The potential of attached growth of microalgae on solid surface for biomass and lipid production

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Abstract. Microalgae cultivation is usually performed via suspended growth system; however, the subsequent harvesting technologies are either costly or energy intensive. Herein, attached growth of *Chlorella vulgaris* was investigated using low-cost supporting materials (polystyrene foam and cotton duct) to ease the microalgae harvesting process. Cotton duct served as a better supporting material than polystyrene foam as it promoted microalgae attachment. Evidently, the attached cultivation performed on cotton duct granted higher biomass yield (16.40 g/m²) than the attached cultivation with polystyrene foam (11.70 g/m²) after 14 days of cultivation. From the studied range of 0.1 – 0.3 v/v%, the optimal inoculum concentration for the attached cultivation of *C. vulgaris* on cotton duct was 0.3 v/v%. After 14 days of cultivation, the optimized attached cultivation of *C. vulgaris* was capable to produce a biomass yield of 18.2 g/m². The lipid content of dried microalgae from optimized attached cultivation (43 wt%) was higher than that of suspended cultivation (32.7 wt%).

1. Introduction

For the past decade, global energy demand underwent most conspicuous growth in 2018, with an increment of circa 2.3% [1]. Due to the upsurge of renewable energy, biofuel is often applauded as the
potential surrogate of exhaustible fossil fuel [2]. Biofuel is readily produced from edible crops, crop wastes, woody parts of plants, and even algae [3]. Despite the potentiality of food-based biofuel, the food versus fuel controversy prompted the birth of third generation biofuel that derived from microalgae [4]. As compared to food crops, microalgae are better biofuel feedstock with faster growth rate, simpler cell structure (tolerate most of the environment), and higher lipid productivity [5]. Besides, microalgae-based biofuel is more environmental benign than fossil fuel in terms of lesser carbon footprint [6].

The commercialization of microalgae cultivation for biofuel production was eventually impeded by high production cost [7], which associated with cost-ineffective microalgae harvesting process (about 30% of total cost [8]). Prevalently, suspended growth system was opted for microalgae cultivation whereby the microalgae dispersed freely over aqueous media [9]. Separation of microalgae from enormous amount of aqueous media could be performed using coagulation-flocculation, filtration, flotation, or centrifugation [10]. Aforesaid separation techniques are either costly or energy intensive. Lately, attached growth system was touted as a promising cultivation system to ease microalgae-media separation by forming biofilm on the surface of substrate. In relative to suspended growth system, the attached growth system necessitates lower water requirement, shorter hydraulic retention time, lower capital cost, and lesser monitoring [11]. Apart from high microalgae yield, the attached growth system allows the microalgae being concentrated in the form of biofilm, thereby guarantees a more effective microalgae separation.

To date, the microbial biofilm designs of attached growth system could be classified into three types, viz. permanently immersed biofilms, biofilms between liquid and gaseous phases, or permeated biofilms, depending on the biofilm arrangement and liquid medium supply [5]. For attached cultivation, environmental parameters that could influence biofilm growth include microalgae strains, supporting material for attachment, liquid medium flow rate, concentration of nutrients, and light intensity [12]. Previous studies demonstrated the potentiality of attached cultivation to replace suspended cultivation; nonetheless, this method had not been comprehensively investigated.

Herein, attached cultivation of *Chlorella vulgaris* microalgae was performed using cheap and readily available supporting materials, namely polystyrene foam and cotton duct. Despite diverse species of high lipid-bearing microalgae, *Chlorella vulgaris* was selected in this work owing to its small cell size and high tolerability to extreme environmental conditions [13]. After the screening of better supporting material (polystyrene foam or cotton duct), the attached cultivation of *Chlorella vulgaris* was further optimized for its biomass yield by varying inoculum concentration (0.1 – 0.3 v/v%). For optimization study, the reported biomass yields were biomass dry weights that quantified after 2 weeks of cultivation. Lastly, the lipid content of microalgae from optimal attached cultivation was assessed before compared with that from suspended cultivation.

