

Mesocosm study of microalgae in different weather conditions.

Estudio del mesocosmos de microalgas en diferentes condiciones climáticas

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ABSTRACT

Microalgae have valuable contributions in carbon dioxide sequestration. There are no much investigations about motivation of mix microalgae productivity in outdoor cultures. This study aims to evaluate microalgae biomass production in outdoor mesocosms under different weather conditions. The experiment was done in Tilapia pond in the hatchery of fisheries of Universiti Putra Malaysia. Weather parameters were recorded daily. Microalgae seeds were obtained from Tilapia pond effluent and added to eight floating aerated mesocosms. Mesocosms were divided into four treatments. Two g triple supper phosphate: 20g Urea were used as fertilizers. Physical and chemical conditions, microalgae primary productivity and biomass, and species composition were measured every two days. Three cycles were categorized as mix, wet and dry cycles based on weather recording scores. Water quality parameters in treatments and controls cultures showed significant variations. Primary production variables were higher in the fertilized non-sheltered mesocosms (treatment 1). Productivity variables were lower in the dry cycle and higher in the mix cycle. The highest value of fixed CO₂ was (3.2) mg/L/d in treatment 1 in the mix cycle, while the lowest value was (0.11) mg/L/d in treatment 3 and control 1 in dry cycle. Changes in weather patterns are seen in the light and temperature values. Microalgae biomass was lower in dry weather conditions because of effect of high air temperature. Weather conditions and different treatments significantly influenced microalgae species composition, due to the sensitivity in some of them to different light intensities. Chlorophytes were the most abundant due to their ability to adapt with different culture conditions.

Key words: Primary productivity, Mesocosms, Climate change, Microalgae biomass, Weather change, Chlorophytes dominance.

RESUMEN

Las microalgas tienen contribuciones valiosas en el secuestro de dióxido de carbono. No hay muchas investigaciones sobre la motivación de mezclar la productividad de microalgas en cultivos al aire libre. Este estudio tiene como objetivo evaluar la producción de biomasa de microalgas en mesocosmos exteriores bajo diferentes condiciones climáticas. El experimento se realizó en un estanque de tilapia en el criadero de pesquerías de Universiti Putra Malaysia. Los parámetros meteorológicos se registraron diariamente. Se obtuvieron semillas de microalgas del efluente del estanque de tilapia y se agregaron a ocho mesocosmos aireados flotantes. Los mesocosmos se dividieron en cuatro tratamientos. Dos g de fosfato triple cena: 20 g de urea se utilizaron como fertilizantes. Cada dos días se midieron las condiciones físicas y químicas, la biomasa y productividad primaria de microalgas y la composición de especies. Tres ciclos se clasificaron como ciclos mixtos, húmedos y secos según las puntuaciones de los registros meteorológicos. Los parámetros de calidad del agua en los cultivos de tratamientos y controles mostraron variaciones significativas. Las variables de producción primaria fueron mayores en los mesocosmos fertilizados no abrigados (tratamiento 1). Las variables de productividad fueron menores en el ciclo seco y mayores en el ciclo de mezcla. El valor más alto de CO₂ fijo fue (3.2) mg / L / d en el tratamiento 1 en el ciclo de mezcla, mientras que el valor más bajo fue (0.11) mg / L / d en el tratamiento 3 y el control 1 en el ciclo seco. Los cambios en los patrones climáticos se ven en los valores de luz y temperatura. La biomasa de microalgas fue menor en condiciones de clima seco debido al efecto de la alta temperatura del aire. Las condiciones climáticas y los diferentes tratamientos influyeron significativamente en la composición de las especies de microalgas, debido a la sensibilidad de algunas de ellas a diferentes intensidades de luz. Las clorofitas fueron las más abundantes debido a su capacidad para adaptarse a diferentes condiciones de cultivo.

Palabras clave: Productividad primaria, Mesocosmos, Cambio climático, Biomasa de microalgas, Cambio climático, Dominio de clorofitas.

INTRODUCTION

World economies, human health, weather patterns were affected negatively by global warming (Adio-Moses & Aladejana, 2016; Damari et al., 2016; Murray & Lopez, 2013). Carbon dioxide capture and sequestration is one of the most serious challenges nowadays for global warming reduction (Fernández et al., 2012). Microalgae as large consumer of CO₂ can sequester carbon dioxide from gases emission in atmosphere (Ma & Gao, 2014; Saharan et al., 2013; Singh & Ahluwalia, 2013), however, the cost of CO₂ sequestration depends on the

microalga biomass and productivity (Farrelly et al., 2013). The high photosynthesis rate and the rapid growth rate are the main characters of CO₂ fixation by microalgae (Wu et al., 2012).

Cultivation methods and conditions influence microalgae biomass production. A combination of light intensity, temperature and nutrient level have profound effect on microalgae growth (Choi, 2014; Fagiri et al., 2013; Khalili et al., 2015). The higher growth rate of microalgae reflects the high light intensity and durations (Harun et al., 2014). The influence of temperature on algal cell structure ranged broadly between algae species, because there is a high correlation between temperature and cell volume (Agrawal, 2009; He et al., 2013; Yu et al., 2014). Temperature impacts significantly on the cellular chemical composition, nutrients uptake, CO₂ assimilation and consequently impacts the growth rates for each species of microalgae. Temperature fluctuation controlled the high rate of microalgae outdoor production (Ras et al., 2013). In addition, the distribution of carbon dioxide and availability can be changed because of pH. The extreme pH levels can alter the availability of essential nutrients, and led to direct physiological effects (Costache et al., 2013; Razzak et al., 2015; Shen et al., 2014). Microalgae growth depends on nutrient level in culture medium, phosphorus and nitrogen are the main important nutrient that enquired for growth (Brito et al., 2013; Kim et al., 2014; Procházková et al., 2014).

Microalgae produced on a larger scale are either grown in open ponds or in photo bioreactors. Using mesocosms for microalgae cultivation and biomass production is also interesting and can give valuable achievements (Hernando et al., 2006; Petersen et al., 1997; Sommer, 2009; Sutherland et al., 2016; Vidoudez et al., 2011). So, the main objectives of current study are record growth and species composition of microalgae in mesocosms in semi-controlled condition under variable weather conditions.

