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Effect On Growth of *Desmodesmus sp* Under Different Incubation Conditions

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Abstract— The aim of the research was to find the best conditions for selected microalgae species, *Desmodesmus. sp* in term of growth productivity in different pH, temperature and intensity of light. *Desmodesmus. Sp* (TRG-D01) was isolated from Setiu Wetlands, Terengganu and cultured using Bold Basal Media (BBM). The species was incubated under different selected parameters for nine days using microplate reader technique through Optical Density (OD) measurement. From the experiment, *Desmodesmus. sp* showed variety of growth pattern. Incubation under different pH, light intensity and temperature showed specific growth rate ranging from 0.21 to 0.47 d^{-1} (maximum OD 0.67−0.93), 0.33 to 0.43 d^{-1} (maximum OD 1.12) and 0.12 to 0.42 d^{-1} (maximum OD=1.18) respectively. *Desmodesmus. sp* was optimum in were observed when incubated under pH in between 7 to 9, 56 to 91 μmol $m^{-2}s^{-1}$ of light intensity and optimum temperature between 23ºC to 27ºC.

Keywords: *Desmodesmus sp.*; Light Intensity; Optical Density; pH; Specific Growth Rate; Temperature

1. Introduction

Single celled green algae are photosynthetic microorganism that have ability to produce biomass from solar energy, CO_2 and nutrients. Nowadays, microalgae become an asset to consumers and industries especially from food industries, aquaculture and pharmaceutical. These products become interest for researcher for deeper understanding due to high nutritional value and potential that can be used in biodiesel, bioremediation human and animal nutrition. Generally stated that most microalgae able to undergoes photosynthesis and cell division process in between 15 to 30ºC but best growth conditions would be in between 20 to 25ºC (Munoz and Guieysse, 2006). Increasing temperature below optimum temperature showed that every 10ºC increment, photosynthesis, cell division and growth should be expected to be double until maximum temperature reached (Difusa *et al*., 2015). Exceeding favorable temperature would make microalgae growth decreased because heat would stress the enzyme or modify the protein that responsible for photosynthesis processes (Gardner *et al*., 2011)

Light also one of the major elements that responsible for microalgae growth. Microalgae used light as source of energy for their metabolic processes. High amount of light exposed also damaged and irreversible damage maybe occurred as excessive amount of photon absorption exceed the electron turnover, thus inhibited the photosynthesis process (Ren, 2014). Light source not only effect the photosynthesis activities but also influenced the metabolic

pathway and cell composition (He *et* al., 2015). Light intensity always dependent on volume and cell density of culture itself. On other words, light intensity needed to be increased if the microalgae cultured in bigger volume and high concentrations so that light able to penetrate entirely through the culture. Another critical conditions that determine microalgae growth is the pH since it determines the solubility and availability of CO_2 and nutrients content that significant for metabolic processes and the suitable range of pH for growth also specific for each microalgae (Rios *et al.*, 2015). pH of culture steadily increased due to utilization of inorganic carbon by microalgae. Depending on the species, microalgae able to adapt variety of environment conditions including pH, temperature and nutrient availability. Hence, the productivity of microalgae really dependent on how microalgae been cultured especially abiotic factor to maximize the production and quality in term of biological and chemical to design efficient photobioreactor (Van Wagenen *et al.*, 2014). This experiment was conducted to determine the optimum culture conditions namely pH, light intensity and temperature maximum growth of *Desmodesmus sp*. This work should be the one of first step for monitoring the best growth conditions before application into biomass production.

2. Materials & Methods

Microalgae Samples

Identified microalgae, *Desmodesmus. sp* (TRG-D01) was isolated from Setiu Wetlands and donated by University Malaysia Terengganu (UMT) in broth stock culture. Bold Basal Media (BBM) based on (Bischoff, 1983) was prepared and used in this experiment. Each macro and micronutrients were made into stock culture at given concentrations to 0.934 L of distilled water. Final volume of media would be 1L. The BBM media final concentrations consist of 2.94×10^{-3} M $NaNO_3$, 3.04×10^{-4} M $MgSO_4.7H_2O$, 4.28×10^{-4} M NaCl, 4.31×10^{-4} M K_2HPO_4, 1.29×10^{-3} M KH_2PO_4, 1.70×10^{-4} M $CaCl_2.2H_2O$, f/2 trace elements solution, 1.85×10^{-4} H_3BO_3, 1.71×10^{-4} $Na_2EDTA.2H_2O$, 1.79×10^{-5} $FeSO_4.7H_2O$, 5.53×10^{-4} KOH, f/2 vitamins solution (B_1, Biotin and Cyanocobalamin).