### 2. Materials and methods

#### 2.1. Stock culture preparation

*Chlorella vulgaris* was sourced from Centre of Biofuel and Biochemical Research (CBBR), Universiti Teknologi PETRONAS. The microalgae were cultivated in Bold’s Basal Medium (BBM). Every 1.0 L of BBM culture medium was made up of (a) 10 mL of NaNO₃ (2.5 g/L), CaCl₂•2H₂O (2.5 g/L), MgSO₄•7H₂O (7.5 g/L), K₂HPO₄ (7.5 g/L), KH₂PO₄ (17.5 g/L), NaCl (2.5 g/L); and (b) 1 mL of anhydrous EDTA (50 g/L), KOH (31 g/L), FeSO₄•7H₂O (4.98 g/L), H₂SO₄ (1 mL), H₂BO₃ (11.4 g/L), ZnSO₄•7H₂O (8.82 g/L), MnCl₂•4H₂O (1.44 g/L), O₃ (0.71 g/L), CuSO₄•5H₂O (1.57 g/L), Co(NO₃)₂•6H₂O (0.49 g/L). For the preparation of stock culture, 0.5 L of mother seed was inoculated into 1.0 L Duran bottle with an initial pH of 3.5, wherein the surrounding temperature was about 25 – 28 °C. During cultivation, compressed air was supplied to aerate the culture medium whereas cool-white fluorescent light (Philip TL-D 36W/865, light intensity of 60 – 70 μmol/m²•s) was used to illuminate the culture medium [14].

#### 2.2. Suspended cultivation
By using optimum condition from previous study [13], suspended cultivation of *C. vulgaris* was carried out in a 5-L Duran bottle to serve as a control. Chicken manure compost was used as the nutrient to further grow *C. vulgaris* from seed culture. A 10 g of compost was dissolved in 0.6 L of tap water, and the mixture was stirred for 24 h. Centrifugation of mixture was then performed to separate insoluble solids from the compost solution. For suspended cultivation in sterilised bottle, a 5-L culture medium was prepared from 0.5 L of stock culture, 0.2 L of compost solution, and 4.3 L of tap water. The initial pH of culture medium was adjusted to 3 while the ambient temperature was around 25 – 28 °C. The culture medium was aerated and light-illuminated simultaneously, similar to the stock culture preparation. Tap water was added daily to compensate the loss of water from the culture medium through evaporation. Figure 1 shows the experimental set-up of suspended cultivation.

**Figure 1.** Experimental set-up of suspended cultivation.

### 2.3. Attached cultivation

Figure 2 illustrates two different experimental set-ups employed for attached cultivation of *C. vulgaris*. For a fair comparison with suspended cultivation, attached cultivation of *C. vulgaris* was performed using identical volumetric ratio of stock culture: compost solution: tap water (2.5: 1: 21.5). Under the illumination of cool-white fluorescent light, the culture medium was aerated with compressed air using air pump and air stones. The initial pH of culture medium was adjusted to 6, and the culture temperature was maintained in between 25 – 28 °C. Tap water was added daily to compensate the loss of water from the culture medium through evaporation.

For screening of supporting materials, set-up Version 1.0 shown in Figure 2 (a) was used to ease the growth observing process. In this set-up, supporting materials (polystyrene foam and cotton duct) were cut into strips (surface area of 3 cm × 9 cm), which later placed in bioreactor. The bioreactors were modified from clear rectangular polypropylene containers, with size of 16.5 cm length × 11.5 cm width × 7.1 cm height. A total volume of 500 mL of culture medium (50 mL stock culture, 20 mL of compost solution, and 430 mL of tap water) was transferred to the bioreactor.

After the identification of suitable supporting material, set-up Version 2.0 shown in Figure 2 (b) was used for the optimization of attached cultivation for biomass yield. The aforesaid optimization was done by manipulating the inoculum concentration from 0.1 – 0.3 v/v%. The selected supporting material was cut into smaller pieces (surface area of 1 cm × 1 cm), and 300 pieces of supporting material were loaded into the bioreactor. Every piece of supporting material was numbered and weighed to facilitate the computation of biomass yield. The bioreactors were 1-L conical flasks instead of the polypropylene containers. For 0.1 v/v% inoculum concentration, the 1-L culture medium consisted of 100 mL of stock culture, 40 mL of compost solution, and 860 mL of tap water. The volume of compost solution was kept constant during the manipulation of inoculum concentration.
Figure 2. Experimental set-ups of attached cultivation: (a) set-up Version 1.0 (b) set-up Version 2.0.

