

## Three types of Marine microalgae and *Nannochloropsis oculata* cultivation for potential source of biomass production

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**Abstract.** Microalgae have been vastly investigated throughout the world for possible replacement of fossil fuels, besides utilization in remediation of leachate, disposal of hypersaline effluent and also as feedstock for marine organisms. This research particularly has focused on locally available marine microalgae sample and *Nannochloropsis oculata* for potential mass production of microalgae biomass. Biomass produced by sample 1 and sample 2 is 0.6200 g/L and 0.6450 g/L respectively. Meanwhile, sample 3 and *N. oculata* has obtained maximum biomass concentration of 0.4917 g/L and 0.5183 g/L respectively. This shows that sample 1 and sample 2 has produced approximately 20% higher biomass concentration in comparison to sample 3 and *N. oculata*. Although sample 3 and *N. oculata* is slightly lower than other samples, the maximum biomass was achieved four days earlier. Hence, the specific growth rate of sample 3 and *N. oculata* is higher; meanwhile the specific growth rate of *N. oculata* is the highest. Optical density measurements of all the sample throughout the cultivation period also correlates well with the biomass concentration of microalgae. Therefore, *N. oculata* is finally selected for utilization in mass production of microalgae biomass.

### 1. Introduction

There is an intense research focus towards production of biofuel from microalgae as an alternative for fossil fuels. Unlike other energy crops, microalgae growth is extremely rapid besides having high photosynthetic efficiency and very high lipid content [1]. Although, the growth of microalgae is species dependent, they effortlessly double their biomass within 24 hour. Moreover, microalgae production provides a solution for the mitigation of carbon dioxide which causes climate changes. Whereby, one ton microalgae are estimated to consume 1.83 ton of atmospheric carbon dioxide [2]. Further, biofuel made from microalgae is non-toxic, biodegradable and renewable resource [3].

On the other hand, microalgae are also vastly being utilized in remediation of leachate from municipal waste and disposal of hypersaline effluent from desalination plants [4,5]). Also, microalgae rich in lipid especially in essential fatty acids are widely cultivated as diet for juvenile fish, crab, shellfish, and rotifers [6]. However, large scale fresh water microalgae production could endanger water availability. Therefore, marine microalgae species are more favourable for sustainable biofuel production [7]. Yet, there is no commercialization of biodiesel from microalgae. This is mainly due to high biomass production cost related to the microalgae cultivation.



A few plans have been strategized in order to reduce costs of microalgae biofuel which include usage of natural seawater or wastewater, reduce energy for cultivation and harvesting, and importantly increase the productivity and oil content [8]. Therefore, this research is undertaken to investigate potential source for mass production of microalgae biomass. This research investigates biomass production rate of locally available microalgae from Straits of Malacca Sea in comparison to pure strain *Nannochloropsis oculata*.

## 2. Material and Method

*Nannochloropsis oculata* purchased from UTAR Microalgae Sdn. Bhd. and three mixed cultures obtained from Malacca Straits Sea were used for all the experiments. The mixed cultures and their source location are listed in the table 1. Source of sample 1 and 2 are directly from seawater, meanwhile sample 3 is brakish water. These sources are chosen in order to compare the growth of microalgae from the seawater and brakish water. As there is higher diversity of microalgae in seawater, two seawater sources were selected.

Table 1: Sample location of wild microalgae obtained from Malacca Straits Sea

Sample	Location	Google GPS coordinate
1	Teluk Batik Beach	N 4° 11' 20.4", E 100° 36' 20.8"
2	Marina Cove Resort	N 4° 12' 48.06", E 100° 36' 08.1"
3	Titi Panjang (Lumut Jetty)	N 4°13' 56.0", E 100° 38' 31.6"

### 2.1. Cultivation

Small scale culture was conducted in 500 ml culture flask containing F2 medium for all the microalgae. During the exponential phase, the microalgae were then transferred into 5.0 L glass bottle containing F2 medium at  $23 \pm 2$  °C. All the cultures were continuously illuminated with fluorescent lamps approximately at 5000 lux and aerated with air at 3.0 L/min.

