FLOW CULTURE OF SCENEDESMUS DIMORPHUS IN FILTERED SAGO EFFLUENT

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</tr>
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<tr>
<td>DCW</td>
<td>dry cell weight</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>g/L</td>
<td>gram per liter</td>
</tr>
<tr>
<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>lux</td>
<td>luxury</td>
</tr>
<tr>
<td>mg</td>
<td>miligram</td>
</tr>
<tr>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>FSE</td>
<td>Filtered sago effluent</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>rpm</td>
<td>rotation per minute</td>
</tr>
<tr>
<td>%</td>
<td>percent</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
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</table>
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Flow Culture of *Scenedesmus Dimorphus* in Filtered Sago Effluent

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**ABSTRACT**

In this study, *Scenedesmus dimorphus*, photosynthetic green algae was cultivated in filtered sago effluent that added with NaHCO₃ and compared with the filtered sago effluent without NaHCO₃ to investigate the effect of the NaHCO₃ to the cultivation of *Scenedesmus dimorphus*. The cultivation was done for 20 days in the flow culture with the speed flow 12.3 ml/sec under natural light. After the cultivation, the lipid extraction was done to determine the lipid content of *Scenedesmus dimorphus*. The result showed that the cultivation in FSE without NaHCO₃ produced the highest dry cell weight which is 395 mg/L on day 10 of cultivation. The cultivation in FSE contain 4g/L produced 315 mg/L. There are another factor that can affect the cultivation of *Scenedesmus dimorphus* in FSE such as pH of medium, light and agitation. In addition, the highest lipid content of *Scenedesmus dimorphus* that was obtained in this study is 18.39%. Cultivation of *Scenedesmus dimorphus* in filtered sago effluent can be improved by providing optimum conditions such as nutrients, light, agitation and environment condition for the cultivation of this alga.

Key words: *Scenedesmus dimorphus*, filtered sago effluent, sodium bicarbonate, lipid extraction

**ABSTRAK**

Dalam kajian ini, *Scenedesmus dimorphus* iaitu alga hijau yang menjalankan proses fotosintesis di kultur di dalam sisa air sagu yang telah di tapis. Sisa air sagu tersebut dimasukkan dengan NaHCO₃ sebanyak 4 g/L untuk mengkalji kesan NaHCO₃ terhadap pertumbuhan *Scenedesmus dimorphus*. Satu kultur lagi tidak dimasukkan dengan NaHCO₃. Kultur tersebut dijalankan selama 20 hari dengan menggunakan sistem kultur aliran dengan kelajuan aliran 12.3ml/saat di bawah sinaran matahari. Selepas pengkulturan selama 20 hari, proses pengekstrakan lipid dijalankan untuk mengkaji kandungan lipid di dalam *Scenedesmus dimorphus*. Hail kalian menunjukkan pengkulturan di dalam sisa sagu bertapis yang tidak ditambah dengan NaHCO₃ menghasilkan biojisim yang paling tinggi iaitu sebanyak 395 mg/L pada hari ke-10 pengkulturan. Pengkulturan menggunakan sisa sagu bertapis yang mengandungi 4 g/L menghasilkan biojisim sebanyak 315 mg/L. Terdapat beberapa faktor yang boleh mempengaruhi hasil pengkulturan *Scenedesmus dimorphus* di dalam sisa sagu bertapis seperti pH, medium, suhu, cahaya dan agitasi. Penghasilan lipid oleh *Scenedesmus dimorphus*, pengkulturan menggunakan sisa sagu bertapis yang ditambahkan dengan 4 g/L NaHCO₃ menunjukkan peghasilan tertinggi iaitu sebanyak 18.39% daripada berat kering sel. Pengkulturan *Scenedesmus dimorphus* menggunakan sisa sagu bertapis dapat diperbaiki dengan menyediakan persekitaran nutrisi, cahaya, agitasi yang optimum.

Kata kunci: *Scenedesmus dimorphus*, sisa sagu bertapis, natrium bikarbonat, pengekstrakan lipid
CHAPTER 1

INTRODUCTION

1.1 General Introduction

Currently, the world faced the crisis on energy resources which is expected to get even worse. This happened due to the high demand for the energy to sustain industrial activities even though the resources of the energy are limited. Most of the energy sources used in the world comes from the sources that are available in limited quantities such as coal, natural gas and petroleum. Besides, these types of energy sources are also highly contaminant to our ecosystems. Therefore the research to find other types of energy sources that is renewable provides environmental benefits and capable to solve this global problem is very important nowadays (Gouveia & Oliveira, 2009).

