FEASIBLITY OF THE OPTICAL DENSITY (OD) IN THE DETERMINATION OF THE MICROALGAL BIOMASS USING PALM OIL MILL EFFLUENT (POME) AS MEDIUM

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Abstract- Microalgal biotechnology has attracted intense research interests in the past decade, and relevant topics of microalgal application in different fields have been extensively explored. Plotting the microalgae growth profile vs culture time period is the intuitive way to help understand the growth performance. Estimation of the microalgal biomass concentration by plotting the standard curve of optical density (OD) at certain wavelength is a rapid and common approach. However, the accuracy of this method might be affected by wavelength, microorganism morphology and culture medium compositions. In this study, the standard curve was generated from the biomass concentration of the microalgal strain Chlorella cultivated in diluted palm oil mill effluent (POME) against OD at 750 nm. The average errors of lag phase, exponential phase and stationary phase, as well as that of the entire culture period, were 9.59%, 8.12%, 3.25% and 6.86%, respectively. The results indicate that it is practical and feasible to use OD to determine the biomass concentration when the microalgae grow in POME medium.

Keywords- Microalgae, Chlorella sp, Optical Density, Biomass, Palm oil mill effluent.

I. INTRODUCTION

Microalgae are useful microorganisms for multiple purposes in different applications. The fatty acids from microalgal biomass could be utilized to produce biodiesel which is considered as third generation biofuels [1]. Microalgae pigments, such as astaxanthin, β-carotene and phycobiliproteins, are widely use in the health industry due to its anti-oxidant, and in the animal feed due to the function of its colorant [2]-[4]. Chlorella, Scenedesmus, Chlamydomonas and other species have been reported to grow in various wastewaters, and have the potential to remove the polluting nutrients efficiently and to produce high yields of microalgal biomass [5]-[7]. In all these processes, direct monitoring of microalgal biomass vs cultivation time shows the microalgae growth performance. Optical density (OD), or absorbance, is a common approach for measuring the microalgal biomass concentration since it is a simple and rapid way [8] [9]. OD at a particular wavelength is correlated to the cell concentration using the standard curve. Thus the standard curve is a quantitative research technique to determine the biomass concentration by using OD [10]. However, the accuracy of this method can be affected by the wavelength used, the cell morphology, and the chemical composition of the medium [11]. The biochemical composition in microalgal biomass is influenced by various parameters of culture conditions, such as the type of medium, temperature, pH, and agitation [12] [13]. A large number of different wastewaters have been used as media for microalgae growth. Palm oil mill effluent (POME) is one of the potential wastewater for microalgae growth due to its high concentration of nitrogen and phosphorus [14]. However, POME is thick brownish in color, and its chemical composition are different from that of the standard medium [15]. Therefore it is important to study the accuracy of the standard curve for measuring the microalgal biomass growing in POME medium. The objective of this study is to investigate the accuracy and feasibility of OD monitoring to determine the microalgal biomass using POME as culture medium.

II. DETAILS EXPERIMENTAL

2.1. Microalgae

Microalgal strain Chlorella sp was collected and isolated from POME sample at East Mill of Sime Darby Plantation in Carey Island, Malaysia. The culture was maintained on the shaker in Bold's Basal Medium (BBM) [16], illuminated under daylight white florescent lamp with light intensity of 4000 lux.

2.2. POME pretreatment

POME was collected from the anaerobic digester at East Mill of Sime Darby Plantation in Carey Island, Malaysia. POME was sterilized in 2L flasks by autoclave at 121°C for 20 min, followed by storage in the cool room at 4°C for three days to settle suspended solids (SS) down to the bottom of the flask. The supernatant of POME was then harvested and diluted six times with distilled water. Afterwards, it was filtered by microfiber filters (Grade GF/C) to remove any remaining SS, followed by TPP vacuum filtration to sterilize it.

2.3. Microalgae growth in POME

The inoculation volume is 10% of the final volume of the culture. 100ml of microalgae is poured into a flask containing 900ml of pre-treated POME medium. All operations were conducted in the biohazard safety cabinet to prevent contamination. The microalgae were bubbled with filtered mixture of 1% CO_2 in air and cultivated for 11 days, under cycles of 12 hours light and 12 hour darkness.

2.4. Biomass measurement

20 ml of microalgae culture was collected every 24 hours and filtered using Whatman glass microfibers (Grade GF/C 1.2µm) under vacuum, and rinsed with distilled water. The biomass was dried at 105°C in the oven for 24 hours and measured by the analytical balance.

2.5. Measurement of OD

2 ml of the microalgae culture was collected every 24 hours and centrifuged at 8000 rpm for 10 minutes. The supernatant was disposed gently and the biomass was rinsed with distilled water and re-suspended in distilled water to a volume of 2 ml. Afterwards, it was diluted four times to a maximum OD less than 1.0. OD was determined at wavelength of 750 nm by spectrophotometer (model: Hach DR 2800). The reading was carried out in triplicate and the average value was reported.

