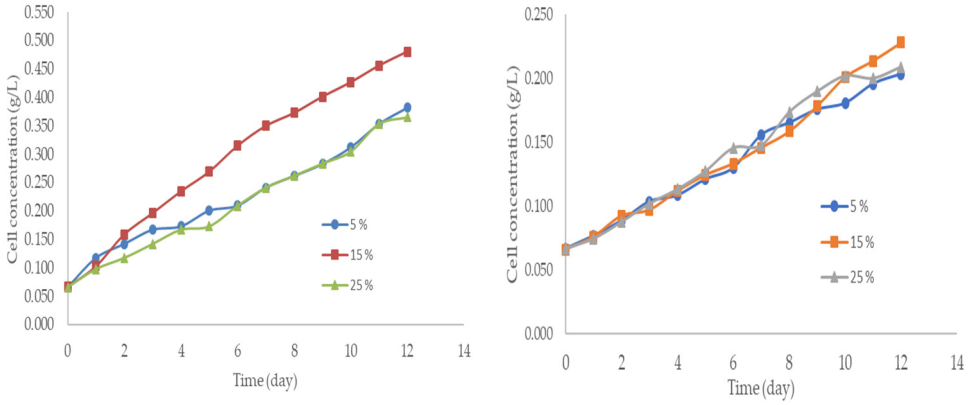
Figure 2 shows the light intensity effect on the growth of *C. vulgaris* in an aerated bottle and an in-house fabricated reactor. The highest cell biomass density was obtained in an aerated vessel with 0.430 g/L under 1000 lux light intensity (Figure 2a). As the light intensity increases, the growth of *C. vulgaris* also increases over time (0–12 days). This shows that light intensity is crucial for the growth of microalgae as it goes through a photosynthesis and respiration process. This light energy will convert into chemical energy in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH). Meanwhile, respiration occurring in dark conditions and the light energy will affect the growth of microalgae [9]. The prime factor of the light/dark cycle in the cultivation of microalgae to improve cell concentration has also been reported by previous researchers [7,10,11]. In the fabricated bioreactor (Figure 2b), we exhibit the comparison between white-light and red-light sources. The white-light source gave a higher cell concentration (0.280 g/L) if compared to red-light sources (0.197 g/L) which are 1.42 times higher. A study by Rai et al. (2015) gave an opposite effect of the light colour effect whereby red-light sources promote a higher biomass production if compared to white, yellow and green-light sources for the growth of *Chlorella* sp. with 26% higher biomass [12]. Even though the results vary, these only applied to the present study using the prototype reactor with LED sources of energy.



(**a**) (**b**)

Figure 2. Effect of different light intensities for cultivation of *C. vulgaris* in (**a**) aerated bottle (**b**) CLPBR.

Figure 3 shows the effect of different concentrations of CO_2 supply to *C. vulgaris* during cultivation (5%, 15% and 25%). From the figure, *C. vulgaris* grow best in 15% of CO_2 concentration for both types of reactor. The highest cell biomass obtained at 15% of CO_2 with 0.481 g/L for an aerated vessel and 0.228 g/L cell biomass for a fabricated reactor. Providing sufficient CO_2 concentration will have a positive effect on the growth of *C. vulgaris* but too much CO_2 concentration can cause inhibition in the growth of *C. vulgaris*. This is due to the CO_2 that dissolves in the water, causing the formation of carbonic acid which can acidify the medium culture over time [13].



(**a**) (**b**)

Figure 3. Effect of different CO_2 concentrations for cultivation of *C. vulgaris* in (**a**) aerated bottle (**b**) CLPBR.

The final stage of the present study was using the best conditions achieved during a parameter screening study and using it to carry out optimization. The conditions selected were pH 10.5, 15% of CO_2 concentration and 1000 lux of light intensity (aerated vessel) and white light (fabricated bioreactor); the results are presented in Figure 4. The highest cell biomass recorded in aerated bioreactor cultivation is 0.485 g/L and for the fabricated bioreactor yield a value of 0.232 g/L for the cultivation of *C. vulgaris*. This shows that the best cultivation vessel for *C. vulgaris* is an aerated vessel with an outer light source using pH medium of 10.5, 15% of CO_2 concentration and a white light having 1000 lux of light intensity. The cell density obtained was two-fold in a tubular-shaped aerated vessel compared to a fabricated centred-light reactor for the cultivation of *C. vulgaris*.

