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Abstract. Harvesting of suspended microalgae biomass will generally incur excessive time and intensive energy due to low biomass density. Microalgae cultivation via fluidized bed bioreactor was introduced to tackle the harvesting process in which the support material was fluidizing within the culture medium, allowing the microalgae to settle onto the surface of fluidized material and grow thereafter. The Central Composite Design (CCD) was adopted to design the experiments for optimization of attached microalgae growth onto the fluidized bioreactor. The optimization condition occurred at 216 µmol/m^2 s light intensity and 9% CO_2 concentration with maximum biomass concentration (X_{max}) and maximum specific growth rate (μ_{max}) of attached microalgae obtained at 0.692 g/L and 0.028 1/h, respectively. The Verhulst logistic kinetic model illustrated the attached microalgae growth from lag to stationary phase, supporting the use of this model to represent the kinetic of attached microalgae growth onto the fluidized bed bioreactor under various condition.

1. Introduction

Microalgae biomass fall in category of the third-generation of biodiesel replacing the first and second generation. The first-generation of biodiesel derived from plant help in minimizing the burning of fossil fuel and CO_2 emission, but adverse the effect of food versus fuel crisis and land utilization. Concern on these problems, researchers start to shift to lignocellulosic feedstock which can be obtained from plant biomass which is also known as the second-generation of biofuel. Although, the second-generation biodiesel is considered as a very cheap and abundant plant waste biomass, the production cost to convert the lignocellulosic to biofuel is much more expensive than petroleum-based fuel. Hence, researchers start to focus on the third-generation biodiesel to overcome the drawbacks faced by first and second-

generation biofuels. Microalgae captured the CO_2 from atmosphere 10 to 50 times faster than first generation of biodiesel due to their high growth rate [1]. Considering this, the microalgae can act as the powerful carbon sink in mitigating the CO_2 emission. Apart from that, certain microalgae species such as *Chlorella vulgaris* have a short life cycle, thus the biodiesel production are expected to be 15 to 300 times higher than plant-based fuel [2].

Although, microalgae are considered as the feasible ways to replace the petroleum-based fuel, the commercialization approaches of this application are still not applicable[3]. Since microalgae are very small in size, the separation of microalgae cells from the culture medium lead to the intensive time and energy requirements causing the cost for harvesting alone covered up to 30% of total production cost. Hence, attached microalgae growth systems are introduced to reduce this cost by replacing the traditional approach on microalgae harvesting such as sedimentation, flocculation, filtration, and centrifugation. In this cultivation system, the support material is introduced inside the culture medium to promote the microalgae growth onto the surface of material. Attached microalgae growth system can be categorized into two, namely, fix bed bioreactor and fluidized bed bioreactor. Several study on fix bed bioreactor shows a tremendous increase on microalgae growth than suspended culture systems with an ease of harvesting process as the microalgae biomass are easily harvest from the support material through scrapping [4, 5]. Since microalgae form a thick layer on the support material for fix bed bioreactor, this system inherent several drawbacks, for instances, nutrient limitation and limited light penetration within the layer [6]. Instead of culturing the microalgae using fixed support material, some researchers started to culture the microalgae using the fluidized support material to tackle the problems faced by fix bed bioreactor [7]. The study thus far only focuses on initial attachment of microalgae onto the support material, hence, further enhancement of the microalgae growth should be implemented to make this system as a promising way to overcome the feedstock shortage for microalgae industry.

Microalgae cultivation via fluidized bed bioreactor was introduced to tackle the harvesting process in which the support material was fluidizing within the culture medium, allowing the microalgae to settle onto the surface of fluidized material and grow thereafter. This cultivation mode enhances the *Chlorella vulgaris* growth and eases the harvesting process as the biomass on support material can be facilely separated from the culture medium. Thus, this study provided an insight on the impacts of light intensity and CO_2 concentration in enhancing the immobilized *Chlorella vulgaris* growth in fluidized bed bioreactor. The microalgal cells assimilate carbon dioxide as a carbon source and utilize light energy for photosynthesis, which make these two factors playing a decisive role in microalgal growth. Thus, it is important to reveal the delivery of light intensity and CO_2 concentration in order to obtain a high yield of microalgal biomass.