Fuels represent around 70% of the total global energy requirements especially for transportation, manufacturing and domestic heating. Microalgae, the third-generation bio-fuel feedstock have been suggested as one of the good candidates for fuel production as they are the only renewable bio-fuel that is capable to solve world energy sources (Silva, Santos, & Reis, 2009). Grima et al. (2002) mentioned that the production of algal-derived metabolites requires the process for culturing the algal, recovery of the biomass and further downstream processing to purify the metabolites from the biomass. Furthermore microalgae lipid production is strongly dependent on environmental factors.
Microalgae have been attracting attention as a source of high-lipid material to produce bio-fuel because photosynthesis conversion is an efficient and alternatively process which is does not compete with the food crops (Chisti, 2007). Grima *et al* (2002) mentioned that the production of algal-driven metabolites requires the process for culturing the algae, recovery of the biomass and further downstream processing to purify the metabolites from the biomass. The hard part about algae production is growing the algae in a controlled way, harvesting it and extracting its oil efficiently. Furthermore, microalgae lipid production is strongly dependent on environmental factors.

According to Dr. Orcutt (n.d), *Scenedesmus* is a very promising strain of microalgae that should be further researched. *Scenedesmus dimorphus* being one of preferred species for oil yield in the production of biodiesel. However, one of the problems with this microalga is that it is heavy and need constant agitation in order to overcome the sedimentation of the cells. Furthermore, this microalga is usually grown in liquid medium which is high in pH such as Chu or Protease medium. But the cost of cultivation in this commercial medium is quite high especially for economical production. Therefore, this study is to develop cultivation in the filtered sago effluent which is lower in cost production. Besides, using this sago effluent as a medium can help in the sago waste treatment which is a can contaminate environment.

The sago palm is a crop that is excellence for encourage and support the agriculture in many countries. Douglas and William (1984) state that, in Malaysia sago starch is the main source of carbohydrates. Sago palm also is excellent starches resource in which can be apply in many industries such as foods, pharmaceuticals and textiles industries.
However, sago waste such as waste water which is usually eliminated into the rivers can harm the environment (Singhal et al., 2007). Therefore the waste water which has the high carbon to nitrogen ratio (105:0.12) in which can be use as a fermentation medium (Phang et al., 2000) should be treat wisely.

1.2 Objectives

The aim of this study is to investigate the best concentration of sodium bicarbonate (NaHCO3) for *Scenedesmus dimorphus* culture in Filtered Sago Effluent (FSE) using flow culture technique. There are four main objectives in this study. They are:

1. To growth *Scenedesmus dimorphus* in Filtered Sago Effluent (FSE)
2. To investigate the effect of adding sodium bicarbonate (NaHCO₃) in FSE to the growth of *Scenedesmus dimorphus*.
3. To test the lipid contents in *Scenedesmus dimorphus* that grown in the FSE.
4. To know the specific parameters for *Scenedesmus dimorphus* growth.
CHAPTER 2

LITERATURE REVIEW

2.1 Microalgae

Microalgae are some of the simplest and oldest organisms ever to grace the planet. There are approximately 8,000 species of green algae estimated to be existence. They are unicellular and grow and multiply through photosynthesis just like plants. Their simple structures allow them to more efficient convert solar to chemical energy. The potential behind them and in large quantities of microalgae is where they become significant as an energy source. Most green algae are aquatic and commonly found in fresh tropical waters. All green algae are photosynthetic, which means they get all their organic carbon (energy) from photosynthesis. Green algae are generally fast growing and sturdy if culture at optimum condition. They reproduce both asexually and sexually (Michael, 2009).

2.1.1 Scenedesmus dimorphus

2.1.1.1 Morphology and characteristic

As members of the order Chlorococcales, green micro-algae of the genus *Scenedesmus* are characterized typically by the well-known two-dimensional arrangement of 2, 4, 8 and rarely 16 cells in regular aggregates called coenobia (Hegewald & Schnept, 1979; Komarek & Fott, 1983). *Scenedesmus dimorphus* shown in Figure 2.1 is a green microalgae, bean shaped of approximately 10μm in size. Categorized as a heavy
bacterium, *Scenedesmus* has a lipid content of 16-40%, being one of the preferred species for oil yield in the production of biodiesel. The cells are heavy and forms thick sediments if not kept in constant agitation.