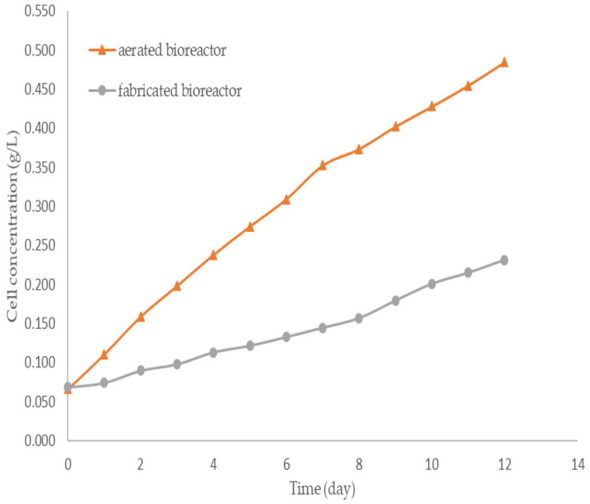


Figure 4. Cell growth of *C. vulgaris* for two different reactors under optimum conditions.

Lipid Production

The lipids extracted from the cell biomass collected were quantified and the values are presented in Table 1. This would be a secondary response that is being monitored in the present study. When varying the pH medium initially, the aerated vessel obtained the highest percentage of lipids (pH 10.5, 23.0%) if compared to the fabricated reactor (pH 10.5, 19.5%). The range of lipids produced showed a rising trend with the increasing pH medium. Rai et al. (2015) observed the same trend with *Chlorella* sp. cultured in Foggs media [12]. For the light intensity effect on the *C. vulgaris* cultivation, the intermediate light intensity (in this case 500 lux) promotes a higher lipid percentage if compared to a high light intensity (1000 lux) for the aerated vessel. The colour of light also produced a difference in lipid accumulation and in this case *C. vulgaris* prefers red lighting over white lighting for the fabricated vessel. Even though previous results on biomass accumulation showed a different result, it can be observed that light intensity is not a proportionally promoted lipid production and a variety in colour sources has an effect on the lipids produced. CO_2 concentration fed to the cultures also helps in promoting the lipids produced, as can be observed in Table 1. Aerated vessels gave the highest lipid percentage for 5% of CO_2 concentration, but for fabricated vessels showing 5% and 15% CO_2 has a better result in lipids produced with less significant difference. The overall profiles for the CO_2 concentration study presenting a lower CO_2 are the best for *C. vulgaris* growth, especially if the later stage targets lipid production. The same observation can be made for *H. rubescens* cultured in a 5-litre tubular reactor where the lipid that produced more than 30% and 15% CO_2 yielded the highest lipid percentage [7]. Lastly, when the cultivation was performed using optimum conditions selected in a previous study, the lipid extracted from aerated vessel gave a higher lipid content than the fabricated reactor with a difference of 1% and these can be considered to be not significant changes for both types of photobioreactor.

Table 1. Summarization of lipid production under different conditions studied.

Factor	**Lipid Content (%)**	
pH Medium	**Aerated Bottle**	**Fabricated Reactor**
pH 6.5	18.20	13.02
pH 8.5	17.12	15.05
pH 10.5	23.00	19.52

Table 1. *Cont.*

Factor pH Medium	Lipid Content (%) Aerated Bottle	 Fabricated Reactor
Light intensity		
200 Lux	17.77	*na
500 lux	18.60	*na
1000 lux	11.93	*na
White light	*na	13.94
Red light	*na	23.00
CO_2 concentration (%)		
5%	27.63	18.63
15%	20.07	19.53
25%	18.06	14.09
Optimum conditions pH 10.5, 15% CO_2, white light, (1000 lux for aerated vessel)	21.05	20.14

*na not available.

4. Conclusions

In conclusion, the present study has successfully cultured *C. vulgaris* using a 5-litre tubular-shaped aerated vessel and oval-shaped vessels with a centred-light source. Both types of photobioreactor produced an acceptable amount of biomass and lipid content. The selected parameters (pH, light intensity/colours, and CO_2 concentration) each contributed positively to the growth of *C. vulgaris*. The optimum conditions were pH 10.5, white light, and 15% CO_2 concentration, resulting in a lipid content of around 20–21%. The tubular-shaped reactor with an outer light source produced a higher biomass compared to the oval-shaped reactor with a central light source. Future modifications can improve the design and operation of each reactor for microalgae cultivation.

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