EFFECTS OF CULTURE ENRICHMENT AND LIGHT SOURCE ON GROWTH OF *Spirulina platensis* IN MODIFIED SAGO EFFLUENT

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DECLARATION

I hereby declare that no portion of the work referred in this project has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

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Effects of culture enrichment and light source on the growth of *Spirulina platensis* in modified sago effluent

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ABSTRACT

Spirulina platensis, which is photosynthetic algae, can serve as food supplement to human, feed to animal and used as a coloring compound in industries. The growth of *Spirulina* in modified sago effluent (MSE) is observed with optical density (560nm) every 2 days and biomass is determined according to standard calibration curve (OD vs DCW). In the effects of light, biomass produced in MSE under artificial light was higher compare to natural light with 268.84 mg/L and 214.85 mg/L respectively after 8 days of cultivation. Amendments on MSE showed that culture added with nitrate produced highest biomass at 447.13 mg/L, with bicarbonate at 354.89 mg/L and with phosphate at 322.27 mg/L with the same period under artificial light. Biomass produced from Zarrouk's medium under artificial light (as control, 8 days of cultivation) was 219.91 mg/L and maximum biomass was achieved in this medium was at 596.18 mg/L on the 16 days of cultivation. The maximum biomass of *Spirulina plantensis* achieved in MSE under artificial light was 284.59 mg/L (4 days of cultivation) and at 214.85 mg/L under natural light (8 days of cultivation). *Spirulina platensis* was successfully cultivated in modified sago effluent and the best parameters for the growth was addition of nitrate on MSE under artificial light with maximum biomass at 519.69 mg/L on 10 days of cultivation. Hence, MSE can be used beneficially without disposing into rivers thus minimizing environmental pollution.

Key words: *Spirulina platensis, sago effluent, light sources, culture enrichments*

ABSTRAK

Spirulina platensis merupakan alga berfotosintesis yang dijadikan sebagai makanan tambahan kepada manusia, makanan ternakan kepada haiwan dan digunakan sebagai pewarna dalam industri. Pertumbuhan Spirulina platensis disemak dengan kepekatan optika pada 560nm setiap 2 hari dan biomass ditentu berdasarkan "standard calibration curve". Dalam kesan sumber cahaya, biomas yang dihasilkan dalam sago efluen terubah dengan lampu buatan adalah lebih tinggi pada 268.84 mg/L berbanding dengan lampu semula pada 214.85 mg/L dalm lapan pengulturan. Manakala dalam kesan pengkayaan kultur terhadap sagu efluen terubah di bawah lampu buatan, culture dengan penambahan nitrat menghasilkan biomas yang paling tinggi, 447.13 mg/L berbanding dengan bikarbonat pada 354.89 mg/L dan pada fosfat 322.27 mg/L dengan masa pengulturan yang sama. Biomas yang dihasilkan daripada kultur Zarrouk's di bawah kesan lampu buatan (sebagai kawalan, pada hari kelapan pengultura) ialah 219.91 mg/L dan maksimum biomas yang dicapai adalah 596.18 mg/L dalam 16 hari pengulturan. Di samping itu, maksimum biomas dihasilkan dalam sagu efluen terubah bawah lampu buatan adalan 284.59 mg/L dalam 4 hari pengulturan manakala pada 214.85 mg/L di bawah lampu semula jadi pada 8 hari penngulturan. Spirulina platensis telah berjaya dikulturkan dalam sagu efluen terubah and parameter yang paling baik untuk pertunbuhan adalah penambahan nitrat ke dalam sagu efluen terubah di bawah lampu buatan dengan pencapaian maksimum biomass pada 519.69 mg/L dalam 10 hari pengulturan. Oleh itu, sagu efluen boleh digunakan dengan bermanfaatnya tanpa dibebaskan ke dalam sungai dan pencemaran boleh dikurangkan.