**Figure 2.1:**  (a) Microscopic picture of *Scenedesmus dimorphus* at 1000x  
(b) Microscopic picture of *Scenedesmus dimorphus* under NIES-93

2.1.1.2 Cultivation of *Scenedesmus dimorphus*

There are many parameters that can affect the cultivation of *Scenedesmus dimorphus* which are nutrients, temperature, pH, agitation and light. Patrick and Sorgeloos (1996) reported that the various factors may be interdependent and in which a parameter that is optimal for one set of conditions is not necessarily optimal for another.
2.1.1.2.1 Influence of temperature

The optimal growth temperature for *Scenedesmus dimorphus* falls between 30-35 °C. Temperature lower than 16°C will slow down growth, while temperature that higher than 35°C are lethal for the some algae species. Other than that, *Scenedesmus dimorphus* use any and all light it is given and should be further researched for use in mass production.

2.1.1.2.2 Effect of light

Light is the source of energy which drives the conversion of the inorganic carbon into organic matter. Too high density of light may result in photo-inhibition (Patrick & Sorgeloos, 1996) which causes the reduction in the photosynthetic capacity of the algae.

2.1.1.2.3 pH of medium

The pH for most culture algae species is between 7 to 9 and the optimum range for *Scenedesmus dimorphus* being 8.2 to 8.7. The disruption of many cellular processes can result in failure to maintain the acceptable pH which can lead to collapse of complete culture of algae. Addition of carbon dioxide allows correcting for the increased pH which may reach the limiting values up to pH 9 during the cultivation (*Scenedesmus dimorphus*-Algae Culture, 2009).
2.1.1.2.4 Mixing or agitation of culture

As mentioned before, *Scenedesmus dimorphus* is heavy cells and can forms thick sediments if there is no constant agitation. Mixing of culture not only to prevent sedimentation of cells but also to ensure an adequate distribution of nutrients, light and to improves the gas exchange between culture medium. In this study, flow culture was used for the cultivation in order ensure the agitation can occur constantly until the end period of cultivation. The speed of the flow from one chamber into another is 12ml/sec.

2.2 Sago effluent

2.2.1. Sago Palm

Flach (1997) as cited (Singhal *et al*, 2008) mentioned that ‘sago palms are economically acceptable, environmentally friendly, and promote a socially stable agroforestry system’. Malaysia is one of three countries that grown sago commercially for production of sago starch, ethanol and many other products. The largest sago plantation and biggest exporter in the state of Sarawak exporting annually about 25,000-40,000 tons of sago products to another country such as Japan, Taiwan, Singapore and other countries. In Sarawak, there are two places where sago plantations have been developed in Sarawak which are the Dalat Sago Plantation and Mukah Sago Plantation.
2.2.2 Sago effluent

Sago palm can produce many valuable products such as starch, ethanol, lactic acid and many more. However, the processign of sago palm utilized the waste. For example the process of separation of stach from the pith can produce solid components known as hampas and waste water. About 10-20 tones of waste produces by each factory everyday and usually release into the rivers. ‘For every kilogram (dry weight) of starch produced, it has been estimated that 20 L of wastewater is generated in the process’ (Bujang et al, 1996).

Bujang et al, (1996) mentioned that approximately 7 tons of sago pith waste was produced daily from a single sago starch processing mill. Recently, these residues were washed off into nearby rivers together with wastewater which can lead to serious environmental problems. Sago wastewater has been reported represents high organic material (hampas), chemical organic demand (COD) and biological oxygen demand (BOD) which agianst the law of standard limit discharge as mentioned in the Environmental Quality Act, 1974.The environment will affected not because of the chemical components of sago effluents but in fact is due to the large amount of production of sago effluent daily (Bujang and Yusop, 2006). Nevertheless, the waste water from the production of sago starch very high carbon to nitrogen ratio (105:0.12) which is suitable as a medium for the growth of microalgae (Phang et al, 2000). Therefore this waste water actually can be manage propelly and at the same time can be use for other purpose which can benefits if use wisely.
CHAPTER 3

MATERIALS AND METHODS

3.1 Microorganism

*Scenedesmus dimorphus* strains UTEX 1237 from the Culture Collection of University of Texas were used in this study.

3.2 Inoculum

The inoculum was obtained by liquid cultivation using Protease’s medium in 1L universal bottle for 5 days. The cultivation is agitated using air pump with temperature around 30°C, pH 8 to 9 and under natural sunlight.

3.3 Sago effluent

Sago effluent was collected from the Herdsen Sago Mill, Pusa, Sarawak. The sago effluent was filtered using a 710 µm stainless filter to eliminate the solid organic materials (*hampas*) in the effluent.
3.4 Characterization of sago effluent

After first filtration, the sago effluent was centrifuged and then filtered using Whatman filter paper. Then, pH of filtered sago effluent (FSE) was adjusted from 4.45 to 8.46 (the optimum pH for *Scenedesmus dimorphus* growth) using 1M NaOH. The sago effluent was characterized for pH, total suspended solid (TSS), glucose and starch contains before used for cultivation.

