Faculty of Resource Science and Technology

EFFECT OF SODIUM NITRATE ($NaNO_3$) IN STATIC CULTURE ON *Scenedesmus dimorphus*

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Final Year Project Report ☑

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LIST OF ABBREVIATIONS

^0C	Temperature
DCW	Dry Cell Weight
g/L	Gram per litre
GHG	Greenhouse gas
Min	Minute
mL/min	Milliliter per minutes
MSW	Modified Sea Water
MTW	Modified Tap Water
$NaNO_3$	Sodium Nitrate
OD	Optical Density
pH	Acidity, neutrality and alkalinity unit
w/w	Weight per weight

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Effect of Sodium Nitrate ($NaNO_3$) in Static Culture of *Scenedesmus dimorphus*

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EFFECT OF SODIUM NITRATE ($NaNO_3$) IN STATIC CULTURE OF *Scenedesmus dimorphus*

ABSTRACT

This study focused on *Scenedesmus dimorphus* cultured in different types of modified water in order to investigate which medium is the best for producing the highest biomass and lipid in shorter time and less expensive media. In this experiment, *S. dimorphus* was lab-cultured in 2 different types of modified water; modified tap water (MTW) and modified sea water (MSW). Each type of modified water was amended with different concentrations of $NaNO_3$ (0g/L, 3g/L, 5g/L, 8g/L) in a static culture. The growth of the alga was compared to proteose medium as positive control. In order to maximize the distribution of nutrient in the modified water, an aeration pump was used. The cultivation of *S. dimorphus* was provided with 12 hour lighting, correct range of temperature and pH. In this study, the highest biomass was in proteose medium with increasing biomass from 100 mg/L on day 0 to 2550 mg/L on day 9. The highest biomass in MTW shows by 5 g/L $NaNO_3$ which is1850 mg/L biomass. Highest lipid extraction was recorded in MTW without any amended at 31% w/w. The biomass gained during cultivation of *S. dimorphus* proved that correct amount of $NaNO_3$ could increase the growth of *S. dimorphus* and by limited the nitrogen to *S. dimorphus* would give stress to the *S. dimorphus* to produce the high lipid extraction.

Key words: *Scenedesmus dimorphus*, Modified tap water (MTW), Modified Sea Water (MSW), biomass, lipid extraction

KESAN SODIUM NITRAT ($NaNO_3$) DALAM KULTUR STATIK *Scanadesmus dimorphus*

ABSTRAK

Kajian ini menfokuskan pengkulturan Scanedesmus dimorphus di dalam medium air yang diubah untuk mengenalpasti medium air yang manakah terbaik dalam menghasilkan biomass dan lipid tertinggi dalam masa yang singkat dan medium yang tidak mahal. Di dalam eksperiment ini, S. dimorphus akan dikulturkan dalam dua jenis air yang diubah iaitu air paip yang diubah (MTW) dan air laut yang diubah.(MSW). Setiap jenis air yang diubah telah dilarutkan dengan beberapa sukatan $NaNO_3$ *yang berbeza iaitu (0g/L, 3 g/L, 5 g/L, 8 g/L) dengan menggunakan kaedah kultur statik. Pertumbuhan S. dimorphus di dalam air yang diubah akan dibandingkan dengan kultur di dalam media Proteose yang merupakan kultur kawalan. Untuk memaksimakan pengagihan nutrisi-nutrisi di dalam medium tersebut, pam pengudaraan digunakan. Pengkulturan S. dimorphus akan dibekalkan dengan cahaya yang cukup iaitu 12jam sehari dan sentiasa berada di dalam julat suhu dan pH yang optimum. Di dalam kajian ini, biomass tertinggi diperolehi di dalam proteose media yang mana biomasnya adalah dari 100mg/L pada hari ke-0 kepada 2550 mg/L pada hari ke-9.Biomass tertinggi di dalam media MTW diperoleh di dalam larutan 5g/L* $NaNO_3$ *iaitu sebanyak 1850 mg/L biomass. Untuk pengektrakan lipid tertinggi dicatatkan oleh (MTW) tanpa sebarang larutan iaitu sebanyak 31% w/w peratus. Data yang diperolehi menunjukkan bahawa penambahan kuantiti* $NaNO_3$ *yang sesuai mampu menaikkan kadar pertumbuhan S. dimorphus. Di samping itu, menghadkan* $NaNO_3$ *kepada S. dimorphus akan memberi stress kepada S. dimorphus seterusnya menyebabkan ianya menghasilkan jumlah lipid yang banyak.*