2. Materials and methods

2.1. Chlorella vulgaris stock
About 0.5 L *Chlorella vulgaris* species was cultured inside 5 L Duran bottle containing 4.5 L Bold's Basal medium as a stock culture which consist of the following chemicals: (1) 10 mL per liter of the following chemicals: $NaNO_3$ (2.5 g/L), $CaCl_2.2H_2O$ (2.5 g/L), $MgSO_4.7H_2O$ (7.5 g/L), K_2HPO_4 (7.5 g/L), KH_2PO_4 (17.5 g/L), NaCl (2.5 g/L) and (2) 1 mL per liter of the following chemicals: EDTA anhydrous (50g/L), KOH (31 g/L), $FeSO_4.7H_2O$ (4.98 g/L), H_2SO_4 (1 mL), H_3BO_3 (11.4 g/L), $ZnSO_4.7H_2O$ (8.82 g/L), $MnCl_2.4H_2O$ (1.44 g/L), MoO_3 (0.71 g/L), $CuSO_4.5H_2O$ (1.57 g/L), $Co(NO_3)_2.6H_2O$ (0.49 g/L) [7]. The *Chlorella vulgaris* species was acquired from Centre for Biofuel and Biochemical Research (CBBR), Universiti Teknologi PETRONAS stock culture. The culture condition of the stock culture was maintained at light intensity of 70 $\mu mol/m^2$ s, aeration rate of 6.5 L/min, and temperature of 24-26°C throughout the experiment.

2.2. Experimental design
Central composite design (CCD) was adopted using Design-Expert version 10 software to design the batch experiments under different light intensities (ranging from 100 to 300 $\mu mol/m^2$ s) and CO_2

concentrations (ranging from 2% to 12%), resulting in a total of 13 experimental runs needed to be conducted as shown in Table 1. The ranges of both light intensity and CO_2 concentration were selected based on the literature studies for *Chlorella vulgaris* species [6, 8]. The maximum growth rate and specific growth rate of the attached growth microalgae onto fluidized bed bioreactor were selected as the response in this study.

Table 1. Batch experimental runs based on central composite design tools.

Run No.	Light Intensity ($\mu mol/m^2 s$)	CO_2 Concentration (%)
1	200	7
2	100	2
3	100	12
4	200	7
5	200	12
6	300	7
7	200	7
8	200	2
9	200	7
10	100	7
11	300	12
12	300	2
13	200	7

2.3. Experimental set up
Each batch cultivation was setup using a conical flask with a working volume of 3 L. Ten percent (v/v) of *Chlorella vulgaris* stock with an initial concentration of 0.129 g/L was introduced into the flask containing 90% (v/v) of Standard Bold's Basal Medium and 6% (v/v) of polyurethane foam (with 96% of void volume). The optimum pH value of medium was adjusted and maintained at 6 throughout the cultivation period to attain high biomass and lipid yields as documented in previous study [9]. All bioreactors were continuously illuminated with six fluorescent lamps and their individual light intensity received was controlled by adjusting the distance of bioreactor against the light source. The carbon dioxide gas and air were controlled by a flow meter and allowed to mix in the T-junction vessel before being injected into each bioreactor. The aeration rate was maintained at 6.5 L/min until the end of experiment. The attached and suspended microalgae biomass was partially sampled every day and dried in the oven until their constant weights were attained.

2.4. Mathematical modeling
Verhulst logistic kinetic modeling was employed to study the kinetic of attached microalgae growth onto polyurethane foam as shown in Eq. (1) [10].

$$\frac{dX}{dt} = \mu_{max} \left(1 - \frac{X}{X_{max}} \right) X \qquad (1)$$

where, dX/dt is the growth rate of attached microalgae (g/L h), μ_{max} is the maximum specific growth rate of attached microalgae (1/h), X_{max} is the maximum biomass concentration of attached microalgae (g/L), and X is the actual biomass concentration of attached microalgae at time, t. The sigmoidal shape of the graph was expected to illustrate the attached microalgae growth from lag to stationary phase under different light intensity and CO_2 concentration [11].