### 2.2. Data Collection

**2.2.1. Optical density.** Microalgae cultures were sampled every day in order to determine its optical density. One ml of culture was diluted four times with distilled water prior to optical density measurement. Measurements were conducted on Shimadzu UV-Vis Spectrophotometer (UV-2600) at wavelength 688 nm.

**2.2.2. Biomass determination.** On daily basis, 20 ml of culture medium was sampled from the microalgae culture and transferred into three glass vials respectively. Culture mediums were then centrifuged at 4000 rpm for 15 min to produce biomass pellets. Supernatants were removed and the microalgae pellets were dried in oven at 105°C for 24 hours before the dry mass is weighed.

**2.2.3. Microalgae growth determination.** The growth rate of each microalgae was characterized based on daily biomass determination. The specific growth rate,  $\mu$  of each microalgae sample was calculated from the slope of the linear regression of time and nature log of biomass concentration in exponential growth phase; as specified by Song et al., 2013.

$$\text{Specific growth rate, } \mu = (\text{Ln } M_0 - \text{Ln } M_1) / (t_0 - t_1)$$

Where,  $\mu$  is the specific growth rate in exponential phase,  $M_0$  is the biomass concentration at the beginning of exponential phase ( $t_0$ ) and  $M_1$  represent the biomass concentration at the end of the exponential phase ( $t_1$ ).

### 3. Result and Discussion

Generally, growth of microalgae can be segregated into four phases which are lag phase, log phase or exponential, stationary phase and finally death phase [9]. Lag phase is the initial phase of cultivation in which the microalgae adapts to the surrounding such as medium, pH, temperature and lighting. Subsequently, the microalgae begin to undergo active cell division and the biomass of the culture will increase usually in exponential order. Thereafter, stationary phase begins which ceases biomass increase. This is due to the equal rate of the cell division and cell death. This phase mainly occurs due to depletion of nutrients in the medium. Lastly, the microalgae death rate will be higher than the cell division rate, hence the graph shows decrease in biomass.