The environment set up for initially growth of Desmodesmus sp were set according to (Komatsu *et al.*, 2019) with slight modification. The inoculated culture was incubated under controlled environment using climate chamber, Memmert HPP110. The initial surrounding condition of culture media was adjusted to pH 7 and inside climate chamber was set at temperature 25°C, 40 μmol $m^{-2}s^{-1}$ of light intensity with photoperiod 12H:12H of light and dark cycle and the humidity was set to 0%rh. The initial optical density before cultivation experiment were kept in between 0.01 to 0.05.

Manipulation of parameters

For optimization purposes, the suitable range were selected based on different journal by (Difusa *et al.*, 2015; El-Sheekh *et al.*, 2017). For pH, the media was adjusted to pH 5, 6, 7, 8 and 9 by using either 1M of Hydrochloric Acid (HCl) or 1M of Sodium Hydroxide (NaOH). For light intensities and temperature, parameters were set according to preference by climate chamber. *Desmodesmus. sp* was cultivated under 5 different light intensities: (105 µmol $m^{-2}s^{-1}$, 91 µmol $m^{-2}s^{-1}$, 73 µmol $m^{-2}s^{-1}$, 56 µmol $m^{-2}s^{-1}$ and 40 µmol $m^{-2}s^{-1}$). The optical length between light source to microplate was about 0.012 m and surrounded by white LED. The microplate arrangement was changed/adjusted every 24 hours after growth analysis to give the microplate equal irradiation. Cultivation under different temperature were set into 20, 23, 25

and 27°C. As the temperature increased, the microplate was wrapped with 2 layers of parafilm and Mili-Q water surrounding the well with algae need to be added daily. The precaution needed to be taken into note as the evaporation can happen as the temperature and light intensity increased.

Microplate Technique

The incubation test started with inoculation of 20 µL of microalgae (10% of total volume per well) taken during exponential phases into 180 µL of cultured media. The total volume per well would be 200 µL. The analysis was conducted using 96 transparent with flat-bottom wells and microplate was divided into several zones as described in Figure 1. The border of microplate well was filled with 200 µL Mili-Q water to prevent evaporation. The border was not suitable to be used in experiment as it exposed to strong air flow though the microalgae able to grow due to more access to CO_2 and light. During 9 days of incubation, the pipetting (mixing) process all well containing microalgae have been conducted for efficient use of light and nutrients.

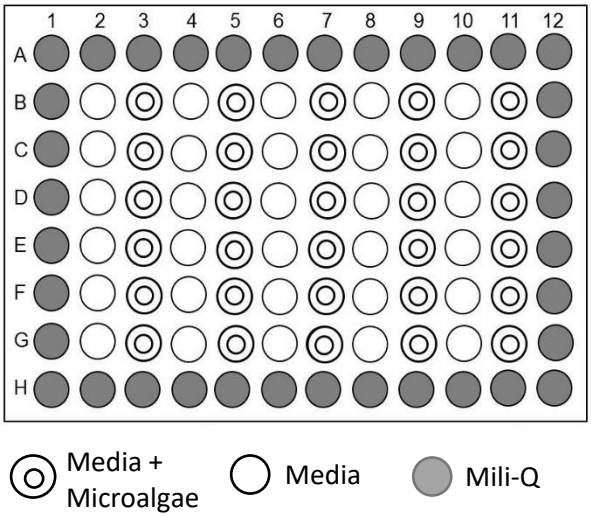

Figure 1: The designated zone of each wells using microplate reader for incubation experiment.

Growth Analysis

Growth of *Desmodesmus. sp* was monitored through Optical Density (OD_{680}) at 680 nm using Microplate reader Tecan M200 for every 24 hours in 9 days. Growth based of absorbance measurements were used in this study to determine microalgal biomass because the method was simple, fast, and proper technique to measure algal culture density (Sharma et al., 2016; Ding et al., 2015; Bohutskyi et al., 2015). For each recorded absorbance on each well, sensitive mean value (SMV) was applied using following formula:

$$SMV = \frac{OD_{tot} - OD_{Max} - OD_{Min}}{4}$$

where,

OD_{tot} = Sum of absorbance at OD_{680} of 6 wells

OD_{Max} = Maximum absorbance at OD_{680} of 6 wells

OD_{Min} = Minimum absorbance OD_{680} of 6 wells

Average OD_{680} of wells containing microalgae were subtracted with well containing media only (control) to obtain exact OD_{680} of microalgae. Meanwhile, the specific growth rate was measured during exponential phases of growth. The specific growth rate (μ) and division rate (k) was measured during exponential phases were calculated using formula as follow:

$$\mu = \frac{\ln(N_2/N_1)}{t_2 - t_1}$$

$$K = \frac{\mu}{\ln 2}$$

Where N_2 and N_1 are the value of OD at given times, t_2 and t_1, respectively.