Equation 1 was integrated to calculate the evolution of biomass concentration, as shown below:

$$X(t) = \frac{X_0 X_{max} e^{\mu_{max} t}}{X_{max} - X_0 + X_0 e^{\mu_{max} t}} \qquad (2)$$

where, X_0 (g/L) represent the initial concentration of the attached microalgae biomass. The experimental data were fitted into Eq. (2) to determine the maximum microalgae biomass concentration (X_{max}) and maximum specific growth rate (μ_{max}) of attached microalgae biomass for each experimental set up.

3. Result and discussion

3.1. Impact of light intensity and CO2 concentration on attached growth Chlorella vulgaris onto the fluidized bed bioreactor

At low light intensity, a further supplied of CO_2 failed to boost the attached microalgae biomass growth due to the low photon flux density penetration into the fluidized beds. This led to low CO_2 intake by the attached microalgae biomass from the culture medium. Chang et al. (2016) also found the similar findings, where the limited supply of light intensity cause self-shading effect on the microalgae, limited the electron transfer into the chlorophyll, and reduced the ability of the microalgae to capture the CO_2, thus reduce the microalgae performance [11]. So, for BR-C, the excess of CO_2 was being used by the suspended biomass for their growth, resulting in high total biomass since the suspended microalgae received more light as compared to BR-A and BR-B (Figure 1). It can be observed that at BR-D, the increase in light intensity to 200 µmol/m^2 s, increase about 15% of attached microalgae biomass from BR-A. This might be due to the increment of photosynthetically active region of the biofilm that enables them to assimilate the CO_2 in the culture medium for their growth. Further raise of CO_2 concentration to 12% causing the suspended biomass to increase simultaneously with the attached biomass, resulting in lower attached growth as the light was blocked by the concentrated suspended microalgae. Light inhibition occurred when attached microalgae biomass have reach the light saturation point, where increase of light intensity from 200 µmol/m^2 s to 300 µmol/m^2 s, decline the biomass growth even at high CO_2 concentration. This might be due to the ability of microalgae to block the extra photons from entering the photosystem II (PSII) and reduce the efficiency of electron transfer from PSII to PSI when the light intensity was to hig h [12].
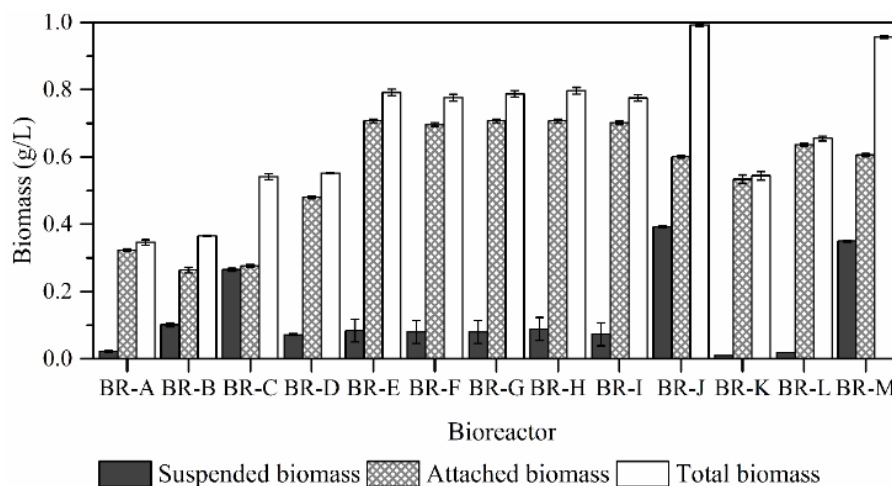

Figure 1. Effect of light and CO_2 concentration on suspended, attached and total microalgae biomass. Following symbols represent: (α_1) light intensity (µmol/m^2 s), and (α_2) CO_2 concentration (%). Where BR represent bioreactor with different light intensity and CO_2 concentration: (BR-A) α_1:100, α_2: 2; (BR-B) α_1:100, α_2: 7; (BR-C) α_1:100, α_2: 12; (BR-D) α_1:200, α_2: 2; (BR-E) α_1:200, α_2: 7; (BR-F) α_1:200, α_2: 7; (BR-G) α_1:200, α_2: 7;(BR-H) α_1:200, α_2: 7; (BR-I) α_1:200, α_2: 7; (BR-J) α_1:200, α_2: 12; (BR-K) α_1:300, α_2: 2; (BR-L) α_1:300, α_2: 7; (BR-M) α_1:300, α_2: 12.

