STATIC CULTURE OF *Scenedesmus dimorphus* IN MODIFIED SAGO EFFLUENTS (MSE)

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DECLARATION

No portions of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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ABBREVIATIONS

BOD	Biochemical Oxygen Demands
COD	Chemical Oxygen Demands
CO_2	Carbon Dioxide
DNS	Dinitrosalicyclic Acids
DCW	Dry Cell Weight
g/L	gram per litre
HCl	Hydrochloric Acids
NaOH	Sodium Hydroxide
$NaHCO_3$	Sodium Bicarbonate
mg/L	milligram per litre
MSE	Modified Sago Effluents
OD	Optical Density
TSS	Total Suspended Solids
UV	Ultraviolet

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STATIC CULTURE OF *Scenedesmus dimorphus* IN MODIFIED SAGO EFFLUENTS (MSE)

ABSTRACT

This study focuses on using sago effluent as the growth medium which will medium minimize the effects of environmental pollution from the sago factories by culturing algae such as *Scenedesmus dimorphus* for biodiesel production. In this study, sago effluent was treated with a commercial microbial amendment (BakWira MP300) to produce Modified sago effluent (MSE). Then, the algae *Scenedesmus dimorphus* was grow in MSE amended with different $NaHCO_3$ concentrations (0g/L, 4g/L and 8g/L) as a static culture. The growth of the algae in MSE was compared to Chu Medium. All cultures were left under sunlight for 20 days. To maximise the distribution of nutrient in the medium, an aeration pump was used. In this study the highest biomass was in MSE with 8g/L $NaHCO_3$ concentration with increasing biomass from 55 mg/L on day 0 to 490 mg/L on day 20. The lowest is in MSE with without $NaHCO_3$ concentration which the increasing of biomass from 40 mg/L on day 0 to 300 mg/L on day 20. For lipid extraction in MSE with 8g/L $NaHCO_3$, percentage of lipid produce by *Scenedesmus dimorphus* was 7.49% w/w. The biomass gained during cultivation of *Scenedesmus dimorphus* proved that additions of bicarbonate salts ($NaHCO_3$) can increased the growth of *Scenedesmus dimorphus.*

Key words: Biofuels**,** *Scenedesmus dimorphus*, Modified sago effluent (MSE), BakWira MP300, Dry Cell Weight.

KULTUR STATIK *Scenedesmus dimorphus* DALAM EFFLUEN SAGO YANG DIMODIFIKASI (MSE)

ABSTRAK

Kajian ini lebih menfokuskan kepada air buangan dari pemprosesan sago. Ini bukan saja mengelak penggunaan terus sumber kanji sago bahkan boleh mengurangkan kesan pencemaran alam sekitar dari kilang-kilang sago dengan memanfaatkan alga seperti Scenedesmus dimorphus. Dalam kajian ini, air buangan dari pemprosesan sago akan dirawat terlebih dahulu dengan campuran microorganisma komersial dikenali sebagai BakWira MP300 untuk menghasilkan effluent sago yang telah di modifikasi (MSE). Kemudian, barulah alga Scenedesmus dimorphus akan di kulturkan dalam MSE dan ditambah beberapa sukatan $NaHCO_3$ (0g/L, 4g/L dan 8g/L) dengan menggunakan kultur statik. Sebagai kultur kawalan, Scenedesmus dimorphus yangn di kulturkan di dalam effluent sago yang telah di modifikasi (MSE) akan dibandingkan dengan kultur dalam media Chu. Kesemua kultur itu akan dibiarkan dibawah sinaran matahari selama 20 hari. Untuk memaksimakan pengagihan nutrisi-nutrisi didalam medium tersebut, pam pengudaraan akan digunakan Dalam kajian ini biomas yang tertinggi diperolehi di dalam MSE dengan 8g/L $NaHCO_3$ dimana kenaikan biomas nya adalah dari 55 mg/L pada hari ke-0 ke 490 mg/L pada hari ke- 20. Manakala biomass yang terendah dicatatkan dalam MSE tanpa $NaHCO_3$ dimana kenaikan biomass dari 40 mg/L pada hari ke-0 kepada 300 mg/L pada hari ke-20. Untuk pengekstrakan lipid dalam MSE dengan 8g/L $NaHCO_3$, peratusan lipid yang dihasilkan oleh Scenedesmus dimorphus adalah 7.49% w/w. Data yang diperolehi juga menunjukkan bahawa penambahan garam bikarbonat ($NaHCO_3$) kedalam medium MSE dapat meningkatkan pertumbuhan alga Scenedesmus dimorphus.