Kata kunci: Spirulina platensis, sagu efluen, sumber cahaya, pengkayaan kultur

LIST OF ABBREVIATION

BOD	Biological Oxygen Demand
BSA	Bovine Serum Albumin
C	Carbon
$CaCl_2.2H_2O$	Calcium chloride-di-water
$CuSO_4.5H_2O$	Copper sulphate-penta-water
COD	Chemical Oxygen Demand
$Co(NO_3)_2.6H_2O$	Cobalt nitrate-hexa-water
DCW	Dry cell weight
DOS	Department Of Statistics
E.coli	*Escherichia coli*
Fe-EDTA	Iron-Ethylene Diaminetetraacetic Acid
FeSO4.7H2O	Iron sulphate-hepta-water
g	Gram
kg	Kilogram
GLA	γ-linolenic acid
g/L	Gram per liter
H_3BO_3	Boric acid
HCl	Hydrochloric acid
HDL	High Density Lipoprotein
JCM	Japanese Collection of Microorganism
K_2HPO_4	Di-potassium hydrogen orthophosphate anhydrous
K_2SO_4	Potassium sulphate

L	Liter
LDL	Low Density Lipoprotein
MSE	Modified sago effluent
Mg/L	Milligram per liter
$MgSO_4.7H_2O$	Magnesium sulphate-hepta-water
mL	Milliliter
mm	Millimeter
nm	nanometer
$MnCl_2.4H_2O$	Manganese chloride-tetra-water
N	Nitrogen
NaCl	Sodium chloride
$Na_2EDTA.2H_2O$	Sodium Ethylene Diaminetetraacetic Acid-di-water
$NaHCO_3$	Sodium hydrogen carbonate
$NaMoO_4.2H_2O$	Sodium molybdate dihydrate
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
OD	Optical density
PO_4	Phosphate
ppb	Part per billion
ppm	Part per million
R^2	Correlation coefficient
Rpm	Revolution per min
tons	Tonnes

Wt.	Weight
$ZnSO_4.7H_2O$	Zinc sulphate-hepta-water
β	Beta
˚C	Degree Celsius
μ	Micron
μm	Micro meter
γ	gamma

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EFFTECS OF CULTURE ENRICHMENT AND LIGHT SOURCE ON GROWTH OF *Spirulina platensis* IN MODIFIED SAGO EFFLUENT

CHAPTER ONE

INTRODUCTION

Sago palm is largely grown in the state of Sarawak in Malaysia. The sago industry not only has contributed as an importance source of starch but also as a source of economical value in Malaysia. However, the generation of sago wastewater from the starch extraction in factories brings about problems to the environment as nearby rivers are contaminated by the sago wastewater (Bujang and Yusop, 2006). Waterways are definitely an important natural habitat for aquatic species and they also provide us an essential source of water for living requirements. Bujang *et al* (1996) reported that more than 1,425 tons of sago effluent is produced per week by a medium sized factory in Sarawak.

The emergence of varied types of functional foods obtained from natural sources and which are high in nutrition are well-known and accepted by people (Sahelian, 2008). *Spirulina* is the best functional food that has obtained attention since many years ago as it possesses high concentration of protein content 60%-70% by weight (Belay *et al*, 1993). Comparing between raw animal and plant tissue, it has the most concentrated natural source of vitamin B-12 in the world (Borowitzka, 1999 cited in Rafiqul *et al*, 2005).

Besides its nutritional value, *Spirulina* is also important in the medical field. Many scientists are interested to investigate the therapeutic effects of *Spirulina* to human beings (Belay *et al*, 1993). According to Hayashi *et al* (1996), *Spirulina platensis* inhibits the replication of viruses such as HIV-1, Influenza A virus and Mumps virus *in vitro* and keeps monkeys and human cell culture away from viral infection (Kozlenko and Ronald, 1998). *Spirulina* also has anti-cancer and immune system effects, maintains intestinal flora and defends against hyperlipidemia (Belay *et al*, 1993). Hence, the demand for *Spirulina* is increasing annually and the production of *Spirulina* for human consumption is mainly in USA, Europe and Japan (Belay *et al*, 1993). However, none of the companies in Borneo is producing *Spirulina.*

1.1. Objectives

From the overview above, the objectives of this study are:

- To develop the cultivation of *Spirulina platensis* in sago effluent in order to minimize cost and environmental pollution.

- To compare the effects of light towards the growth of *Spirulina* between natural light source and artificial light source.

- To compare growth of *Spirulina* in modified sago effluent and modified sago effluent amended with enrichments.