MATERIAL AND METHODS

Experimental design and algal culture techniques: The experimental was done in Tilapia pond (TPU) in the hatchery of fisheries of Universiti Putra Malaysia (UPM) from March to May 2016. Ten litres of microalgae seeds (10% of culture volume) were added to eight (110 L) capacity floating mesocosms as four treatments, treatment 1: non- sheltered with fertilizers, treatments 2: non- sheltered without fertilizers, treatment 3: sheltered with fertilizers, treatment 4: sheltered without fertilizers. The sheltered mesocosms covered by shelter with black orchid plastic netting of 50% light transmission. Microalgae seeds were obtained from Tilapia pond effluent. Two g triple supper phosphate: 20g Urea were used as fertilizers (personal communication with Dr. Hishamuddin Omar). Aeration (2 hours on/ 2 hours off) throughout the day was performed. Sample collection was done in alternative days; one cycle period was 10 days.



Figure 1: The floating mesocosms filled by microalgae culture in fish pond.

Weather conditions recording: Air temperature (Bing weather free application), light intensity by using light meter Licor model (L1-250), and weather state (rainy, cloudy, sunny, haze) were recorded daily three times a day at 8-9 am, 12-1pm, and 4-5 pm. Currently weather data of Bing online weather application comes from multiple providers such as Weather.com, Foreca and AccuWeather.com.

Physical and chemical conditions: The pH was measured by pH meter, water temperature and dissolved oxygen measured by dissolved oxygen meter (YSI MODEL 58). Light intensity measured by light meter Licor model (L1-250), conductivity measured by AST meter. Alkalinity was measured by titration of 100 ml of sample with 0.02 N sulphuric acid using few drops of mixed reagent (methyl red and bromocresol green) as an indicator to determine the end of the titration, the colour change from blue to colourless (VL Snoeyink & D Jenkins, 1980). Total nitrogen and Nitrate-nitrogen analyses were carried out according to Kitamura *et al.*, (1982). Ammonium concentration was analysed based on phenol hypochlorite method described by Weatherburn (1967). Phosphate-phosphorus ($\text{PO}_4\text{-P}$) was determined according to the ascorbic acid method (Ademoroti, 1996). Total phosphorus was measured according to Parsons *et al.* (1984).

Quantifying algal biomass: Water samples were measured immediately by Hitachi UC-1900 UV visible spectrophotometer at 680 nm three times for each sample and the average was taken to determine optical density. For dry weight measuring, 50 ml of water sample was filtered by predried Sartorius glass filter paper in oven dried at 60°C for 24h (Borowitzka *et al.*, 1991). To measure Chlorophyll a, 30 ml of water sample from each bottle was filtered by MS® cellulose acetate membrane filter (0.45 μm). Chlorophyll a was extracted with 5ml (90%) acetone overnight at 4°C. The extraction was homogenized by driller. After centrifugation, the absorbance of the supernatant was measured by spectrophotometer (Hitachi UC-1900)

(Gertraud Hötzel & Roger Croome, 1999). Chlorophyll a was calculated by the equation of Jeffrey and Humphrey (Jeffrey & Humphrey, 1975):

$$[\text{Chl. a}] \text{ extract} = 11.85A664 - 1.54A647 - 0.08A630$$

Microalgae primary productivity: Primary productivity measured for each mesocosm in day 6 and day 10 of each cycle by incubation of one light bottle and one dark bottle for two hours, then dissolved oxygen measured using the Azide modification method by titration (Ademoroti, 1996). Initial dissolved oxygen measured immediately by portable probe. Gross primary productivity, net primary productivity and fixed carbon were calculated by the following equations:

$$\text{Community respiration (R)} = \text{initial} - \text{dark}$$

$$\text{Gross primary productivity (GPP)} = \text{Light} - \text{dark}$$

$$\text{Net primary productivity (NPP)} = \text{GPP} - \text{R}$$

$$\text{Carbon fixed} = \text{NPP} * 0.375$$

Since (0.375) is the factor comes from differences in atomic mass (12/32)

Microalgae Productivity in (g/L/d)

Productivity was calculated using the following equation according to (Eliane Dalva Godoy Danesi et al., 2011):

$$P_x = (X_m - X_i)(T_c)^{-1}$$

where: P_x = productivity (g/L/ day)

X_i = initial biomass concentration (g/ L)

X_m = maximum biomass concentration (g/ L)

T_c = cultivation time related to the maximum biomass concentration (days)

Cell density and species composition study: One hundred ml water samples collected from each mesocosms, and then two drops of glutaraldehyde were added for microalgae preservation. Settlement and counting methods (Edler & Elbrächter, 2010).

Statistical analysis: Factorial ANOVA statistical analysis from SPSS version 21 was used to indicate the significant of variance among microalgae culture treatments, weather conditions and days of microalgae growth. Daily air temperatures and light intensities were analysed statistically by one way ANOVA SPSS version 21.

RESULTS

Weather conditions scoring and categories: Weather conditions in Malaysia is a very complicated event because it considers about daily fluctuation of sunlight, cloud cover, haze and rainfall. Thus, it is important to classify the interpreted weather conditions. Weather conditions during current study period have been scored and categories, since each cultivation cycle was under one type of weather conditions, mix, wet and dry. Each culture cycle is for 10 days. Weather conditions during the mix characterized by dense cloud cover, mix cloudy sky, heavy haze, light rains. In the wet cycle, weather conditions characterized by heavy rains many times a day or daily, and cloud cover. In the dry cycle, weather conditions characterized by sunny sky, no rains or light rain.

Table 1: Summary of weather scoring and categories for three microalgae cultivation cycles : 1 to 4 indicate to wet weather conditions, 5 to 7 indicate to mix weather conditions, 8 to 10 indicate to dry weather.