Kata Kunci: Biofuels**,** *Scenedesmus dimorphus*, effluent sago yang telah di modifikasi (MSE), BakWira MP300, berat kering sel (DCW).

CHAPTER 1

INTRODUCTION

1.1 General Overview

The sago palm (*metroxylon sago*) in Malaysia usually grows in freshwater swamps. It is important sources of starch where most of it can be found in state of Sarawak where sago is a staple food. It has high starch yield where one palm may produce between 150 to 300kg of starch. Reportedly, there are total areas of 24,000 ha cultivated with the sago palm with 32 factories producing almost 67000 tan yr^{-1} starch (Chew & Shim, 1993). The whole trunks of the sago palm are cut and bring back to factory, where after debarking the pith is mashed to extract the sago starch. To separate the starch from the rest of the pith, it will wash with water followed by settling in tanks. Only the starch powder is used and then spray dried. The rest which are in the form of bark, the solid component known as *hampas* and wastewater or effluent (sago starch factory wastewater) become wastes. *Hampas* may be used as animal feed, compost for mushroom culture hydrolysis to confectioner's syrup or for particleboard manufacture. What was become problem is to manage the waste in form of bark and the wastewater. Bark usually burn or thrown to the river, while the wastewater will discharged into nearby river without proper treatment. More than 1245.6 tons of sago effluent is produced in a week from a single medium sized sago mill (Bujang , 1996). This problem has become environmental issue as the wastewater inevitably contributes to river pollution when there no treatments apply.

As members of the order Chlorococcales, green micro-algae of the genus *Scenedesmus* are characterized based on two-dimensional arrangement of 2, 4, 8 or rarely 16 cells in regular aggregates called coenobia, surrounding mucilaginous matrix present or absents. Its shape is nearly spherical to ellipsoidal, elongated or fusiform to elongated fusiform, cell poles capitates, obtuse, acute or long tapering. Cell arranged linearly, alternating or in 2-3 rows, touching with the lateral wall or in subpolar region only. It has cell wall with hemicellulosic and sporopolleninic layer. Physiology and biochemistry relatively uniform,with all 28 investigated strains having hydrogenase and producing secondary carotenoids in nitrogen deficient conditions; species differ in ability to hydrolyze starch, growth under acidic conditions and guanine-cytosine content of DNA varies. For this project, *Scenedesmus dimorphus* has been selected.

Current interest in the possible commercial use of algae resulting from the investigations of few scientists, as well as continued recognition of their value as tools in many fields of research has emphasized the desirability of an understanding of the factors which may limit algal growth under conditions of mass culture. As the result, each growth parameter of *Scenedesmus brasiliensis* need to study for maximise biomass yield from Modified Sago Effluent (MSE). This study will manipulate light parameter and bicarbonate salts such as $NaHCO_3$ to maximise biomass yield. Similar with plants, light is the source of energy which drives photosynthesis reaction in algae. For this regard intensity, spectral quality and photoperiod need to be considered. Although light intensity is very important for photosynthesis process, its requirement is vary accordingly to culture depth and intensity of the cultures. In outdoor mass cultures, carbon supply will usually be either in the form of CO_2-enriched air or as bicarbonate salts. In this study we used $NaHCO_3$ as bicarbonate salts that will supply carbon for photosynthesis.

1.2 Objectives

The objective of this study is:

1. To maximise growth of algae *Scenedesmus dimorphus* in Modified Sago Effluent (MSE).
2. To find specific parameter to maximize growth of algae *Scenedesmus dimorphus in* Modified Sago Effluent (MSE).
3. For economic large-scale culture of *Scenedesmus dimorphus* for producing biodiesel.