3.2. Kinetic of attached Chlorella vulgaris onto fluidized bed bioreactor

The kinetic characteristics of attached microalgae growth onto the fluidized bed bioreactor were experimentally investigated under different light intensity and carbon dioxide concentration. The experimental data were fit in into Eq. 2 to obtain the maximum biomass concentration (X_{max}) and maximum specific growth rate (μ_{max}) of the attached microalgae growth. The experimental data of the attached microalgae growth onto the fluidized bed bioreactor fit well the logistic model with R^2 value more than 0.95 and RMSE value less than 1.0. Figure 2 shows the representative growth curve of attached microalgae under various light intensities and carbon dioxide concentration.
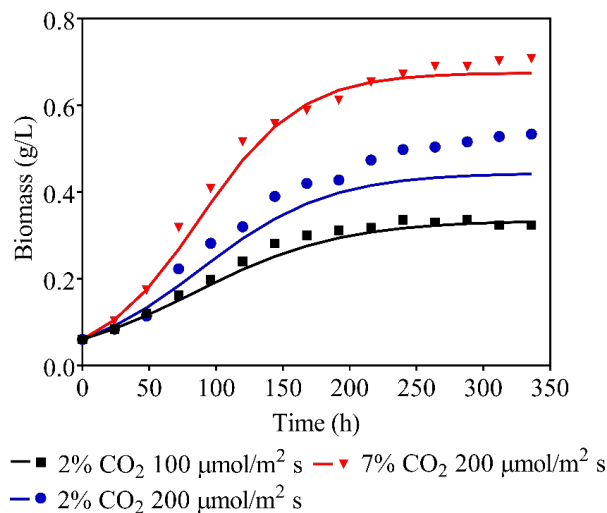

Figure 2 legend: — ■ 2% CO_2 100 µmol/m^2 s   — ▼ 7% CO_2 200 µmol/m^2 s
— ● 2% CO_2 200 µmol/m^2 s

Figure 2. Comparison of model prediction with experimental data. Line represent the model prediction and symbols represent the experimental data.

3.3. Optimization

The interactions between light intensity and CO_2 concentration were manipulated by CCD tools to identify the value of response at the maximum X_{max} and μ_{max} for optimization of attached microalgae growth onto the fluidized bed bioreactor. By narrowing the gap between the independent variables, the optimum combination of the mixture was achieved at light intensity and CO_2 concentration of 216 µmol/m^2 s and 9%, respectively as illustrate in Table 2. The optimum condition predicted by CCD occurred at optimum condition with maximum attached microalgae biomass concentration attained at 0.692 g/L and maximum specific attached microalgae growth rate at 0.028 1/h. Based on this study, light intensity and CO_2 concentration plays a significant role in enhancement of immobilized *Chlorella vulgaris* onto the fluidized bed bioreactor with the ease of microalgae separation process from the culture medium.

Table 2. Optimization of maximum growth rate (X_{max}) and maximum specific growth rate (μ_{max})

Optimization	Variable		Result		Percentage error
	Light intensity (µmol/m^2 s)	CO_2 concentration (%)	Predicted	Experiment	
X_{max}	216	9	0.692 g/L	0.695 g/L	0.3
μ_{max}	216	9	0.028 1/h	0.027 1/h	0.1

4. Conclusion

This study provides an insight into the kinetic characteristics and optimization of light intensity and carbon dioxide concentration in enhancing the attached microalgae biomass onto the fluidized bed bioreactor. The optimization condition occurred at 216 µmol/m^2 s light intensity and 9% CO_2 concentration with maximum biomass concentration (X_{max}) and maximum specific growth rate (μ_{max}) of attached microalgae obtained at 0.692 g/L and 0.028 1/h respectively. The Verhulst logistic kinetic model illustrate the attached microalgae growth from lag to stationary phase, supporting the use of this model to represent the kinetic of attached microalgae growth onto the fluidized bed bioreactor under various condition.

Acknowledgement

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