2.4. Measurement of suspended biomass concentration
Several known volume of culture media were measured for their optical density at a specified wavenumber of 688 nm (OD$_{688}$) with the aid of UV-Vis spectrophotometer (Shimadzu UV-1280). Concurrently, these culture media were oven-dried at 100 °C to determine their suspended biomass concentration. From this preliminary work, the correlation between OD$_{688}$ and suspended biomass concentration was purposed as Eq. 1. Hence, the suspended biomass concentration of culture media could be estimated from OD$_{688}$ via Eq. 1.

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\text{Biomass concentration (g/L)} = 0.5315(\text{OD}_{688}) + 0.0333; R^2 = 0.9721 \quad (1)
\]

2.5. Microalgae harvesting
Different microalgae harvesting methods were used for suspended and attached cultivation, but aeration was stopped before any microalgae harvesting. For suspended cultivation, sedimentation method was applied wherein the microalgae were allowed to settle down naturally in 2 days’ time. After settling, excess water was discarded, and the concentrated microalgae were oven-dried at 105 °C for 24 h. The dried microalgae were scrapped from the beaker and kept for further use. For attached cultivation, it was impractical to collect all the dried microalgae from supporting material by scrapping. Therefore, wet pieces of supporting material (microalgae and supporting material) were retrieved and oven-dried at 105 °C for 24 h. The biomass yield of attached cultivation was expressed as the weight of dried microalgae per unit surface area of supporting material (g/m$^2$).

2.6. Microalgae lipid extraction
The method of microalgae lipid extraction was adopted from the work of Bligh and Dyer [15]. Briefly, 0.2 g of dried microalgae (from suspended cultivation) and 50 pieces of dried supporting materials with microalgae (from attached cultivation) were transferred to pre-weighed beakers. A 60 mL of methanol-chloroform solution (volumetric ratio of 2:1) was poured into each beaker. Both mixtures were stirred at 400 rpm for 24 h in ambient temperature. The resulting mixture was filtered with SMITH filter paper (mean pore size of 20 – 25 µm) using a funnel. Then, the filtrate was air-purged at room temperature inside fume hood to evaporate the solvent before oven-dried at 105 °C for 24 h. Lastly, the dried microalgae lipid was weighed, and its lipid content was reported on the mass basis of dried microalgae.

3. Results and discussions
3.1. Screening of supporting material
In this study, polystyrene foam and cotton duct were purposed as low-cost supporting materials for attached cultivation of C. vulgaris. They were selected owing to their reusability and high availability [16]. Figure 3 depicts the transient profile of suspended biomass concentration for attached cultivation of C. vulgaris on polystyrene foam and cotton duct over 14 cultivation days. After 1 day of cultivation, the suspended biomass concentration dropped significantly, indicating the occurrence of C. vulgaris...
microalgae attachment on supporting materials. The increment of suspended biomass concentration after day 1 eventually discloses that some microalgae detached from supporting materials as the initial adhesion was not firm. As compared with polystyrene foam, the use of cotton duct as supporting material recorded a lower suspended biomass concentration throughout 14 days of cultivation. A good supporting material should generate a low suspended biomass concentration as it favours the attached cultivation. Thus, cotton duct was preferred over polystyrene foam as a supporting material.

Figure 4 shows the biomass yield for attached cultivation of \textit{C. vulgaris} on polystyrene foam and cotton duct on 10\textsuperscript{th} and 14\textsuperscript{th} cultivation day. The biomass yield of both attached cultivation increased from day 10 to 14, proving the microalgae growth in attached growth system. The attached cultivation of \textit{C. vulgaris} performed with cotton duct granted a higher biomass yield (16.40 g/m\textsuperscript{2}) than that of polystyrene foam (10.05 g/m\textsuperscript{2}). This finding corroborates the microalgae attachment was more prevailed over cotton duct. Hence, cotton duct was employed for subsequent attached cultivation since it serves as a better supporting material as compared to polystyrene foam.

The possible reason for cotton duct outperformed polystyrene foam was the unique texture of cotton duct, which created protective clefts that shielded the microalgae biofilm from shear force in aerated liquid media [16]. By using cotton duct as supporting material, the biomass yield obtained in the current
work (16.40 g/m²) was comparable with that of previous research (16.20 g/m²) conducted by Gross et al. [16]. Gross et al. [16] performed attached cultivation of *C. vulgaris* with a rotating algal biofilm system while this study examined attached cultivation of *C. vulgaris* with a simple 1-L conical flask. Surprisingly, the utilization of cotton duct gave similar biomass yield regardless of reactor configuration.