Days of cycle	Cycle 1	Cycle 2	Cycle 3
D1	5	10	1
D2	6	10	3
D3	5	9	4
D4	4	8	2
D5	6	9	3
D6	5	7	4
D7	7	7	1
D8	4	10	3
D9	5	8	4
D10	6	9	2
Average	5.3	8.7	2.7
category	Mix	Wet	Dry

Table 2: Mean \pm SE of air temperature and light intensity at three times a day (n= 30) and rain fall gauging. letter in bold indicate to a significant difference ($p < 0.05$) within rows.

Parameters	Cycles in different weather conditions		
	Mix cycle	Wet cycle	Dry cycle
Air temperature (C°)	29.2 \pm 0.52 a	28.8 \pm 0.23 a	30.9 \pm 0.38 b
Light intensity ($\mu\text{molm}^{-2}\text{s}^{-1}$)	349.2 \pm 68.1a	452.9 \pm 40.6 b	461.1 \pm 58.6 b
*Rain fall (mm)	178 \pm 42.7	384 \pm 20.4	69.0 \pm 23.0
* pH of Rain water	6.0 \pm 0.31	6.51 \pm 0.17	5.67 \pm 0.58
* Nitrate (NO ₃) of Rain water mg/L	0.60 \pm 0.02a	0.27 \pm 0.00b	0.89 \pm 0.01a

Physico-chemical parameters: Water quality parameters in all treatments were monitored over 10 days for three cycles and showed significant variations among the variables.

Water temperature changed in the three cycles. There was significant difference in water temperature between different cycles, and between treatments ($p < 0.05$), while there was no significant different within cultivation days. The highest value was 32.7°C recorded during dry

cycle for treatment 1, while the lowest value was 28.1°C also recorded during dry cycle for treatment 3.

Electric conductivity of the treatments fluctuated within cultivation days. There were no significant differences within cycles and within treatments, while there were significant differences within cultivation days. Values of pH in three cycles were continually on the alkaline side and ranged from 7.2 to 10.05. pH values increased obviously with culture time. There were significant differences in pH values within treatments and cultivation days ($p < 0.05$), while there were no significant differences in pH values between cycles.

Dissolved oxygen fluctuated clearly by weather conditions, there was significant differences between cycles and cultivation days ($p < 0.05$), while there was no significant difference between treatments.

There was wide range of difference in light intensity between the shaded and non-shaded treatments. The range of light intensity in treatment 1 was (377.5 - 781) $\mu\text{mol}/\text{m}^2/\text{s}$, in treatment 2 was (406 - 765) $\mu\text{mol}/\text{m}^2/\text{s}$, in treatment 3 was (56 - 291) $\mu\text{mol}/\text{m}^2/\text{s}$, in treatment 4 was (87.5-323) $\mu\text{mol}/\text{m}^2/\text{s}$ respectively. There were significant differences between treatments ($p < 0.05$) but there were no significant differences between cycles.

Alkalinity range was (44.5 - 67.5) mg CaCO_3/L in treatment 1, and (31 - 55.5) mg CaCO_3/L in treatment 3. There were significant differences in alkalinity within treatments, cycles and culture days ($p < 0.05$). The highest averages of alkalinity were in the 4th and the 10th day in all cycles. Generally, alkalinity of treatment 1 higher than treatment 3 and higher in dry cycle than other cycles.

Nutrients concentration: Nitrate and ammonium decreased gradually with increasing in time of cultivation, as shown in Table 7.5. Comparison in the differences between the three cycles and treatments showed in (Figure 7.2 x & Figure 7.3). Nitrate concentration ranged between (0.01- 0.9) mg/L in treatment 1, (0 - 1.1) mg/L in treatment 2, (0.01 - 4.23) mg/L in treatment 3, and (0.01 - 1.49) mg/L in treatment 4. The lowest value was in treatment 2 in the 6th & 8th of the dry cycle, while the highest was in treatment 3 in the 2nd day of the wet cycle. There were significant differences ($p < 0.05$) within treatments and within different cycles, while there were no significant differences within cultivation days. Ammonium concentration ranged between (0.01 - 0.74) mg/L in treatment 1, (0 - 0.15) mg/L in treatment 2, (0.03- 1.73) mg/L in treatment 3, and (0 - 0.06) mg/L in treatment 4. The highest value was in treatment 3 in the 2nd day of the mix cycle, while the lowest was in treatment 2 in 6th, 8th & 10th days of dry and wet cycles, and in treatment 4 in the 8th day of the wet cycle. There were significant differences in ammonium concentrations within culture days, treatment and within weather conditions ($p < 0.05$).

Total nitrogen decreased gradually in all treatments and in all cycles. The ranged was between (0.06 - 2.75) mg/L in treatment 1, (0.03 - 1.64) mg/L in treatment 2, (0.08 - 1.72) mg/L in treatment 3, and (0.05 - 1.43) mg/L in treatment 4. There were significant differences

in TN within culture days and within weather conditions ($p < 0.05$) but there were no significant differences within treatments.

Phosphate (PO_4^-) concentrations decreased gradually during cultivation days. Its values ranged from (0.00 – 0.25) mg/L in treatment 1, (0.00 – 0.17) mg/L in treatment 2, (0.01 – 0.35) mg/L in treatment 3, and (0.01 – 0.17) mg/L in treatment 4. There were significant differences in phosphate concentration within treatments and within the weather conditions ($p < 0.05$), while there were no significant differences within culture days. Total phosphorus decreased gradually until the end of all cycles. The value of total phosphorus ranged from (0.09 – 0.52) mg/L in treatment 1, (0.01 – 0.27) mg/L in treatment 2, (0.09 – 0.54) mg/L in treatment 3, and (0.04 – 0.24) mg/L in treatment 4. There were significant differences ($p < 0.05$) in TP within treatments, culture days, and weather conditions. TN: TP ratio ranged from (1.2:1 – 8.6:1) in treatment 1, (1:1 – 14:1) in treatment 2, (1:1 – 10:1) in treatment 3, and (1:1 – 17:1) in treatment 4. TN: TP fluctuated during culture time. There were significant differences in TN: TP within culture days, and weather conditions ($p < 0.05$), but there were no significant differences within treatments.