3.4.1 Total Suspended Solid (TSS)

Firstly, the centrifuge tube and filter membrane was weighed. Then, 20 ml of sago effluent (sample) was poured into centrifuge tube. After that, the sample was centrifuged at 6000rpm with 4°C for 15 minutes. After centrifugation, the supernatant was filtered by using 0.45µm filter membrane. Then, the centrifuge tube containing the pellet and the filter membrane which is used to filter the supernatant was dried in an oven at 60°C for 24 hours. After that, the biomass of total suspended solid was obtained by weighed the dried centrifuge tube and filter membrane.

The calculation of total suspended solid is as below:

\[
\text{mg of Total Suspended Solid/L} = \frac{[(A-B)+(a+b)] \times 1000}{\text{Sample volume (ml)}}
\]

Where,

A= weight of centrifuge tube with pellet

B= weight of empty centrifuge tube
3.4.2 Analysis of Reducing Sugar

Analysis of reducing sugars was based on DNS method by Miller (1959). About 3 ml diluted sample was mixed with 3 ml of DNS solution (Appendix A) in a test tube. The mixture was then boiled for 15 minutes and cooled before adding with 1 ml 40% Rochelle salts. The measurement was made using UV/Visible spectrophotometer (Ultraspec 1100-Pro) at 575 nm. A standard curve was plotted using glucose as standard to read glucose equivalent values.

3.4.3 Starch Analysis

Starch analysis was performed based on iodine-starch complex dinitrometric method test (Nakamura, 1981). The effluent sample was heated to 60-70°C until the residues are dissolved. 1 ml sample was transferred into a test tube and added with 100µl iodine solution. Then, the mixture was made to 10 ml with distilled water and mixed well. The OD was taken using UV/Visible spectrophotometer (Ultraspec 1100-pro) at 590nm. A standard curve was plotted using starch as standards to read starch equivalent values.
3.5 Chu’s medium

For preparation of 1L total medium, 10 mL of Chu Stock Solutions added into 950 mL sterile distilled water. After that, final volume was made to 1L before autoclaved.

**Table 3.3 Chu Stock Solution**

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(Na$_3$)$_2$</td>
<td>20</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>2.5</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>12.5</td>
</tr>
<tr>
<td>Na$_2$CO$_3$</td>
<td>10</td>
</tr>
<tr>
<td>Na$_2$SiO$_3$</td>
<td>12.5</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>0.4</td>
</tr>
<tr>
<td>Trace mineral solution</td>
<td>x</td>
</tr>
<tr>
<td>Vitamin solutions</td>
<td>y</td>
</tr>
</tbody>
</table>
A trace mineral solution was prepared by introducing 950 mL of distilled water individually to dissolve 1 mL of each component. Then the total volume was made to until 1L with distilled water.

Table 3.4 Trace mineral solution (x)

<table>
<thead>
<tr>
<th>Components</th>
<th>Amounts (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_3$BO$_3$</td>
<td>2.48</td>
</tr>
<tr>
<td>MnSO$_4$·H$_2$O</td>
<td>1.47</td>
</tr>
<tr>
<td>ZnSO$_4$·7H$_2$O</td>
<td>0.23</td>
</tr>
<tr>
<td>CuSO$_4$·5H$_2$O</td>
<td>0.10</td>
</tr>
<tr>
<td>(NH$_4$)$_6$MO$_7$O$_2$·4H$_2$O</td>
<td>0.07</td>
</tr>
<tr>
<td>Co(NO$_3$)$_2$·6H$_2$O</td>
<td>0.14</td>
</tr>
</tbody>
</table>
3.6 Lab-scale culture

The *Scenedesmus dimorphus* that grown in 1 L universal bottle (Figure 3.1) containing Protease medium (for optimal growth in laboratory) was transferred to chamber for flow culture to investigate the effect of NaHCO$_3$ in FSE to *Scenedesmus dimorphus* growth. The cultivation of *Scenedesmus dimorphus* in flow culture chamber is show in **Figure 3.2**.

![Figure 3.1: Scenedesmus dimorphus in Proteose medium](image)
The experiment was carried out using Perspex boxes flow culture with speed of the flow is 12.3 ml/sec. The pH was initially adjusted to around 8.50 using 1M NaOH solution and thereafter uncontrolled. The alga was cultivated using FSE which is one with addition of 4 g/L NaHCO$_3$ (Velea et al., 2009) and another one without addition of NaHCO$_3$.

3.8 Sampling

20 ml of sample was taken every 2 days for analysis of starch, glucose and dry cell weight (DCW).