CHAPTER TWO

LITERATURE REVIEW

2.1. Sago

2.1.1. Sago starch

"Sago starch is extracted from sago logs through several processing steps such as debarking, pulping, starch extraction, dewatering, drying and packing" (Bujang and Yusop, 2005). It is mainly produced in the state of Sarawak in Malaysia. Sago palms occupy over three quarters of the peat land of Sarawak and it is the only plant that is able to grow well and vigorously in swampy areas (Bujang and Yusop, 2006).

Sago palm does not need much care and pesticide. Each of the mother palm can produce many suckers making replanting not necessary after the mother trunk is cut. As a result, sago starch has a high commercial value and according to Bujang and Yusop (2005), the export of sago starch is 61,000 tonnes annually and valued at US$9.15 million for the year 2005.

2.1.2. Sago effluent

The waste produced from the extraction of sago starch can be in solid form or wastewater. Sago effluent generated from the extraction process can cause pollution to our environment as sago effluent is released into nearby rivers and waterways without any proper treatment. Pollution that occurs is not because of the chemical components of sago effluent but in fact is due to the large amount of production of sago effluents daily (Bujang and Yusop, 2006). "For every kilogram (dry weight) of starch produced, it has been estimated that 20L of wastewater is generated in the process" (Bujang *et al*, 1996). Moreover, according to DOS (2002), Sarawak has over 30 large sago factories, thus enhancing the possibility of water pollution from the sago industries.

Previous researches (Bujang *et al,* 2004) showed that sago effluent can be aerobically treated with enzyme and microbial amendment such as Bakwira where the mixed liquor is aerated before being released into the waterways with a reduction in Chemical Oxygen Demand (COD) by 96% after 32 days.

Phang *et al* (2000) reported that sago effluent contain high ratio in carbon to nitrogen by which this ratio can be increased by carried fermentation in anaerobic digester. "This digested effluent with an average C: N: P ration of 24: 0.14: 1 supported growth of *Spirulina platensis* with an average specific growth rate (μ) of 0.51 per day compared with the average μ of 0.54 per day in the organic Kosaric medium in a high rate algal pond" (Phang *et al*, 2000)

2.2. *Spirulina*

2.2.1. Morphology and Characteristic

"*Spirulina* is a single-celled, unbranched, helicoidal, and filamentous blue-green alga" (Belay *et al*, 1993). It is a cynobacterium that contains chlorophyll a, carotenoids and some unusual accessory blue pigments and red pigments. Blue pigments are phycobilins and phycocyanin while red pigment is phycoerythrin. The name of '*Spirulina*' is actually derived from Latin word which means spiral in shape. This can be proven by referring to **Figure 2.1** and **Figure 2.2** below which is the structural analysis of *Spirulina platensis* that had been done by using light microscope. Binary fission reproduction method is used by *Spirulina* for cell division (Sanchez *et al*, 2002).

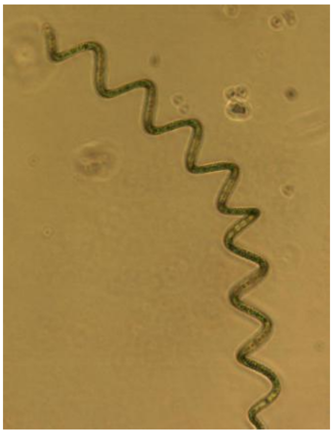

Figure 2.1: Structural analysis of *Spirulina platensis* under Olympus light microscope at 20X.

Figure 2.2: Structural analysis of *Spirulina platensis* under Olympus light microscope at 40X.

2.2.2. Nutritional value of *Spirulina*

2.2.2.1. Protein

Spirulina is of benefits to human as these algae contains high quality and quantity of protein at about 60%-70% by weight (Chronakis *et al*, 2000). "*Spirulina* contains essential amino acids; the highest values are leucine (10.9% of total amino acids), valine (7.5%), and isoleucine (6.8%)" (Cohen, 1997 cited in Sanchez *et al*, 2002).

Essential amino acids cannot be produced by humans' body and hence have to be consumed in the form of supplements. According to Henrikson (1994), "*Spirulina* can grow quickly and produces 20 times more protein by surface unit than that of soya beans" (Sanchez *et al*, 2002).