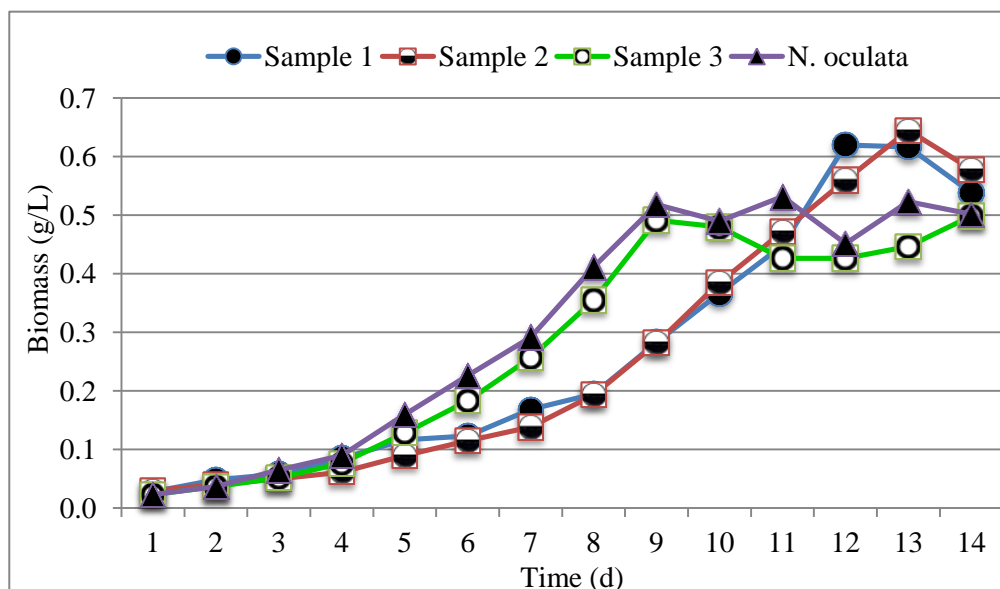


Figure 1: Biomass concentration of microalgae cultivated in F2 medium

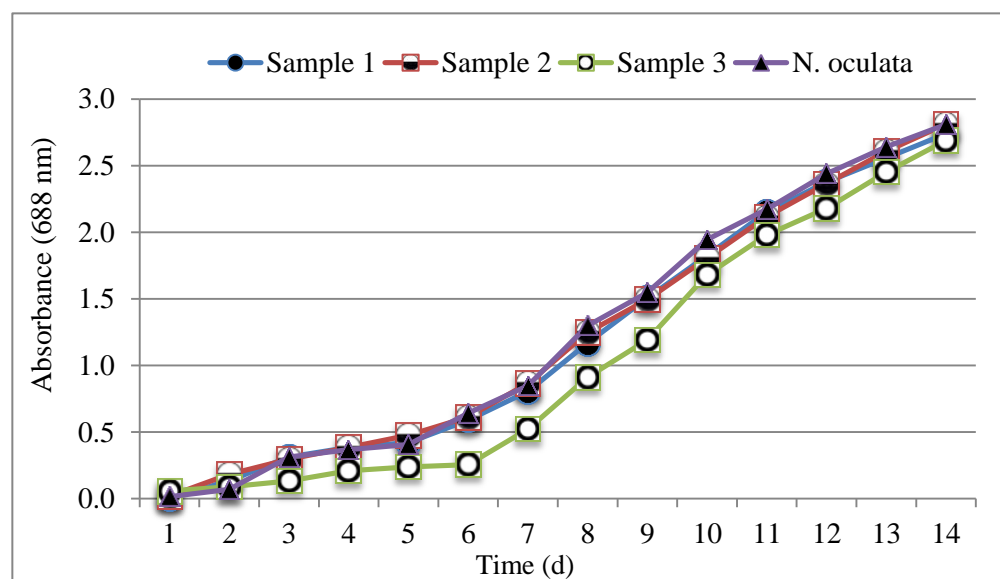


Figure 2: Absorbance measured at 688 nm for microalgae cultivated in F2 medium

Sample 3 and *N. oculata* reached its maximum biomass concentration on day 9 with 0.4917 g/L and 0.5183 g/L respectively. On the other hand, sample 1 and sample 2 reaches its maximum biomass concentration on day 12 and 13, later than sample 3 and *N. oculata*. Sample 1 and sample 2 have recorded biomass concentration of 0.6200 g/L and 0.6450 g/L respectively, which is higher than sample 3 and *N. oculata* approximately by 20%. Therefore, sample 3 and *N. oculata* is considered to have higher growth rate compared to other microalgae culture. Microalgae that produce high biomass concentration in short period of time is vital for high production of biodiesel or other valuable products. The trend of growth for sample 1 and sample 2 is similar, this may be due to the similar sampling location and therefore the microalgae biodiversity is also the comparable.

Occasionally, optical density is also used to measure the growth of microalgae. Optical density is used to measure the intensity of chlorophyll pigments in the microalgae cells. Highest absorbance of *N. oculata* was recorded at 688 nm, this is due to the fact that *N. oculata* contain chlorophyll  $\alpha$ . Therefore the optical density was measured at wavelength of 688nm. However, it does not accurately represent the growth of microalgae. In figure 2, absorbance of all microalgae cultures were exhibited. In actual microalgae growth based on biomass determination, the exponential phase begins from day 5, whereby absorbance diagram shows exponential phase on day 7. This is mainly due to the colour intensity of the culture which begins to become intense on day 7 onwards. The dark green colour of the microalgae culture explains the rapid growth of the microalgae. In addition, all microalgae culture exhibit similar trend of absorbance. It is also important to note that, the gradient of the curves reduces on day 13 to day 14, indicating that the intensity of the green colour is becoming plateau.