CHAPTER 2

LITERATURE REVIEW

2.1 *Scenedesmus sp.*

2.1.1 Morphology and Characteristic

As members of the order Chlorococcales, green micro-algae of the genus *Scenedesmus sp.* are characterized based on two-dimensional arrangement of 2, 4, 8 or rarely 16 cells in regular aggregates called coenobia. Basically most *Scenedesmus sp* shape is nearly spherical to ellipsoidal, elongated or fusiform to elongated fusiform, cell poles capitates, obtuse, acute or long tapering. Cell arranged linearly, alternating or in 2-3 rows, touching with the lateral wall or in subpolar region only. It has cell wall with hemicellulosic and sporopolleninic layer. Physiology and biochemistry relatively uniform, with all 28 investigated strains having hydrogenase and producing secondary carotenoids in nitrogen deficient conditions; species differ in ability to hydrolyze starch, growth under acidic conditions and guanine-cytosine content of DNA varies. For *Scenedesmus dimorphus,* its shape was in colonies of 4 cells and arranging linearly. Their cell body spindle-shaped or elongated ellipsoidal. Its size is about 10-22 μm long and 3-8.5 μm wide, longitudinal ridges on both sides, 2-3 short dented projections (1-1.5 μm long) at both ends. It's surrounding mucilaginous matrix present or absents.

Figure 1: *Scenedesmus dimorphus* (Illustrations from www.algaedepot.com)

2.1.2 Growth Parameters

2.1.2.1 Light

Similar with plants, *scenedesmus sp* need light as source of energy which drives photosynthesis reaction. For this regard intensity, spectral quality and photoperiodism need to be considered. Photoperiodism is defined as 'the control of some aspect of a life cycle by timing of light and darkness'. Although light intensity is very important for photosynthesis process, its requirement is vary accordingly to culture depth and intensity of the cultures. At high depth and cell concentration the light intensity must be increased to penetrate through the culture. However, too high light intensity might cause may result in photoinhibition. Effects of light on the respiration of autotrophic green cells also have repeatedly been described. Hoch, Owens, and Kok (1963) have shown that especially with red light the so-called Kok effect is due to an inhibition of respiration which is saturated at low light intensities.

2.1.2.2 CO_2 or Bicarbonate

The inorganic carbon source for algal photosynthesis has a special role in intensive microalgal cultures. In outdoor mass cultures, carbon supply will usually be either in the form of CO_2-enriched air or as bicarbonate salts. In this study we used $NaHCO_3$ as bicarbonate salts that will supply carbon for photosynthesis. Choosing the right method might have a great impact on algal production. Osterlind (1951) was first found that a lag period in HCO_3 utilization by Scenedesmus quadricauda and ascribed it to an activation period of either bicarbonate absorption or carbonic anhydrase formation. Then Raven (1970) reviewed the literature and concluded that carbonic anhydrase is involved specifically in bicarbonate use by converting the carbon species entering the cell to the form used in carboxylation. In addition, he also suggested an active bicarbonate transport in some algal cells. Findenegg (1976) demonstrated three steps in the adaptation of $C0_2$-grown Scenedesmus obliquus to the use of HCO_3; all three are involved in the production of carbonic anhydrase by this alga. He showed that the affinity of CO_2-adapted S. obliquus to CO_2 is low and increases only after over 4 hours of aeration with low CO_2 air. The low affinity of CO_2-adapted S. obliquus was also shown by Radmer and Ollinger (1980), who suggested active assimilation of HCO_3- by air-adapted algae. Lehman (1978) reconfirmed the well-established opinion that free CO_2 is the only direct substrate for the Calvin cycle and suggested that bicarbonate serves as a vehicle for the transport of inorganic carbon into the cell.