III. RESULTS AND DISCUSSION

3.1. Standard curve

Fig 1 shows the standard curve between microalgal biomass concentration and OD. The equation of linear regression was derived by Eq(1)

Biomass concentration = 1.0461×OD (R^2 = 0.984) (1)

The wavelength of 750 nm was selected to measure the OD of the microalgal biomass growing in the brownish POME. Griffiths et al (2011) suggested that at wavelength 750 nm the error due to the effect of changes in pigments content is minimized, and thus give a higher accuracy of correlation to estimate the biomass concentration [17]. In their study, the error between the actual value and that predicted by the standard curve in the whole growth period at wavelength 750nm is between 5% and 13%, which is lower than the corresponding error at wavelength 680nm of between 9% and 18% [17].

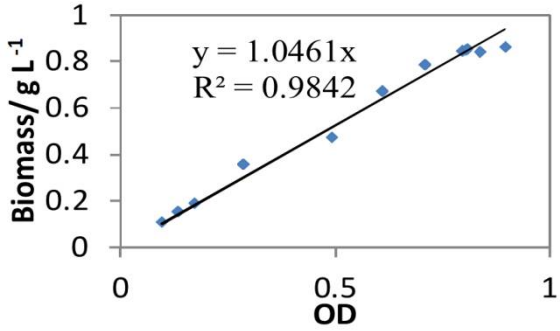


Fig. 1. Standard curve between biomass concentration and OD

3.2. Error introduced from standard curve at different growth phases

The predicted results of biomass concentration can be calculated from the Eq(1), and are presented in Table 2. Fig 2 shows distinctly the microalgae growth profile using both actual and predicted values in three different phases of lag phase, exponential phase and stationary phase. Error between actual and predicted values is then analyzed separately for each of the different growth phases.

Table.1. OD and biomass concentration in each day

Period	Day	OD_{750}	Actual Biomass (g L^{-1})
Lag phase	0	0.095	0.111
	1	0.132	0.156
	2	0.171	0.192
Exponential phase	3	0.285	0.359
	4	0.491	0.475
	5	0.608	0.674
	6	0.707	0.787
	7	0.836	0.842
Stationary phase	8	0.803	0.851
	9	0.794	0.847
	10	0.895	0.864
	11	0.806	0.855

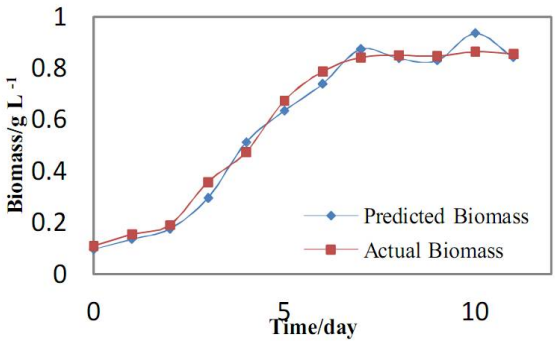


Fig.2. Microalgae growth profile of actual and predicted values

Table 2: Errors in predicted value from OD at 750 nm, relative to actual biomass measurements

Period	Actual Biomass (g L^{-1})	Predicted Biomass (g L^{-1})	Error	Error%
Lag phase	0.111	0.0993795	0.011621	10.47
	0.156	0.1380852	0.017915	11.48
	0.192	0.1788831	0.013117	6.83
Average				9.59
Exponential phase	0.359	0.2981385	0.060862	16.95
	0.475	0.5136351	0.03864	8.13
	0.674	0.6360288	0.037971	5.63
	0.787	0.7395927	0.047407	6.02
	0.842	0.8745396	0.03254	3.86
Average				8.12
Stationary phase	0.851	0.8400183	0.010982	1.29
	0.847	0.8306034	0.016397	1.94
	0.864	0.9362595	0.07226	8.36
	0.855	0.8431566	0.011843	1.39
Average				3.25
Average for all				6.86

Overall, the accuracy of the predicted value generated by standard curve in the entire growth period is

considered as generally acceptable due to the small average error of 6.86. The average errors generated in the lag phase and exponential phase are slightly different, but each is larger than that in the stationary phase. The previous research reported that standard curve gives more accurate result during the periods of exponential phase and early stationary phase, since the errors are less than 10% [17].

Thus, the results in this study are consistent with the data reported in that research. It indicates that the accuracy of estimation of microalgal biomass concentration using standard curve at wavelength of 750 nm is not likely to be impacted due to the use of POME as the culture medium.

CONCLUSIONS

The use of optical density measurement at wavelength of 750 nm to evaluate the biomass concentration of microalgae culture in POME medium is feasible. Error between the actual value and the predicted value is always in accepted range for biomass measurement.

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