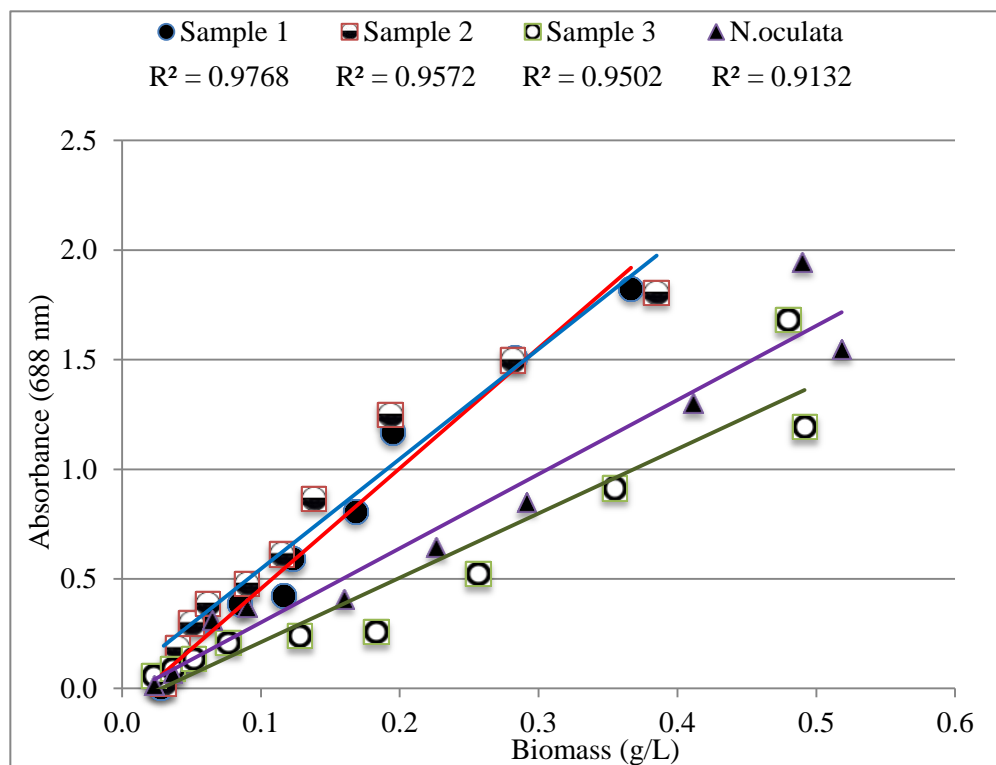


Figure 3: Correlation between absorbance measured at 688 nm and biomass concentration of microalgae

On the other hand, correlation between absorbance and biomass concentration were presented on figure 3. The absorbance correlates well with biomass concentration, especially during the exponential phase. As the microalgae reaches stationary phase, the curves bends upwards showing less correlation to the biomass concentration. Hence, absorbance measurement can be used to represent biomass concentration with a limitation, that it can only be used during the exponential phase. Therefore, absorbance measured at lag phase and stationary phase has been omitted from the diagram. The regression value obtained is reasonable, whereby the minimum  $R^2$  value obtained is 0.9132 for all microalgae culture conducted. Figure 3 also shows that, at same biomass concentration of different samples, the absorbance measured was different. This is mainly due to the presence of high chlorophyll content per unit cell which result in high optical density of microalgae cells. In addition, optical density can be utilized for a rapid estimation of microalgae biomass.