3. Results and Discussion

The maximum OD, specific growth rate and division rate were recorded and tabulated into Table 1 and growth curve of each different parameters were shown in Figure 3. Figure 3A shows the growth curve based on different pH tested on *Desmodesmus sp*. Among the studies of pH range of cultured media, it was observed that the microalgae favour the neutral to alkaline state, pH 7 to 9. The microalgae Within this range, optical density recorded ranging from 0.67 to 0.93 with specific growth rate of 0.39 to 0.44 d^{-1} with division rate of 0.57 to 0.63. Within incubation period, no exponential phases or growth occurred when microalgae cultured under pH 5 and 6 of cultured media. Difusa *et al* (2015) reported that both isolated *Scenedesmus sp.* showed optimum growth at pH 7 to 9 in term of biomass productivity and lipid yield. Another study by (Bakuei *et al*., 2015) also suggest that optimum growth for *Scenedesmus sp* ranged between 8.2 to 8.7. The nutrient uptake was rapidly efficient and released as ammonia during this condition most likely to be the reason for the significant increase in the alkaline state for growth in the presence of CO_2.

Based on Figure 3B, all levels of light intensity showed significant growth of *Desmodesmus* species. Result of study revealed that with maximum light intensity observed the exponential phases at day 4 with maximum OD_{680} at day 9 was 1.12 and specific growth rate of 0.43 d^{-1}. In current study, *Desmodesmus sp*. seems able to grow varies of light intensity from 56-105 μmol $m^{-2}s^{-1}$ and lowering the light intensity directly slowed the growth of algae. This is due to the culture become denser and cell concentrations concentration become higher thus requires increasing light to penetrate throughout the culture depth (Wahidin *et al*., 2013). as the microalgae grow and replicate, light shading must be taken into consideration as cell tend to block each other (Maynardo *et al*., 2015). Incubation using microplate compared with flask or photobioreactor may be differ to one another as the volume and space of culture

exposed differently. Incubation under different light intensity in relation with growth rate can be applied in repeated batch cultures and relate the output to those large culture vessels and suitable for screening purposes (Van Wagenen *et al*., 2014). Based on (Difusa *et al*., 2015), both *Scenedesmus* species exhibit maximum biomass productivity when uniform increased of light intensity from 27 µmol $m^{-2}s^{-1}$ to 81 µmol $m^{-2}s^{-1}$ while increasing light intensity beyond 81 µmol $m^{-2}s^{-1}$ caused the biomass productivity became limited at 91 µmol $m^{-2}s^{-1}$. However, the author found out that the lipid content achieved highest as light intensity increased.

The growth curve under different temperature showed in Figure 3C. At temperature at 30°C notified highest OD_{680}, 1.18 with specific growth rate of 0.54 d^{-1} and the exponential phase could be observed at day 4. Compared to other temperature points, the specific growth rates were ranging from 0.12 to 0.44 d^{-1} and the OD_{680} measured in between 0.34 to 1.18. Subsequently, the exponential phases of these temperature except for 27°C could be seen towards day 5 to day 6. However, at 20°C, the growth slowly increased at day 7 and maximum OD_{680} was 0.34 only till day 9. *Scenedesmus acutus* showed maximum growth and biomass productivity (0.42 g L^{-1} d^{-1}) 30°C Another study also reported that highest biomass productivity between temperature 25°C to 30°C and and in between 25°C to 30°C with no significant variation but the growth inhibit when cultivated over 30°C (El-Sheekh *et al*., 2017). Higher temperature which is favour for microalgae in current study able to supply thermal energy that influenced cellular enzymatic activities, nutrient uptake and CO_2 fixation cycle (Karlo *et al*., 2015). Vanags *et al* (2015) reported that optimum temperature in between 23°C to 27°C (25°C ±) for *Desmodesmus communis* in shake flask culture with no significant difference between the range. The author also stated that 21°C and 29°C inhibited the growth thus not suitable for cultivation conditions. The contradiction of result maybe resulted from the cultivation technique used in study.