Biomass and productivity-Growth performance: The growth performance of the mix microalgae in fertilized and non-fertilized mesocosms from three culture cycles are expressed by optical density, cell dry weight and chlorophyll- a concentrations. The growth rate was slightly increased with culture time. Optical density values of mix microalgae shown in (Figure 2). Optical density in treatment 1 were significantly better ($p < 0.05$) than treatment 2 and controls. The highest value for treatment 1 was (0.64) in the 10th day of the mix cycle, and (0.19) in 10th of the wet cycle and (0.15) in the 10th of the dry cycle. There were also significant differences within culture days and weather conditions. Optical densities values generally lower in dry cycle. Chlorophyll a contents of mix microalgae is shown in (Figure 2). Chlorophyll a concentrations of mix microalgae in treatment 1 were significantly different ($p < 0.05$) with treatment 2 and controls. The highest value for treatment 1 was ($9.3 \mu\text{g L}^{-1}$) in the 10th day of the mix cycle, ($6.6 \mu\text{g L}^{-1}$) in 8th of the dry cycle and ($5.7 \mu\text{g L}^{-1}$) in the 10th of the wet cycle. There were also significant differences within culture days and weather conditions ($p < 0.05$). Dry weight measurements increase during the 10 experiment days are shown in (Figure 2). The maximum value of dry weight was in treatment 1 (145) mg/L in the 6th day of the mix cycle, while the maximum value of dry weight in treatment 2 was (100) mg/L in 6th day of the dry cycle. There were also significant differences within culture days and weather conditions ($p < 0.05$).

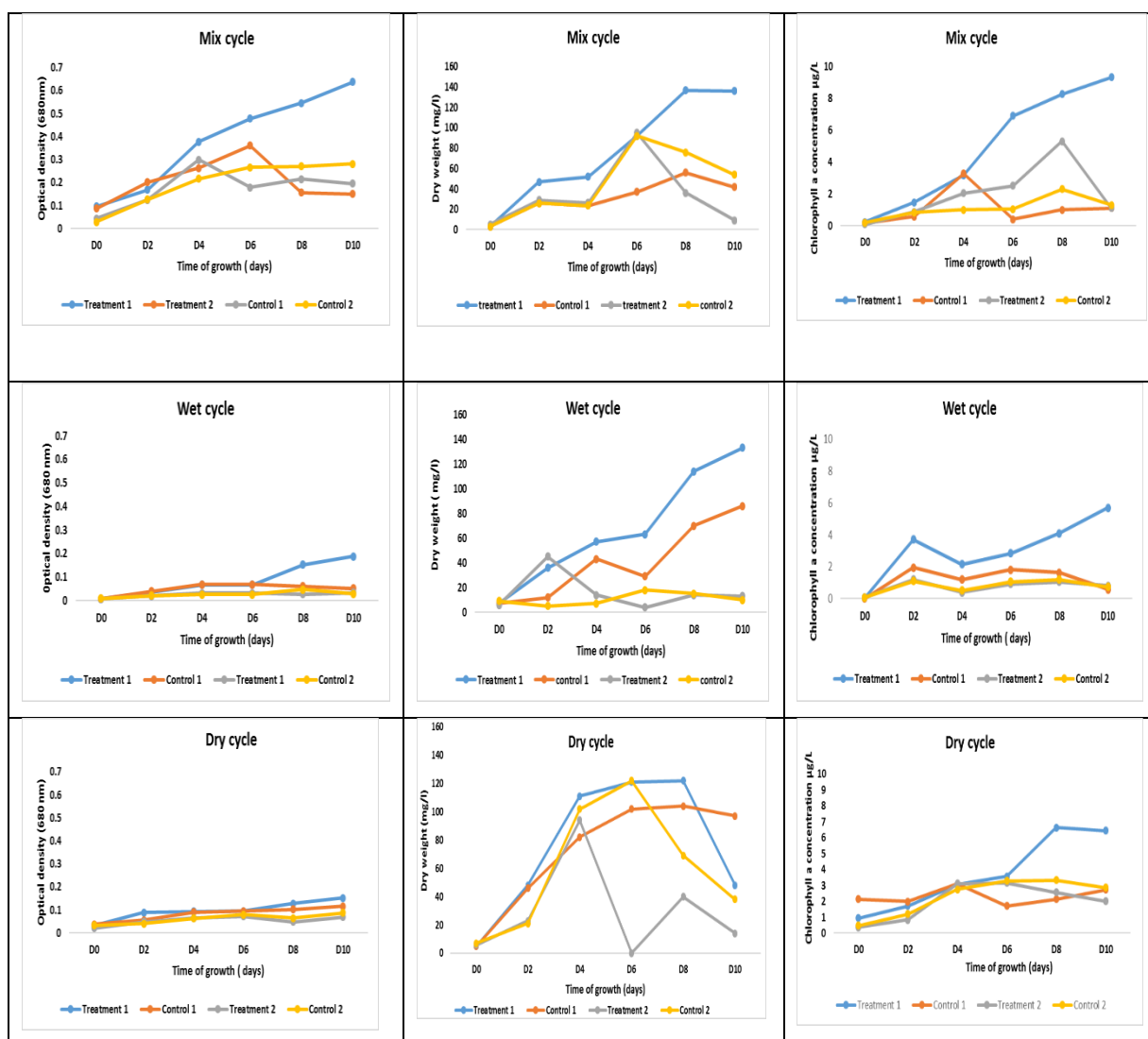


Figure 2: Growth performance of microalgae in controls and treatments in Mix, Wet and Dry cycles categorized based on weather conditions.

Microalgae Productivity in (g/L/d)- Microalgae Productivity in (g/L/d) is shown in Table 3.

Table 3: Mean \pm SE of Productivity (g/L/d) of mix microalgae in all treatments in different weather conditions, (n=6), letter in bold indicate to a significant difference ($p < 0.05$) within columns.