Kata kunci: *Scenedesmus dimorphus*, Air paip yang diubah (MTW), Air laut yang diubah (MSW), biomass, pengektrakan lipid

INTRODUCTION

1.1 General Overview

1.1 Background Research

Nowadays, our energy crisis is expected to get even worse with the shortages of fossil fuel. The limitation of energy resources which come from the three main sources; coal, natural gas and petroleum are unsustainable resources. Due to that, fossil fuel is now highly priced and causes economic crisis around the globe. Usage of fossil fuel also contributed to climate change and pollution due to accumulation of GHGs in the environment that have already exceeded the dangerous level. In order to overcome this problem, many researches have been study to find the alternative renewable sources which are friendlier toward environment. According to Frac *et al*. (2013), many studies have concluded that using biofuel can be part of climate-energy solution.

Farrell *et al.* (2006) reported that biofuel has been considered as candidate of alternative energy of fossil fuel. In order to be categorized as biofuel, the fuel must contain over 80 percent renewable material. First generation biofuels are derived from edible biomass such as corn, sugar cane and soybeans. According to Asada *et. al*. (2012) these biofuels have caused the remarkable rise of price of the food globally as they are used as food. McMichens & Pledge (2009) mentioned that there is not enough available farmland to plant them and the total emission of growing; harvesting and processing them are not environmentally friendly.

Second generation of biofuel came from lignocellulosic material such as wood, straw, construction debris which does not compete with food (Lavoie & Lee, 2013). However, the cost for carried out the process is quite high and the extraction process is harder as most of them are complex carbohydrates which are closed in the lignin.

Nowadays, algae as in a third generation biofuel have gaining wide attention as alternative sources for production of biofuel (Nguyen, 2012). This is because of their faster growth rate, higher photosynthetic efficiency and high polysaccharide production compared with other energy biomass (Asada *et. al.*, 2012). Furthermore, it helps to reduce the economic crisis and at the same time provide the better environment by reducing the pollution. As they grow, they use less water compared to terrestrial crops. Besides, it can be cultivated in brackish water or on non-arable land and reduce environment impact. Therefore, micro alga such as *S. dimorphus* is one of the potential alga for the production of biodiesel due to large lipid content per cell (16-40%), faster growth rate, and high photosynthesis rate (McMichens & Pledge, 2009).

1.3 The objectives of this study are to:

i. Maximize the growth of *S. dimorphus* in 2 types of modified water with difference concentration of $NaNO_3$ for production of bioethanol.

ii. Find the best modified water that produce the highest biomass and lipid production in shorter time and less expensive media.

iii. Study specific parameters involves in the growth of *S. dimorphus.*

CHAPTER 2

LITERATURE REVIEW

2.1 World Production of Biofuel

Biofuels are fuel obtained from biomass of plant, animal or microorganism. According to Kanani (1999), Europe biodiesel is produced mainly from sugar beet and cereals while USA and Brazil mainly produced from maize and sugar cane. As shown in **Figure 1**, United State shows the highest production of biofuels followed by Brazil, European Union and other countries. According to the latest studies, the consumption of fossil fuels for production of petrol has increased by 90% and will be depleted by 2050 (Chena *et al.*, 2013). This shows that biodiesel production plays an important role as alternative energy for fossil fuel. This is a big step for facing the economic crisis due to depleting of fossil fuel in the future.