2.2.2.2. Carbohydrates

Spirulina consist 15%-25% of carbohydrates by dry weights which are constituted mainly by simple carbohydrates such as glucose, fructose and sucrose (Falquet, 1997). The cell wall structure of *Spirulina* is quite similar to Gram-positvie bacteria (Falquet, 1997). These algae actually composed of four layers in their cell wall (Sanchez *et al*, 2002). One of the four layer is made up from β-1,2-glucan which is a type of polysaccharide that is hard to be digested by human beings (Sanchez *et al*, 2002). However, the concentration of this layer is low (less than 1%) and its thickness is only about 12nm (Sanchez *et al*, 2002). According to Kozlenko and Ronald (1998), *Spirulina* is more easily to digested as compare to others algae as it has a soft cell wall complex which is composed of sugars and protein. This is because the cell walls of *Spirulina* are fragile and easily digested by enzyme (Falquet, 1997). By comparing *Spirulina* to *Chlorella* and *Scenedesmus*, these two algae are hard to harvest and digest as they contain cellulosic membrane which has low ability being digested (Danesi *et al*, 2002). Hence, this high digestibility makes *Spirulina* an ideal supplement for human.

2.2.2.3. Lipids

Lipids constitute 4-7% of the dry weight of *Spirulina* (Sanchez *et al*, 2002). It contains essential fatty acids linoleic acid (LA) and γ-linolenic acid (GLA) which cannot be secreted in the body (Sanchez *et al*, 2002). According to Falquet (1997), "GLA are the precursor of the postglandins, leukotrienes and thromboxanes that serve as chemical mediators of inflammatory and immune reactions". Cohen (1997) reported that GLA are able to lower low-density lipoprotein (LDL) cholesterol more efficiently than LA (Sanchez *et al,* 2002). This is one of the reasons why *Spirulina* is a valuable pharmaceutical product. Moreover, this can be proven by the research that had been done by Nakaya *et al* (1988) which is explained in the **Section of 2.2.3.1.1**.

2.2.2.4. Vitamins

Spirulina contains pro-Vitamin A (beta-carotene), vitamin E, water soluble-vitamin and also vitamin B_{12} (Falquet, 1997). Belay *et al* (1993) reported that *Spirulina* posses 10 times higher in the content of β-carotene as compared to the concentration of β-carotene in carrots by comparing dry weight. Research had been done by Annapurna *et al* (1991) stated that the carotenoids of *Spirulina* can be utilized by human excellently. "Beta-carotene accounts for 80% of the carotenoids present in the *Spirulina*" (Falque, 1997).

2.2.3. Application of *Spirulina*

2.2.3.1. Benefits of *Spirulina* as health products

2.2.3.1.1. Effects against Hyperlipidemia

The study done by Nakaya *et al* (1988) was conducted among 30 patients that had mild hyperlipidemia and mild hypertension (Belay *et al*, 1993). They were split into two groups and the consumption of *Spirulina* was the same for each group, 4.2g per day for four weeks (Nakaya *et al*, 1988 cited in Belay *et al*, 1993). After that, the consumption of *Spirulina* was stopped for four weeks to observe any significant of differences (Nakaya *et al*, 1988 cited in Belay *et al*, 1993). The result showed that LDL-cholesterol in 30 patients of both group were found to be lowered but increased from its baseline value after continuous consumption of *Spirulina* was terminated (Nakaya *et al*, 1988 cited in Belay *et al*, 1993). The level of the HDL-cholesterol did not show any changes (Nakaya *et al*, 1988 cited in Belay *et al*, 1993).

2.2.3.1.2. Effects on intestinal flora

According to Kozlenko and Ronald (1998), consumption of *Spirulina* can increase beneficial intestinal flora such as *Lactobacillus* and *Bifidus*. Previous research done by Tsuchihashi *et al* (1987) showed that the number of *Lactobacillus* in the caecum of rats increased 3 times compared with those rats that were not supplied with a 5% *Spirulina* in their diet (Belay *et al*, 1993). Increasing beneficial intestinal flora can prevent problems arising from pathogen such as *E.coli*. (Kozlenko and Ronald, 1998).

2.2.3.1.3. Effects against Vitamin A deficiency

Vitamin A deficiency is mainly caused by the lack of the beta-carotene content and this might cause blindness if the situation is serious. Clinical studies conducted by Seshadri (1993) on 5000 Indian pre-school children proved that supplement of one gram of *Spirulina* daily was effectively against chronic Vitamin A deficiency among the children