Treatments	Culture cycles in different weather conditions		
	Mix cycle	Dry cycle	Wet get cycle
Treatment 1	1.259 \pm 0.06 c	0.690 \pm 0.03 a	1.079 \pm 0.02 b
Treatment 2	0.855 \pm 0.06 ab	0.569 \pm 0.01 a	0.556 \pm 0.01 a
Treatment 3	0.763 \pm 0.01 b	0.683 \pm 0.06 a	0.603 \pm 0.07 a
Treatment 4	0.053 \pm 0.02 a	0.557 \pm 0.03 a	0.423 \pm 0.02 a

Primary productivity and carbon fixation: Community respiration, gross production, net production, CO₂ fixation recorded in the 6th day and the 10th day of each cycle. Primary production variables were higher in the fertilized non-sheltered mesocosms (treatment 1). In general, productivity variables were lower in the dry cycle and higher in the mix cycle as can be seen in Table (4) & Figure (3). The highest value of fixed CO₂ was 3.2 mg/L/d in treatment 1 in the 6th day of the mix cycle, while the lowest value was 0.11 mg/L/d in treatment 3 in the 6th day and treatment 2 in the 10th of the dry cycle. There were no significant differences in CO₂ values between treatment 3, treatment 2 & treatment 4, while treatment 1 was significantly different in CO₂ with other treatments. Net production and gross production were no significantly different between treatment 2, treatment 3 and treatment 4 ($p > 0.05$), while treatment 1 was significantly different with other treatments, also they were no significantly different between mix and wet cycles, while dry cycle was significantly different with the other cycles ($p < 0.05$). Community respiration values were very varied during cultivation days in all cycles. They were lowest in the 10th day than the 6th day for each cycle. There was no a significant difference between treatments in community respiration ($p > 0.05$), while their values were significantly different between cycles ($p < 0.05$).

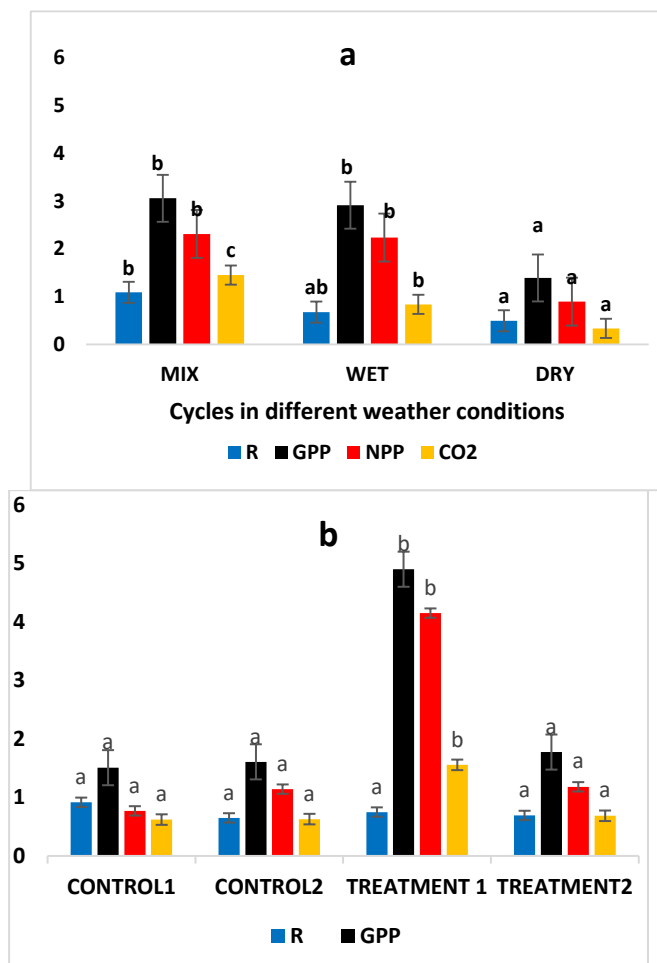


Figure 3: Mean \pm SE of Community respiration, gross primary production, Net primary production, and fixed carbon dioxide in different weather conditions. a: between weather conditions, b: between treatments. Different letters indicated to significant at ($p < 0.05$).

Table 4: Mean \pm SE of Primary productivity parameters for treatments and controls in the 6th day and the 10th day of all cycles in different weather conditions.

Parameters	Treatments	Mix cycle		Wet cycle		Dry cycle	
		D6	D10	D6	D10	D6	D10
Community respiration	Control 1	2.1 \pm 0.8	0.5 \pm 0.0	1.0 \pm 0.1	0.6 \pm 0.1	1.0 \pm 0.1	0.4 \pm 0.1
	Treatment 1	1.3 \pm 0.0	0.5 \pm 0.1	0.3 \pm 0.0	1.3 \pm 0.7	1.1 \pm 0.1	0.2 \pm 0.0
	Control 2	2.0 \pm 0.4	0.2 \pm 0.1	0.9 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0
	Treatment 2	0.8 \pm 0.1	0.7 \pm 0.0	1.0 \pm 0.1	0.3 \pm 0.1	0.5 \pm 0.1	0.6 \pm 0.2
Gross Primary Production	Control 1	1.8 \pm 0.6	1.4 \pm 0.5	1.6 \pm 0.1	1.9 \pm 0.2	1.6 \pm 0.0	1.1 \pm 0.1
	Treatment 1	8.3 \pm 1.0	6.4 \pm 1.2	6.5 \pm 1.4	5.0 \pm 0.3	2.9 \pm 0.2	0.9 \pm 0.1
	Control 2	2.3 \pm 0.5	1.2 \pm 0.1	2.2 \pm 0.1	1.8 \pm 0.0	1.5 \pm 0.0	0.9 \pm 0.1
	Treatment 2	1.1 \pm 0.1	2.6 \pm 0.2	2.3 \pm 0.4	2.6 \pm 0.2	1.2 \pm 0.1	2.0 \pm 0.0
Net Primary Production	Control 1	0.7 \pm 0.1	1.0 \pm 0.4	0.6 \pm 0.0	1.3 \pm 0.2	0.5 \pm 0.1	0.7 \pm 0.2
	Treatment 1	7.0 \pm 0.9	6.0 \pm 1.2	6.1 \pm 1.3	3.8 \pm 0.9	1.9 \pm 0.4	0.8 \pm 0.1
	Control 2	0.4 \pm 0.0	1.0 \pm 0.4	1.4 \pm 0.1	1.6 \pm 0.1	1.3 \pm 0.1	0.9 \pm 0.1
	Treatment 2	0.8 \pm 0.0	1.9 \pm 0.2	1.3 \pm 0.5	2.4 \pm 0.3	0.7 \pm 0.2	0.5 \pm 0.2
Fixed carbon dioxide	Control 1	0.3 \pm 0.0	0.4 \pm 0.1	0.2 \pm 0.0	0.5 \pm 0.1	0.2 \pm 0.0	0.3 \pm 0.1
	Treatment 1	2.6 \pm 0.3	2.2 \pm 0.5	1.7 \pm 0.5	1.4 \pm 0.4	0.7 \pm 0.1	0.3 \pm 0.0
	Control 2	0.1 \pm 0.0	0.4 \pm 0.0	0.5 \pm 0.0	0.6 \pm 0.0	0.5 \pm 0.0	0.3 \pm 0.0
	Treatment 2	1.1 \pm 0.5	0.7 \pm 0.1	0.5 \pm 0.2	0.9 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.1