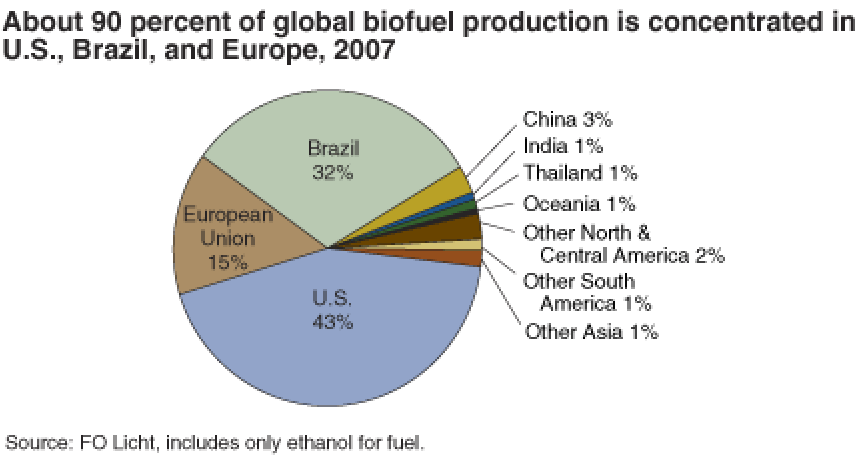

Figure 1: World biofuel production

(illustration from http://www.csa.com/discoveryguides/biofuel/review.php**)**

According to Jon cited by Kanani (1999), more than 50% of the oil has been imported each year in United State. Considering the average spent of imported oil, which currently is above $110 per barrel, the United States spent over $460 billion on imported foreign oil in 2011, and consequently the investment of these funds in algae biofuel could have significant benefit (Kanani 2013). Biodiesel also offer the new perspective on agriculture industry, academic research, and also microalgae industry. This project not only provides an economic value by developing the microalgae industry at the same time providing job opportunity which may reduce the numbers of unemployed graduates.

2.2 Biodiesel from Algae

In first and second generation of biofuel, production of biodiesel has been come from plantation of food crops and lignocellulosic materials (Brennan & Owende, 2009). Then, microalgae, as a third generation biofuels have gaining wide attention replacing them for the biofuel production. According to Grosmann & Martin (2012), algae are considered as alternative technologies for the biodiesel synthesis and bioethanol synthesis. Biodiesel are made from algae oil while bioethanol are made from algae starch. Based on Frac *et al.* (2010) algae are the main raw materials from which such biofuel can be produced in high efficiency levels and low investment. In facts, algae are cost effective and provide relatively high yield of biofuel (Chand *et al.*, 2012).

Table 1 shows the production of oil from different crops. Micro-algae shows the highest gallons of oil per year per acre which is 5000 until 15000 gallons compared to oil palm which just 635 gallons. According to Benítez *et al.* (2010) there are some main advantages of using algae to produce biodiesel. Firstly, the yield of oil and fuel from algae are much higher (10 -100 times) than competing energy crops like corn and sugar cane. Secondly, algae can grow practically anywhere, thus ensuring that there is no competition with the food crops. Thirdly, algae are excellent bioremediation agent which can absorb massive amount of CO_2 and play vital role in sewage and wastewater treatment. Lastly, certain algae technologies have been designed to absorb CO_2 from smokestacks.

Table 1: Production of oil from different crops (Benítez *et al*, 2010)

Oils	**Gallons of Oil per Year per Acre**
Corn	18
Soybeans	48
Safflower	83
Sunflower	102
Rapessed	127
Oil Palm	635
Microalgae	5000-15000

Cultivating microalgae reduces the usage of arable lands that can be used to grow food crops instead of biomass crops. Besides that, microalgae are fast growing species since it has short life cycle. Moreover, culturing microalgae can tolerate in huge range of temperature and salinity (Watson, 2001). Generally, anything that contains lipid is potential for biodiesel production. Biodiesel can be produced through the extraction of lipid which later on will be converted into biodiesel as shown in **Figure 2**.