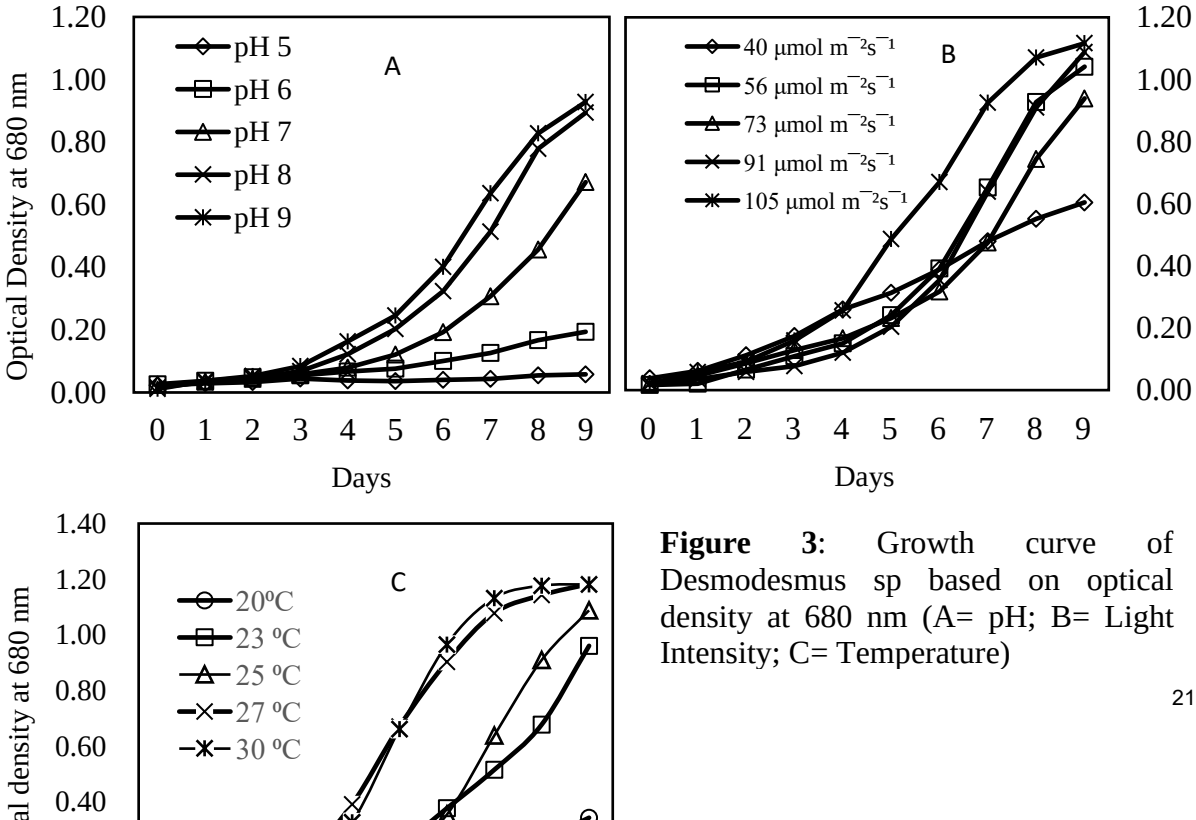

Figure 3: Growth curve of Desmodesmus sp based on optical density at 680 nm (A= pH; B= Light Intensity; C= Temperature)

Table 1: The maximum OD, specific growth rate and division rate of *Desmodesmus sp* at different incubation conditions

Parameters		Maximum OD at 680 nm	Specific Growth Rate, d^{-1}	Division rate, *k*
pH	5	0.06 ± 0.01	NA	NA
	6	0.19 ± 0.03	NA	NA
	7	0.67 ± 0.04	0.39 ± 0.02	0.57 ± 0.03
	8	0.89 ± 0.02	0.44 ± 0.02	0.63 ± 0.03
	9	0.93 ± 0.02	0.40 ± 0.02	0.58 ± 0.02
Light Intensity (µmol $m^{-2}s^{-1}$)	40	0.60 ± 0.08	0.18 ± 0.07	0.26 ± 0.10

	56	1.04 ± 0.04	0.43 ± 0.02	0.62 ± 0.03
	73	0.94 ± 0.05	0.34 ± 0.01	0.50 ± 0.02
	91	1.09 ± 0.04	0.46 ± 0.09	0.67 ± 0.12
	105	1.12 ± 0.02	0.43 ± 0.02	0.62 ± 0.02
Temperature (°C)	20	0.34 ± 0.08	0.25 ± 0.07	0.36 ± 0.11
	23	0.96 ± 0.07	0.30 ± 0.05	0.43 ± 0.07
	25	1.09 ± 0.04	0.44 ± 0.09	0.63 ± 0.12
	27	1.18 ± 0.05	0.42 ± 0.02	0.42 ± 0.03
	30	1.18 ± 0.04	0.54 ± 0.02	0.78 ± 0.04

NA=Not Available; no exponential phases of growth observed within incubation period
Note: The value represent means ± SD (n=6)

4. Conclusions

Desmodesmus sp (TRG-D01) able to grow in different conditions, at least in the experiment conducted. Present experiment clearly observed that the species favours in neutral to alkaline state (pH 7, 8 and 9) of cultured media, irradiance levels in between 56 µmol $m^{-2}s^{-1}$ to 105 µmol $m^{-2}s^{-1}$, and the temperature ranging from 23°C to 27°C give the steady increased of growth in term of optical density. Since the current study only cover the growth aspect and limited study due to incubation technique, it could be useful in future to investigate biochemical composition such as lipid content based on microplate technique. This groundwork could be useful as a first step towards biomass production.

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