Species composition and cell density: The mix microalgae were not much divers in the culture. Twenty-six species were recorded in mix cycle, 6 species were dominant, 29 species were recorded in dry cycle, 8 species were dominant, and 26 species were recorded in wet cycle, 8 species were dominant. Chlorophyta was the main dominant group during all cycles, it formed 80%, 83%, and 85% during mix, dry and wet cycles respectively, while Cyanophyta formed 12%, 10%, and 7.6% during mix, dry and wet cycles respectively. Bacillariophyta formed only 8%, 6%, and 7.6% during mix, dry and wet cycles respectively. Cell density was significantly higher during mix cycle ($p < 0.05$). There was a clear obvious variation of microalgae population among the different treatments. During mix cycle, *Monoraphidium contortum* was the most dominant species with the highest cell densities. Its cell density reached (1224.4×10^3 cell/ L) in treatment 1 (non-sheltered with fertilizer) in the 10th day of the mix cycle, on the other hand, its lowest density was (0) recorded at control 2 in the 8th day of the same cycle. *Chlorella vulgaris* (20×10^3), *Chlamydomonas reinhardtii* (18×10^3), *Oocystis borgei* (17.8×10^3) and *Monoraphidium griffithii* (12.8×10^3) were the most dominant with the highest cell densities (cell/L) in treatment 3 in the 10th day respectively. *Ulothrix aequalis* cell density reached to (51×10^3) cell/L in treatment 3 in the 6th day. During dry cycle, the highest cell density was for *Merismopedia punctata* (115.5×10^3 cell/ L) in treatment 1 followed by *Coelastrum microporum* (105×10^3 cell/ L) in treatment 4, *Golenkinia radiata* (15.1×10^3 cell/ L) in treatment 1 and *Oocystis borgei* (11.6×10^3 cell/ L) in treatment 4 in the 10th day respectively. While the highest density of *Monoraphidium griffithii* in the dry cycle was (61.5×10^3) cell/ L in the treatment 1 in the 2nd day and *Ulothrix aequalis* (18.75×10^3 cell/L) in treatment 2 in the D0. During the wet cycle, the highest cell densities was also for *Coelastrum microporum* (20.3×10^3 cell/L) in treatment 1 in the 10th, followed by *Monoraphidium contortum*, *Scenedesmus quadricauda*, *Scenedesmus acuminatus*, and *Merismopedia punctata*.

The highest cell density of *Chlamydomonas reinhardtii* was generally in the sheltered mesocosms (Treatment 3 & Treatment 4). The highest cell density was (18×10^3) cell/ L in the treatment 2 in the 10th day of the mix cycle, (13.1×10^3) cell/L in treatment 3 in the 2nd day of the dry cycle, (7×10^3) cell/L in the treatment 4 in the 8th day of the wet cycle. The highest density of *Chlorella vulgaris* was (20.4×10^3) in the treatment 3 in the 10th day of the mix cycle, (19×10^3) cell/L in treatment 3 in the 2nd day of the dry cycle, and (5.4×10^3) cell /L in treatment 1 in the 4th day of the wet cycle. *Scenedesmus* genus was the more divers with 8 species. *Scenedesmus quadricauda* was not abundant during the mix cycle, while it had high cell density (17.8×10^3) cell/L in treatment 1 in the 4th day of the wet cycle, and (3.6×10^3) cell/L in treatment 1 in the 10th day of the dry cycle. The highest density of *Golenkinia radiata* was (15.1×10^3) cell/L in treatment 1 in the 10th day of the dry cycle, and (1.8×10^3) cell/L in treatment 4 in the 10th day of the wet cycle. The highest density of *Oocystis borgei* was (17.8

$\times 10^3$) cell/L in treatment 3 in the 10th day of the mix cycle, and (8.9×10^3) cell/L in control 1 in the 6th day of the wet cycle.

Table 5: Microalgae species recorded in all mesocosms in different weather conditions.

Division	Species	order in Figure 4
Chlorophyta	<i>Coelastrum microporum</i> Nägeli	1
	<i>Monoraphidium contortum</i> Komárková-Legnerová	2
	<i>Monoraphidium griffithii</i> M Komárková-Legnerová	3
	<i>Oocystis borgei</i> J.W. Snow	4
	<i>Pandorina morum</i> Bory	5
	<i>Scenedesmus quadricauda</i> Brébisson	6
	<i>Scenedesmus ellipticus</i> Corda	7
	<i>Scenedesmus opoliensis</i> P. Richter	8
	<i>Scenedesmus dimorphus</i> Kützing	9
	<i>Scenedesmus acuminatus</i> Chodat	10
	<i>Scenedesmus subspicatus</i> Chodat	11
	<i>Chlamydomonas reinhardtii</i> P.A. Dangeard	12
	<i>Scenedesmus abundans</i> (O. Kirchner) Chodat	13
	<i>Golenkinia radiata</i> Chodat	14
	<i>Scenedesmus apiculatus</i> (West & G.S. West) Chodat	15
	<i>Pediastrum duplex</i> Meyen	16
	<i>Chlorella vulgaris</i> Beyerinck	17
	<i>Zosterocarpus oedogonium</i> (Meneghini) Bornet	18
	<i>Tetraëdron minimum</i> Hansgirg	19
	<i>Ulothrix aequalis</i> Kützing	20
	<i>Dictyosphaerium pulchellum</i> H.C. Wood	21
	<i>Lagerheimia ciliate</i> Chodat	22
	<i>Kirchneriella obesa</i> West & G.S. West	23