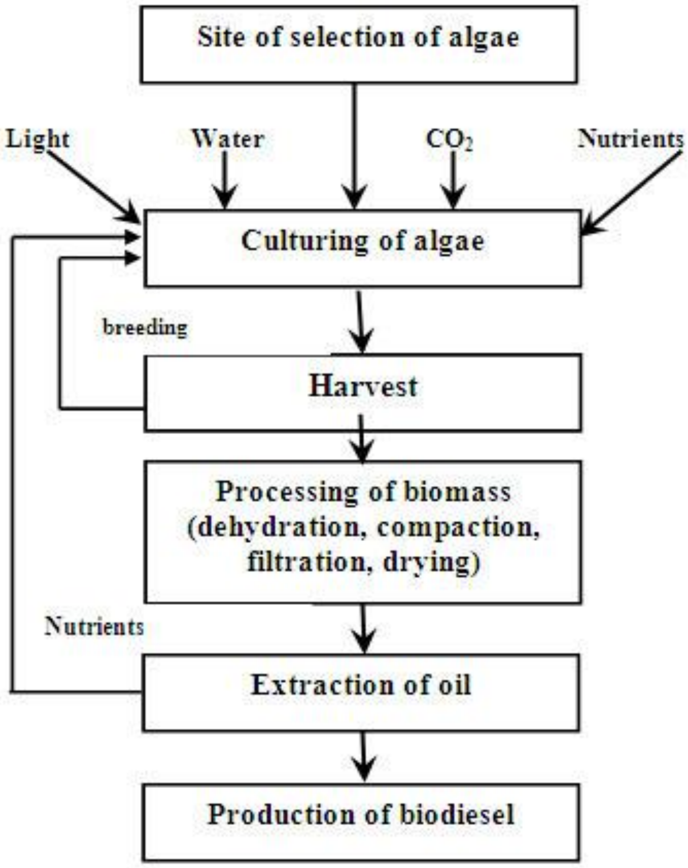

Figure 2 The stage of biodiesel production from microalgae (Frac *et al*., 2010)

2.3 *Scanedesmus dimorphus*

2.1.1 Morphology and Characteristic

Based on **Figure 3**, *S. dimorphus* is a genus of alga, specifically of the Clorophyceae and it was non-motile and colonial. According to Velichkova *et al.* (2013), *S. dimorphus* contains 16-40% lipid, 21-52% carbohydrate and 8-18% protein on dry weight basis. *S. dimorphus* are freely floating and able to form filaments and colonies which have the ability to adapt of extreme ecological habitats (Oncel, 2013). *S. dimorphus* colony usually in 4 cells, the two inner cells are oval to box shaped, whereas the two outer cell have spines and crescent-shaped. However, on some species, the shape of the cell may granulate, dented or ridged. Cell length of *S. dimorphus* is 12.5 micron and width 5 microns..

Figure 3: *S. dimorphus*
(Source: http://www.shigen.nig.ac.jp/algae/images/strainsimage/nies-0093.jpg)

Based on **Table 2**, lipid content in *S. dimorphus* strain is 16-40% which is the best alga strains that have the highest lipid content. Followed by *Prymnesium parvum* that have 22-38% of lipid content. Others algae strains have lipid content below 25%. This is the main reason why *S. Dimorphus* have been choosen from abundant number of algae strains. The higher the lipid content in the algae, the higher biodiesel will be converted from the lipid.

Table 2: Lipid content in algae (McMichens & Pledge, 2009)

Strain	Lipids percentage (%)
Scenedesmus obliquus	12-14
Scenedesmus quadricauda	1.9
Scenedesmus dimorphus	16-40
Chlamydomonas reinhardtii	21
Chlorella vulgaris	14-22
Chlorella pyrenoidosa	2
Spirogyra sp.	11-21
Dunaliella bioculata	8
Dunaliella salina	6
Euglena gracilis	14-20
Prymnesium parvum	22-38
Tetraselmis maculata	3
Porphyridium cruentum	9-14
Spirulina platensis	4--9
Spirulina maxima	6-7
Synechoccus sp.	11
Anabaena cylindrica	4-7

2.3.2 Growth Parameters

Growth parameters are important to be regulating as it will determine the algal growth. The most important parameter are involved includes light, pH, temperature, agitation and CO_2

2.3.2.1 Light

Similar with plants, *S. dimorphus* needs light as source of energy which drives photosynthesis reaction (Velichkova *et al.*, 2013). *S. dimorphus* prefer highly light environment. However, too much light will result in photoinhibition. Photoinhibition is light-induced reduction in the photosynthetic capacity of the alga (Benitez *et al*.,2010). Therefore, photoperiodism needs to be considered. Photoperiodism is defined as the control of some aspect of a life cycle by timing of light and darkness. According to Benitez *et al*. (2010), direct sunlight or light is often strong for alga. So, it is important to agitate the water so that it does not remain on the surface, which would cause it to be over exposed.