2.1.3 Lipid production

Due to their simple cellular structure, algae have higher rates of biomass and oil production than conventional crops (Becker, 1994). Because of that, producing biodiesel from algae has become one of the most efficient ways of generating biofuel. In nature, increasing microalgal accumulation of lipid depends on certain conditions which are the main factor for high biodiesel production. Known to grow more abundantly in nutrient-rich (eutrophic) which can lead to algal bloom (Paerl , 2001), microalgal populations will reach to its limit and a large number of algal cell die either cause by nutrient depletion or high cell densities that limit light penetration. Different depth in lake can be group into different tropic status. The tropic status of lakes has been defined based on primary production and can be measured by the amount of organic carbon assimilated by photosynthesis. In certain tropic status, nutrient especially nitrogen and phosphorus are limited. However, cell densities are not too high and photosynthesis is still possible. During these limiting conditions, cell still able to fix CO2 and accumulate photo-assimilates in the form of starch or lipids as important storage functions for survival under unfavourable conditions such as low nutrients, low or high light and other conditions. According to Peer M. Schenk (2008), Algae that accumulate lipids in large amounts are therefore often found where environments and microclimates alter frequently between optimal growth conditions and survival under suboptimal conditions.

2.2 Sago Effluent

The sago palm (metroxylon sago) in Malaysia usually grows in freshwater swamps especially in state of Sarawak where sago is a staple food. It has high starch yield where one palm may produce between 150 to 300kg of starch. Reportedly, there are total areas of 24,000 ha cultivated with the sago palm with 32 factories producing almost 67000 tan yr^{-1} starch (Chew & Shim, 1993). During starch extraction process, it will wash with large amount of water followed by settling in tanks where only the starch powder is used and then spray dried. This will generates wastewater or effluent which become problem to manage it. Usually the wastewater will discharge into nearby river. For each kilogram of sago starch, it has estimated that 20L of wastewater is generated from extraction process (Bujang , 1996). According to Chew and Shim (1993), this sago effluent contain high Biochemical Oxygen demands (BOD), Chemical Oxygen Demand (COD) and Suspended Solid in the range of 1650-3444 mg/L, 2632-11428mg/L and 366-12936mg/L respectively.