Cyanophyta	<i>Merismopedia punctata</i> Meyen	24
	<i>Microcystis aeruginosa</i> Kützing	25
	<i>Chroococcus turgidus</i> Nägeli	26
Bacillariophyta	<i>Navicula rhynchocephala</i> Kützing	27
	<i>Cyclotella meneghiniana</i> Kützing	28
	<i>Synedra ulna</i> (Nitzsch) Ehrenber	29

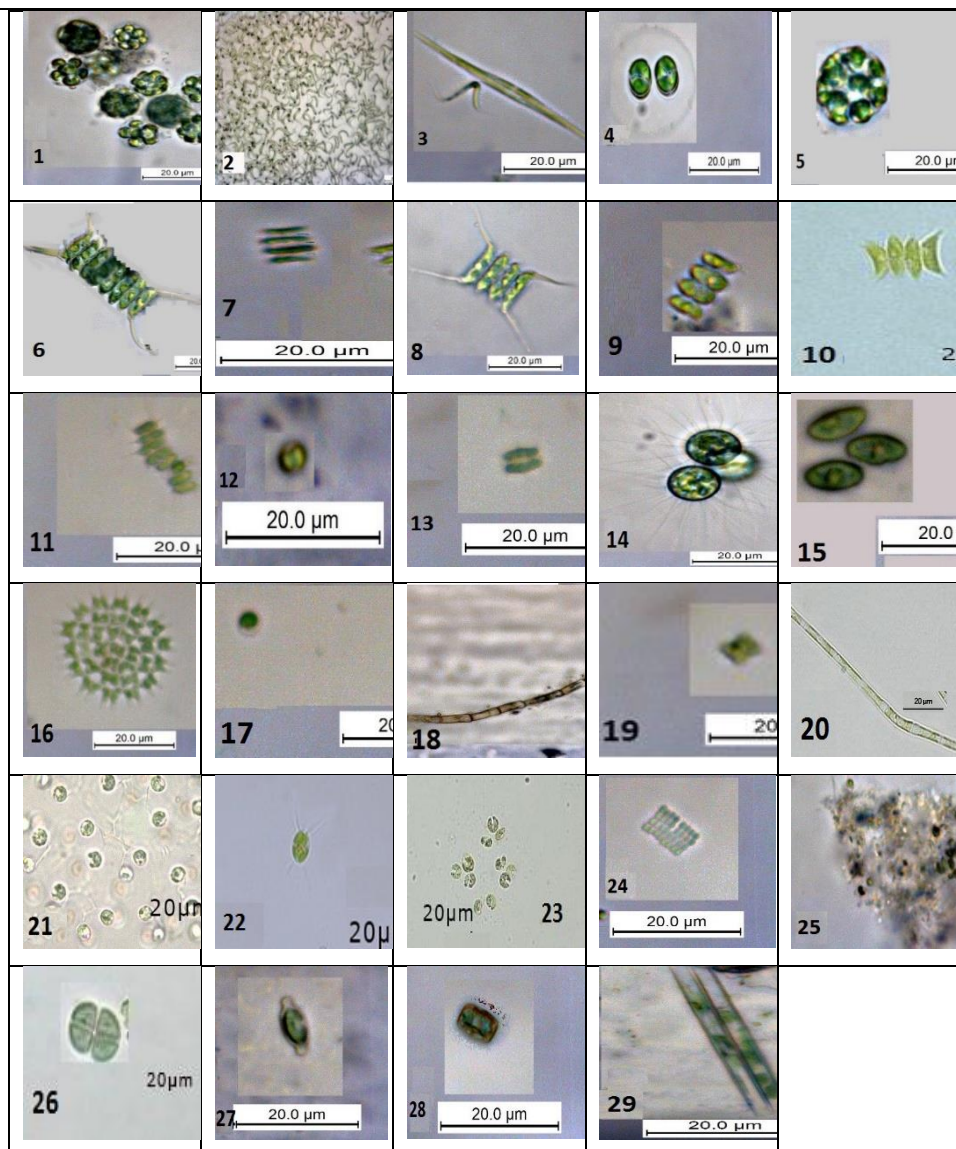


Figure 4: Microalgae species cultivated in all treatments in different weather conditions. The scientific names mentioned with their affiliation in (Table 5).

DISCUSSION

During the three outdoor culture cycles, the changes in weather patterns are seen in the light, temperature and rain fall values in (Table 1). The mass culture of microalgae in

various production systems is primarily concerned with maximising daily yield of microalgal biomass in the conditions of light limitation. To achieve an ideal condition for microalgae production, two main aspects are generally considered, the physicochemical environmental factors such as temperature, light intensity, pH and aeration, and the other is selection of a suitable nutrient medium (Esra Imamoglu et al., 2007).

Mix microalgae cultures can withstand and tolerate a wide range of variations in physical and chemical parameters. Dissolved oxygen, light intensity, and temperature are the factors that can impact microalgae growth and morphological features (Vamadevaiah, 2010). Light and nutrients are most vital environmental factors that affect photosynthesis in photosynthetic organisms such microalgae and plants. From this term, the experiment was designed to test the influence of these parameters on productivity of microalgae. In case of Malaysia, the daily light intensity fluctuated accordance to the sky status and weather condition. Light plays an important role in growth rate of microalgae. The growth rate of microalgae can vary under different light exposures, durations and exposure frequencies. In non-sheltered mesocosms (Treatment 1& Treatment 2) the growth of microalgae was better than the sheltered mesocosms (Treatment 3&Treatment 4).