Table 2: Specific growth rate of various microalgae sample cultivated in F2 medium

Microalgae	Specific growth rate, $\mu$ ( $d^{-1}$ )
Sample 1	0.2558
Sample 2	0.2488
Sample 3	0.3386
<i>N. oculata</i>	0.3445

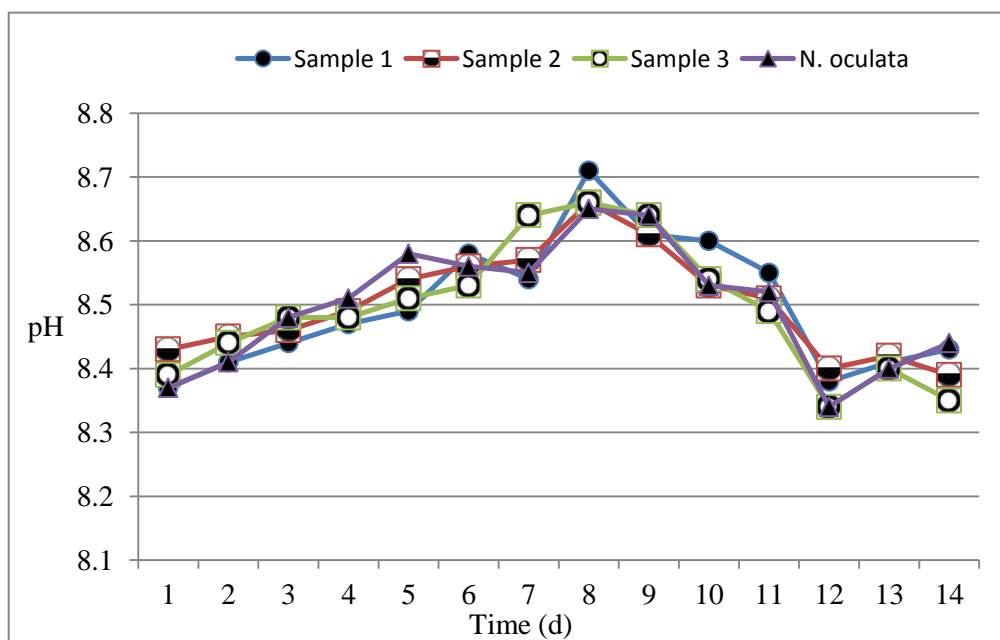


Figure 4: pH changes observed during microalgae cultivation in F2 medium.

Specific growth rate of microalgae were affected by cultivation of nutrient in culture medium and also the reproduction rate of the microalgae itself [9]. Sample 1 and sample 2 exhibited low specific growth rate that is 0.2558 and 0.2488 respectively (Table 2). In comparison, sample 3 and *N. oculata* exhibited significantly higher specific growth rate which is 0.3387 and 0.3445 respectively. Hence, it is obvious that *N. oculata* exhibit the highest growth rate among all samples investigated and it also reaches maximum biomass concentration earlier than other microalgae.

Microalgae growth are influenced by a few factors namely, nutrient concentration, carbon dioxide concentration, light intensity, aeration rate and pH. On a daily basis, pH of the microalgae culture was measured. Initial pH of nutrient medium was adjusted to  $8.00 \pm 0.05$ . Changes of pH in nutrient medium were presented in figure 4. All microalgae cultures exhibit similar trend that is increasing continuously until late exponential phase thereafter the pH gradually decreases. Maximum pH was observed on day 8 of cultivation which ranges from 8.66 to 8.71.

#### 4. Conclusion

Three types of locally available marine microalgae and *N. oculata* were successfully cultivated in F2 medium supplemented with artificial seawater. Among all samples investigated, *N. oculata* exhibited the highest specific growth rate and high biomass concentration in a shorter duration of cultivation time. Therefore, high biomass productivity can supply demand for high biodiesel production or other value added products which on the other hand will reduce the production cost. Due to that reason, *N. oculata* is selected for mass production of microalgae biomass.

#### 5. Acknowledgements

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