CHAPTER 3

MATERIALS AND METHODS

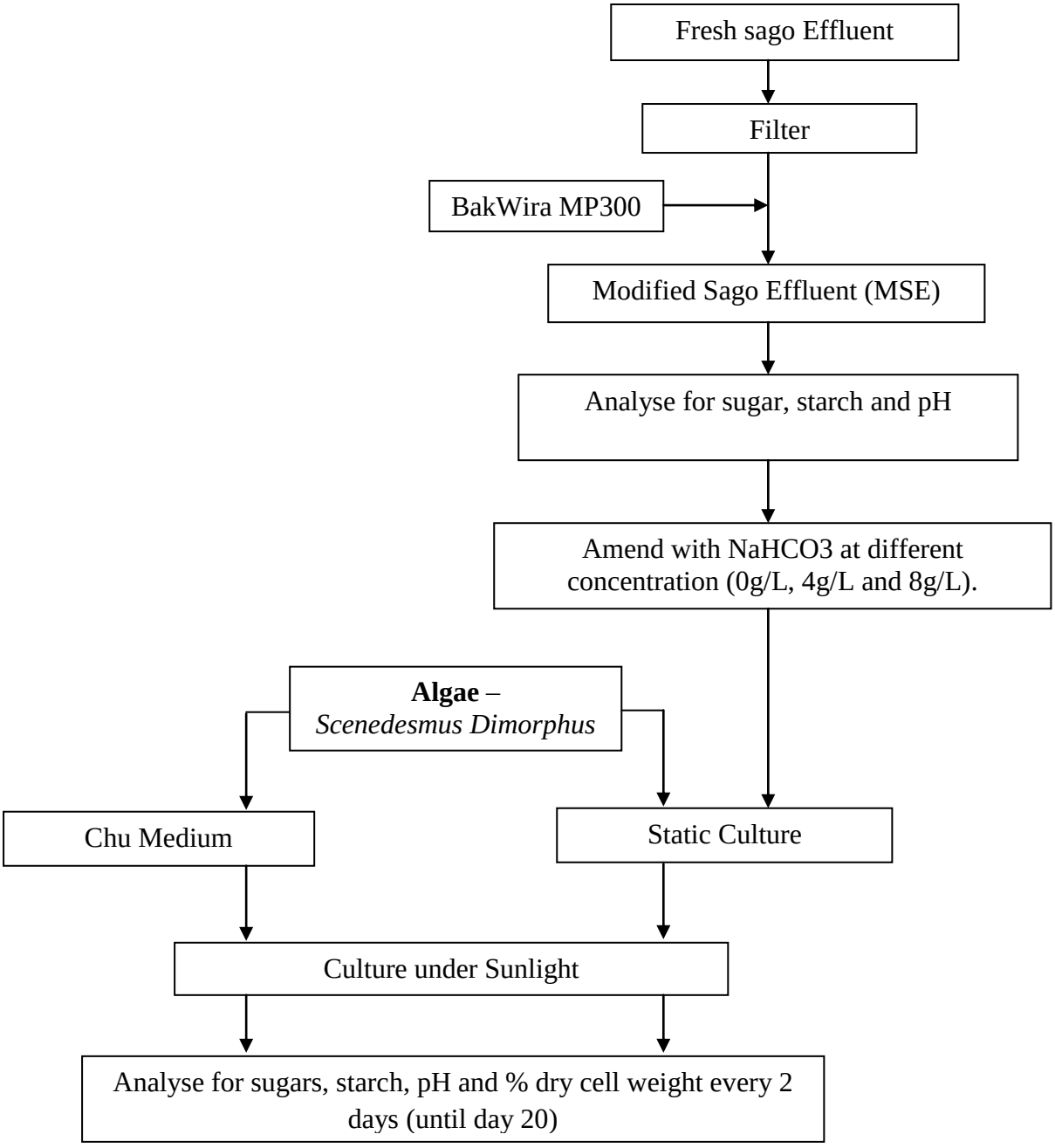

Figure 2: Flow diagram of static culture of *Scenedesmus Dimorphus* in modified sago effluent (MSE).

3.1 Microorganism - algae

Algae use in this study is *Scenedesmus dimorphus.* It was supplied by Aquatic Laboratory, Faculty of Resource Sciences and Technology, UNIMAS.

3.2 Inoculum

Inoculums of algae *Scenedesmus dimorphus* was prepared by culturing in protease medium (**Appendix A**). For preparing 1 L algae inoculums, 10% or 100 mL of *Scenedesmus dimorphus* was cultured into 900 mL protease medium. The cultivation conditions were agitation at 120 rpm, temperature 30°C, and luminosity 1125 lux shown in **figure 3.** The inoculums are matured and ready for the experiment after 14 days of cultivation.