pH is an important factor that effects on microalgal biomass production. pH increased with increasing of culture growth because photosynthesis can cause pH rising in algal culture (García et al., 2006). Since the initial pH was around (7.5 to 8.0). Meanwhile, alkaline pH indicated to higher algal biomass productivity. Microalgae are sensitive to acidic conditions due to inhibition of cellular enzymes and biological processes in low pH conditions (Skjånes et al., 2013). Consequently, the optimal pH of most of the algal species has been found in the range of (7 to 9). In addition, CO₂ fixation depends on pH values, because availability of bicarbonate and carbonate materials that resulted from CO₂ dissolving in culture water to microalgal cells depends on the pH of the culture. Increasing pH in treatment 1 and treatment 3 can be also due to presence of nitrogen sources (Urea) in the medium, because consumption of nitrate from the medium helps in increase of alkalinity (Horn, 2008). Majority of microalgae require nitrogen in a soluble form, since urea being the best source (Bejarano et al., 2011). According to (Boyd & Tucker, 2012), 45% of urea is nitrogen, and 19 – 24 % of triple superphosphate is phosphorus. The augmented amount of nitrogen in the culture medium leads to increase microalgae growth. Temperature and pH influence Hydrolysis of urea to ammonia and carbon dioxide in the culture medium. Nutrients uptake has gone in the opposite direction with increasing of biomass. Nitrogen is most important nutrient after carbon to microalgae. Nitrogen occurs in several forms, and the most nitrogen compounds are assimilated by microalgae are ammonium (NH₄⁺) and nitrate (NO₃⁻) (Oliver & Ganf, 2000). Optimal phosphorus concentration that is favourable to growth of microalgae is not less than 0.045mg/L and not higher than 1.65 mg/L according to (Ren, 2014), this agreed with current result, however, he stated that when TP equals to 0.02mg/L, microalgae can grow well, but the concentration of phosphorus has no

promotion to growth rate of algae when TP \geq 0.2mg/L, while (Becker, 1994) stated that the tolerant range of microalgae to phosphorus is from 0.05 to 20 mg/L. Phosphate (PO_4^-) is the major required form of phosphorus by microalgae.

Optimization of mix microalgae biomass production involves an understanding how it can be affected by various factors individually and also their multiple interactions in the whole complex process. Growth performance was influenced significantly by the different light exposition and fertilizer addition. The highest growth rate was in treatment 1 because of the light availability in comparison with treatment 3.

Microalgae biomass was lower in dry weather conditions because of effect of high air temperature that was clear especially for non-fertilized treatments (Treatment 2 & Treatment 4). Meanwhile, temperature led to an effect on the physiological characters of microalgae in the mesocosms because of the small volumes of culture medium. Water temperature correlated negatively with axis 1 which represented biomass and productivity variables. Temperature has a strong impact on the cell chemical structure, nutrients and CO_2 uptake, and the growth rates for every species of microalgae. Cell density, pH, and ammonium (NH_4^+) concentrations correlated positively with biomass and productivity variables. Phosphate (PO_4^-), Nitrate (NO_3^-), and total phosphorus correlated positively with axis 2 and negatively with optical density, cell density and dissolved oxygen.

Species existence along an environmental gradient often follows Shelford's law of tolerance (Braak & Verdonschot, 1995), each species grows in optimal way at a particular value of environmental parameters and cannot flourish when the value increases or decrease from this range. So, this limited correlation between the species and the variable is called species niche, however, some species may prefer extreme environmental conditions or their optima may fall outside the environmental region.

In the present study, the weather conditions and different treatments (Sheltered & non-sheltered) significantly influenced microalgae species composition, probably due to the sensitivity in some of them to different light intensities. Chlorophytes were the most abundant due to their ability to adapt with different culture conditions.

Species distributed based on their high cell densities in treatments that are located near them. Some species tended to be most present and abundant the non-sheltered mesocosms (Treatment 1 & Treatment 2), while others leaned to be abundant in the sheltered mesocosms (Treatment 3 & Treatment 4). Chlorophytes have a maximum growth in a wide range of light intensity from (129 to 773.8 $\mu\text{molm}^{-2}\text{s}^{-1}$). The Chrysophytes (*Navicula rhynchocephala* and *Cyclotella meneghiniana*) had their optimal growth at light intensity values 49.50 & 184.6 $\mu\text{molm}^{-2}\text{s}^{-1}$ respectively. This result agreed with (Fadel et al., 2015), since diatoms prefer low light intensities. *Monoraphidium contortum* is commonly found in meso to eutrophic environment. It was considered by (Bogen et al., 2013) as promising algae for liquid biofuel production, because of its high biomass productivity. (Latala, 1991) stated that *Monoraphidium*

griffithii grows best at (150 -270 $\mu\text{E m}^{-2} \text{s}^{-1}$), same with the current result, this robust species withstood with medium nature light intensities (152 $\mu\text{E m}^{-2} \text{s}^{-1}$). *Chlorella vulgaris* is very common in fresh waters, show great adaptability to various environmental conditions, grow and divided faster when CO_2 and nutrient are available (Singh & Singh, 2015). It is a commonly cultivated for its high dry weight production and it is commercially cultivated worldwide for nutritional, cosmetic purposes. *Chlamydomonas reinhardtii* can be cultivated photoautotrophically and heterotrophically as well (Kliphuis et al., 2012), in aerobic and anaerobic medium. It has been cultivated worldwide for industrial purpose specially hydrogen production in anaerobic conditions. Species belonging to the genus *Oocystis* are relatively common in different freshwater water bodies and predominant in small lakes and ponds. (Stoyneva et al., 2007) mentioned that *Oocystis* sp. contributed significantly in microalgae biomass in Tanganyika lake. *Microcystis aeruginosa* was flourished with high water temperature (30 °C) and low nutrient concentrations, same findings were achieved by (Parrish, 2014).

In Conclusion, reduced light intensity may have impacted microalgae populations. The amount of available light is closely correlated to survival and growth, and too much light can lead to light inhibition for the surface layer of microalgae. There was a significant decline in abundance of species cell density, and a corresponding change in species composition in all treatments during the dry cycle due to the high air temperature during the dry weather conditions. Mesocosm cultures indicated that some species can adapt well to large scale production.

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