2.3.2.2 Temperature, and pH

According to Lupi *et. al.* cited in Velichkova *et al. (*2013) *S. dimorphus* optimum temperature for growth is at room temperature which is 25-27 ^{o}C. According to Benitez *et al.* (2010), microalga can be tolerate temperature in arrange of 16 and 27 ^{o}C. Temperature which is below 16 ^{o}C will slow down the growth whereas those higher than 35 ^{o}C are lethal for some species. Like other microalga, *S. dimorphus* is sensitive to pH. Therefore, maintenance of an acceptable pH range throughout culturing is vital as it may impacts all aspects of media biochemistry. The pH range for *S. dimorphus* is between 7-9.

2.3.2.3 Agitation and Carbon Dioxide

Agitation is not only to prevent sediment of the alga but also to ensure all the cell are equally exposed to the light and nutrients and also improve the gas exchange between the culture medium and air. For carbon dioxides in its experiments, it was originates from air that may create by vacuum pump and air.

2.4 Utilization of $NaNO_3$

According to Puzz (2001), nutrients provision in a culture medium is important for optimal alga growth. Nutrients mainly carbon, nitrogen, and phosphorus, along with various types of macro- and micronutrients are prerequisite for high growth rates of microalga. Deficiencies in any nutrients will cause physiological changes, disturbance in metabolism and decrease productivity. There are various types of common media used for microalga strains such as Zarrouk medium, Chu medium, Bold's Basal medium and Beijerinck medium. However, the common media is not cost effective. According to Bujang & Manggi (2010), Sodium Nitrate ($NaNO_3$) is a prerequisite nutrient for optimal alga growth as tested on *Spirulina platensis*. In this study, *S. dimorphus* will be cultured in modified water amended by different concentration of $NaNO_3$ in order to investigate effect of $NaNO_3$ on *S. dimorphus* which give ethe highest production of biomass and lipid in shorter time and less expensive media instead of using common media or standard medium. According to Rani *et al*. (2011), nitrogen content may enhance the growth of alga. However, in order to enhance the lipid production in alga, nitrogen limites situation is needed.

MATERIAL AND METHOD

3.1 Material

3.1.1 Microalga

Alga *Scanedesmus dimorphus* was used in this study. The inoculum for this project was supplied by Aquatic Laboratory Faculty Resource Science and Technology.

3.1.2 Standard medium

Standard medium acted as positive control in this experiment. Standard medium for this experiment was proteose medium (**Appendix A**).

3.1.3 Modified Water

2 type of water which are tap water and sea water. Each of them was amended with sodium nitrate with different concentration (0g/L, 3g/L, 5g/L, 8g/L).

3.2 Methods

3.2.1 Microalga inoculum

Inoculum of *S. dimorphus* was prepared by culturing in Proteose medium **(Appendix A)**. 2L of inoculum was prepared by adding 10% of *S. dimorphus* into 1800mL proteose medium. The cultivation condition was agitated at 120rpm, temperature 27^0C and luminosity 3000lux as shown in **Figure 4**. After 14 days of cultivation the inoculum will be matured and ready for the experiment.

Figure 4: Inoculum of *S. dimorphus*

3.2.2 Cultivation of microalga

During cultivation of microalga, all the variables were kept controlled such as temperature, pH, and light supply. The culture experiments were carried out at 27^{o}C, pH 7-9. The carbon will be supplied by aeration pump. A light level of 3000 lux will be 12 hours on/off.

3.2.2.1 Culture of *S. dimorphus* in Standard Medium (control)

Standard medium was prepared using the formula in **Appendix A**. 1600ml of standard medium will be mix with 20% v/v (400ml) of *S. dimorphus* in a 2L plastic tubing. Aeration pump was used to provide aeration so the nutrient shall be distributed evenly. All the variables such as temperature, nutrients supply and light supply were controlled.