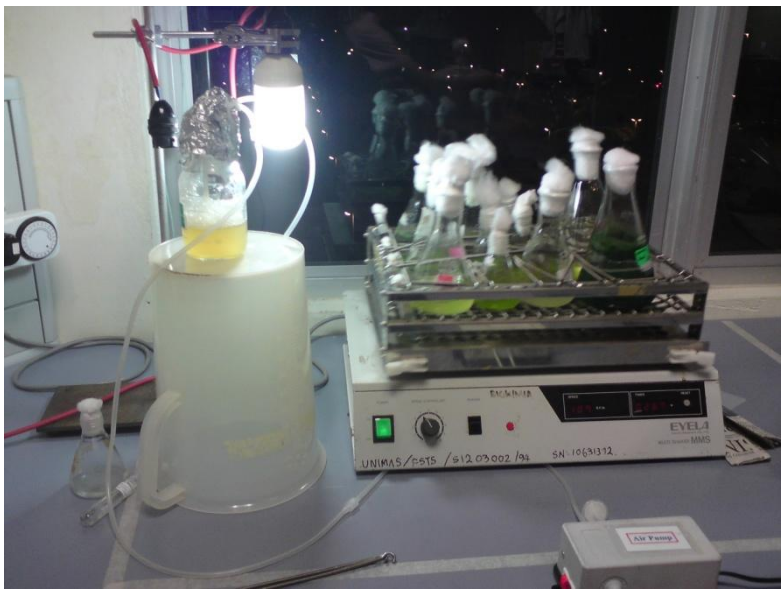

Figure 3: The cultivation conditions for *Scenedesmus dimorphus.*

3.3 Modified sago effluent (MSE)

Process of treatments for sago effluent was shown in **Figure 2.** Sago effluent was collect from Hardsen sago mill in Pusa, Betong. The effluent was filtered first using a 710 micrometre stainless filter. Then, the sago effluent was treated with a commercial microbial amendment (BakWira MP300) for 2 to 3 days. It involved few processes. 1L of filtered sago effluents was treated first with 300mg Bakwira and bubble for 2 hours before added into another 9L of filter sago effluent. The final mixture (10L) was bubbled for 3 days to become Modified sago Effluent (MSE). Subsequently, tests were carried out for sugar using Dinitrosalicyclic (DNS) method and for starch based on iodine test. In addition, initial pH of the MSE also was recorded.

3.4 Chu Medium

As control in this experiment, algae s *Scenedesmus sp.* was cultured in Chu medium. Chu medium must be preparing first. Chu medium must contain Ca $(NO_3)_2$, K_2HPO_4, $MgSO_4.7H_2O$, Na_2CO_3, Na_2SiO_3, FeCl3, Trace Mineral Solution and Vitamin Solution. Each component of Chu medium must be prepared separately first as shown in table 1 before mixed together.

Table 1: Component of Chu Medium

Component	Stock Concentration, g/L
$Ca(NO_3)_2$	20.0
K_2HPO_4	2.5
$MgSO_4.7H_2O$	12.5
Na_2CO_3	10.0
Na_2SiO_3	12.5
$FeCl_3$	0.4
Trace Mineral Solution	Refer to table 1.2
Vitamin Solution	Refer to table 1.3

* 950ml of distilled water was introduced and dissolved with 1ml of each component individually. Then final volume was brought to 1L and sterile using syringe filter.

Table 1.2: Components for Trace Mineral Solution

Compound	Concentration, g/L stock
H_3BO_3	2.48
$MnSO_4.H_2O$	1.47
$ZnSO_4.7H_2O$	0.23
$CuSO_4.5H_2O$	0.10
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.07
$Co(NO_3)_2.6H_2O$	0.14

*****950ml of distilled water was introduced and dissolved with 1ml of each component individually. Then final volume was brought to 1L.

Table 1.3: Components for Vitamin Solution

Compound	Concentration, g/L stock
Thiamine-HCl	2.0
Biotin	2.5
Cynocabalamin	2.5

* 950ml of distilled water was introduced into a flask. 50mg of Thiamine-HCl was dissolved and then 1ml of each primary stock was added into the distilled water. Then final volume was brought to 1L. The components was filtered sterilizes and stored frozen.

3.5 lab-scale culture of *Scenedesmus dimorphus* in

3.5.1 Chu Medium (control)

1800ml of Chu medium was prepared and it was transfer into a clean 2 L bottle. 10 % v/v (200ml) algae *Scenedesmus dimorphus* was taken out from stock culture and Optical Density (OD) of the algae solution was taken. Then, the algae were transferred into the bottle. The bottle then was put under sunlight. Aeration pump was setup in the bottle to provide aeration in the container and to ensure nutrient in MSE equally distribute. The bottle then was put under sunlight.

3.5.2 Modified Sago Effluent (MSE) medium

1800ml of Modified Sago Effluent (MSE) that has been treated with BakWira was prepared and transfer into a clean transparency 2 L bottle shows in **Diagram 3.5.2**. 10 % v/v (200ml) algae *Scenedesmus dimorphus* was take out from stock culture and Optical Density (OD) of the algae solution was taken. Then, the algae were transferred into the bottle. 3 replicates (containers) of *Scenedesmus dimorphus* growth in MSE with same quantity was prepared where 0g/l, 4g/l and 8g/l (w/v) of $NaHCO_3$ was added into each of the bottle. Aeration pump was setup in the container to provide aeration in the container and to ensure nutrient in MSE equally distribute. The Container then was put under sunlight.