### 3.2. Inoculum concentration

Inoculum concentration represents the concentration of stock culture in the cultivation medium. The attached cultivation of *C. vulgaris* on the cotton duct was optimized for its biomass yield by altering the inoculum concentration from 0.1 to 0.3 v/v%. According to Huang et al. [17], inoculum concentration could influence the light penetration to the biofilm, so optimal inoculum concentration should be used to maximize the microalgae growth. A dilute inoculum could result in fewer participating microalgae in photosynthesis whereas a concentrated inoculum might hinder the light transmission to the biofilm [18].

Figure 5 presents the influence of inoculum concentration on attached cultivation of *C. vulgaris* on cotton duct over 14 days of cultivation. The positive growth trend reveals that the microalgae could be cultivated via attached growth system. Figure 6 summarizes the final biomass yield for attached cultivation of *C. vulgaris* on cotton duct using different inoculum concentrations. In overall, the final biomass yield increased as the inoculum concentration increased from 0.1 – 0.3 v/v%.

![Figure 5](image)

**Figure 5.** Influence of inoculum concentration on attached cultivation of *C. vulgaris* on cotton duct over 14 days of cultivation.

![Figure 6](image)

**Figure 6.** Final biomass yield for attached cultivation of *C. vulgaris* on cotton duct using different inoculum concentrations.

In present study, the biomass yield did not decline with greater inoculum concentration used, positing the cool-white fluorescent light was approachable by the microalgae biofilm for photosynthesis even at highest inoculum concentration of 0.3 v/v%. For inoculum concentration range of 0.1 – 0.3 v/v%, the increase of inoculum concentration enhanced the biofilm growth on cotton duct, which reduced the
lengthy lag-phase and improved the survival rate of microalgae over other microbes [15]. Therefore, the optimal inoculum concentration for attached cultivation of *C. vulgaris* on cotton duct was 0.3 v/v% in this project.

3.3. Lipid content

Lipid is the most precious part of microalgae since it could be converted into biodiesel, viz. a potential substitute for petroleum-based diesel [2]. The dried microalgae samples used for lipid extraction were collected from optimized suspended cultivation [13] and attached cultivation. Figure 7 compiles the lipid contents of dried microalgae from current work and previous studies. Under optimized conditions, the lipid contents of dried microalgae from suspended and attached growth systems were 32.7 wt% and 43 wt%, respectively. Therefore, the employment of attached cultivation on cotton duct could enhance the lipid content of *C. vulgaris* by circa 1.31 times. As compared to previous studies on attached cultivation, the lipid content of dried microalgae from optimized attached cultivation was nearly 3.64 – 4.83 times of literature lipid contents [11, 16]. This finding evinces the feasibility of optimization to maximize the lipid content of dried microalgae from attached cultivation. For cultivation of *C. vulgaris*, the attached growth system seems to be more effective than suspended growth system.

![Figure 7. Lipid contents of dried microalgae from current work and previous studies](image)

4. Conclusion

The present work investigated the potentiality of attached growth system to cultivate *Chlorella vulgaris* on low-cost supporting material like polystyrene foam and cotton duct. The declined suspended biomass concentration after 1 day of cultivation corroborated the microalgae attachment on supporting material. As compared to polystyrene foam, the cotton duct was more suitable to be used as supporting material since it attained lower suspended biomass concentration and higher biomass yield throughout 14 days of cultivation. For attached cultivation of *C. vulgaris* on cotton duct, the biomass yield was optimized by altering the inoculum concentration from 0.1 – 0.3 v/v%. The parallel trend between biomass yield and inoculum concentration suggested the light penetration was suffice for 0.1 – 0.3 v/v% inoculum concentration. In the specified range, the optimal inoculum concentration was 0.3 v/v%, which achieved highest biomass yield (18.2 g/m$^2$ on day 14). Herein, the lipid content of dried microalgae from optimized attached cultivation (43 wt%) was greater than that of suspended cultivation (32.7 wt%). Aforesaid finding shows the feasibility of attached cultivation for *C. vulgaris*, but further investigations should be conducted to scrutinize the effect of other environmental parameters on the biofilm growth.

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